Thesis submitted for Doctor of Philosophy

Phytochemical Studies of Selected Medicinal and Aromatic Plants of Nepal and Effect of γ-Irradiation on Volatile Organic Compounds of *Glycyrrhiza uralensis* F.

by
Rajendra Gyawali

Department of Applied Science
Major: Food Science and Biotechnology
Graduate School of Chosun University, Korea

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ABBREVIATIONS

AchE	Acetylcholinesterase
CAST	Council of Agricultural Science and Technology
EI	Electron Impact
FCC	Food Chemical Codex
FDA	Food and Drug Administration
Fig.	Figure
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometery
HMGN	His Majesty's Government of Nepal
ICGFI	International Consultative Group on Food Irradiation
IAEA	International Atomic Energy Agency
IIP	Isopentenyl Pyrophosphate
JECFA	Joint Expert Committee on Food Additives
MAP's	Medicinal and Aromatic Plants
MS	Mass Spectrometry
MVA	Mevalonic acid
RI	Retention Index
RT	Retention Time
SDE	Simultaneous Steam Distillation and Extraction
Temp.	Temperature
UV	Ultraviolet and Visible Specpectroscopy
VOC's	Volatile Organic Compounds
WHO	World Health Organization

APPENDICES

ABSTRACT

Phytochemical Studies of Selected Medicinal and Aromatic Plants of Nepal and Effect of γ-Irradiation on Volatile Organic Compounds of *Glycyrrhiza uralensis* F.

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This study was performed to enumerate the phytochemicals alkaloids, anthocyanosides, cardiac glycosides, carotenoids, coumarins, glycosides, flavonoids, saponins, tannins, triterpenoids and essential oils from medicinal and aromatic plants (MAP's) of Nepal. The screening tests were carried out on aqueous and alcoholic extracts using standard procedures. The essential oils were extracted using simultaneous steam distillation and extraction (SDE) and the volatile organic compounds (VOC's) were analyzed by gas chromatography/mass spectrometry (GC/MS). Effect of γ-irradiation on VOC's of *Glycyrrhiza uralensis* F. was studied to ascertain its threshold limit and acceptability. Effect of different plant extracts on spontaneously induced rat uterine smooth muscle cell contractility was also evaluated.

Phytochemical screening of forty-seven MAP's of Nepal belonging to 45 genera and 39 families revealed the presence of plant secondary metabolites in all species with the various concentrations. Glycosides, tannins, terpenoids, flavonoids, alkaloids and saponins were the major secondary metabolites present in most of the plants while cardiac glycosides and carotenoids were rarely detected. Amongst the investigated plants, 81% plant species contained glycosides, 70% showed the presence of tannins, 66% terpenoids, 62% alkaloids, 60% flavonoids, 57% saponins, 45% volatile oils, 43% coumarins, 30% anthocyanosides, 17% cardiac glycosides and 15% carotenoids. Flowers and roots were rich in alkaloids, flavonoids,

tannins and saponins. Most of the plants can be seen as a potential source of useful drugs particular reference to glycosides, flavonoids, saponins, tannins, and terpenoids. However total 8 species *Asparagus racemosus*, *Bergenia ciliata*, *Daphne bholua*, *Rhododendron arboretum*, *Schima wallichii*, *Terminalia chebula*, *Tinospora cordifolia* and *Woodfordia fructiosa*, out of 47 species containing high concentrations of diverse phytochemicals are confirmed the potential species of medical value.

Study on the essentials oils of 8 MAP's of Nepal revealed that all the plants have the existence of essential oils but their concentration varies. The yields of essential oils obtained from *Acorus calamus*, *Asparagus racemosus*, *Bergenia ciliata*, *Centella asiatica*, *Dipsacus mitis*, *Swertia chirata*, *Terminalia chebula* and *Woodfordia fruticisa* were 0.7, 0.005, 0.006, 0.1, 0.006, 0.024, 0.004 and 0.019% respectively. Similarly, numbers of VOC's tentatively identified in essential oil of above species were 53, 49, 44, 53, 53, 77, 53 and 81 respectively. Aldehyde was detected as a dominant group in *T. chebula* and *W. fruticosa*. Similarly ketone and alcohol were dominant in *A. calamus, B. ciliata, S. chirata, A. racemosus* and *D. mitis* respectively and hydrocarbon group was dominant in *C. asiatica*. Compounds *β*-asarone, borneol, 5,6-dihydro-2-pyranone, [*Z*]-*β*-farnesene, 2-butenal and undecanoic acid, were detected as a major compounds in *A. calamus, A. racemosus, B. ciliata, C. asiatica, D. mitis and S. chirata*, respectively while furfural was commonly dominant in both *T. chebula* and *W. fruticisa*. Some of the compounds such as linalool, farnesol, α-terpeniol were common among many species. Majority of compounds detected in those species were monoterpenes. More than 9 monoterpene hydrocarbons viz: [*Z*]-ocimene, *β*-phellandrene, *β*-myrcene, *β*-pinene, *α*-pinene, camphene, thujene, limonene, 3-carene were prevalent constituents of species *A. calamus, A. racemosus* and *C. asiatica.* Sesquiterpene hydrocarbons such as α-copaene, *β*-elemene, junipene, [*E*]-caryophyllene, *α*-humulene, *β*-farnesene etc were highly distributed in *A. calamus,* and *C. asiatica.* Some of them were detected in *S. chirata* and very few were detected in *T. chebula* and *A. racemosus*. Oxygenated terpenes were higher in *S. chirata* and *W. fructicisa*. Essential oils obtained from *C. asiatica. A. calamus, A. racemosus* and *S. chirata* can offer good source for terpenoids, much wanted aromatic chemicals in perfume, flavour and pharmaceutical industries. However, due to low concentrations of essential oils in plants *D. mitis, B. ciliate, A. racemosus* and *T. chebula*, can not be recommend for further studies in course of extraction and separation of essential oils. Species *W. fruticisa* and *S. chirata*, offer new interest whereas essential oil content of *C. asiatica* and *A. calamus*, were verified.

Study on the effect of γ-irradiation on the VOC's of licorice (*Glycyrrhiza uralensis* F.) showed that low irradiation doses did not affect the yield and number of compounds. Sixty-one volatile organic compounds of the essential oil were tentatively identified in licorice. Above the dose of 1 kGy, one more compound of aldehyde group detected and a few kinds of compounds detected upto 10 kGy irradiated samples were disappeared at 20 kGy irradiated sample. Though the 10 kGy dose of irradiation induced the maximum yield of essential oil of licorice by 12.12%, the maximum dose given at 20 kGy inhibited the total yield by 6.11%. Highest numbers of the compounds highly enhanced at 10 kGy doses resulted that the total yield of volatile oil was found maximumly increased at this dose. Though the content of several VOC's increased after irradiation, the content of major compounds 4-terpineol, myrtenal, tetramethylpyrazine, hexanoic acid, azulene and *p*-cymene were decreased by the process. Alcohol group was detected as major volatile chemical class (44.12~51.71%) of irradiated samples like in non-irradiated sample. The relative content of total alcohol compounds from volatile oil of irradiated licorice was increased by 5.47~11.44% from 1~10 kGy but decreased by 4.91% at 20 kGy dose of irradiation. The contents of functional groups identified from volatile oil of licorice were changed after irradiations but their proportions were variable in dose dependent manner. We conclude that γ-irradiation upto 20 kGy causes only slight differences in the content and composition of VOC's of licorice. Therefore, the application of irradiation is feasible as it did not undergo major qualitative and quantitative loss of VOC's when subjected to such irradiation doses.

Biological activity of different plant extracts on the smooth muscle cell contraction was also evaluated. Uterine smooth muscle tissues were obtained from non-pregnant rats (n=21). Dramatic muscular relaxation on spontaneous contractility was obtained by methanol extract of *Dipsacus mitis* at concentration of 6500 μg/ml and slight relaxation on spontaneous contractility was obtained upto concentration of 20000 μg/ml of *Woodfordia fructicosa*. These results appear to justify their traditional uses.

CHAPTER I

Phytochemical Screening of Medicinal and Aromatic Plants of Nepal

1. Introduction

1.1. Plant as a resource for medicinal remedies

Ethnobotanical uses of plants and plant products in the form of crude drugs such as tinctures, teas, poultices, powders and other herbal formulations have been in practice in various cultures for the thousand of years (1-10). In an age when toxic drugs are increasingly unwelcome and when conscious people are using viable alternatives, plant base remedies have established their importance globally. Indeed up to the 20^{th} century, much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples (11). Current trends have shown that people are willing to try natural medicine especially those of plant base because they are natural and have negligible or no side effects. People of developing countries are mostly using the plant-based medicine because plants are easily available and sometime only source of health-care possible to the poor. The importance of medicinal plants is demonstrated by fact that 60% of the population of world and 80 % of the population in developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs (12). Therefore the interest lies in plants and their phytochemical constituents as likely source of new commercial drugs. But the knowledge of plant constituents gained so far is still meager, considering the huge number of species available in the world. Out of nearly 300,000 species of higher plants, about 10,000 species are considered to be medicinal one and only a small proportion has been investigated for medicinal properties and still small percentage of all plants have been investigated from the phytochemical and/or pharmacological point of view (13,14). Thus, even if very small percentage of medicinal plants has been investigated, the medicinal plants and herbs continue to be the source of proven medicaments and revolutionary drugs. Medicinal plant trade is a booming business worldwide. Consequently, increase of consumption of medicinal plants and herbs enhances the responsibility of researchers in order to assuring the chemical profile of these herbs. As per the estimates, the global market of the medicinal

plants and herbal products is about 62 billion US $ and is expected to increase at the level of 5 trillion US $ by the year 2050 (15).

Drug resistance development and adverse side effects of synthetic medicines have additionally encouraged many people to look for safe alternative source of treatment. While there have been a grate deal of progress made in understanding plant natural products, a general lack of knowledge and much misinformation remain about natural products in plants. However, World Health Organization (WHO) has emphasized the need for utilization of indigenous system of medicine based on the locally available medicinal plants in the developing countries. During the last two decades, the western societies are increasingly realizing that the drugs from natural sources are safer. Therefore, an upsurge in the use of products based on plants is expected, especially in the field of health care products. In this process, researchers are concentrating on age-old traditional source of medicinal formulations. Many of the medicinal plants offer us new source of drugs that have been used effectively for centuries in traditional medicine. It is so because; medicinal plants synthesize and accumulate various chemical compositions or different metabolites such as alkaloids, glycosides, steroids, flavonoids or other group displaying diverse pharmacological activities. In traditional medicine, plant drug are mostly prescribed on the crude essence forms and the crude drugs not only contains an active principle but also other phytochemicals; some of them are synergistic, some antagonistic, some toxic and some inactive (16). Hence, there have been global interests in scientifically validating the chemistry of plant drugs for dissolving the actual value of folkloric remedies, their efficacy and safety (17). Several phytochemical surveys have been published which involved some plant accessions collected from many parts of the world. The major chemical substances of the interest in these surveys have been the alkaloids and steroidal saponins, however other diverse groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, essential oils etc. have also been reported. Phytochemical surveys on medicinal plants are seen as the first step towards the discovery of new drugs, giving a better indication of the usefulness of the plants and will be benefited in ensuring the isolation of the bioactive principles. The majority of traditional medicines used in developing countries have not been evaluated for quality, safety, efficacy to same standards as those in developed countries. Nevertheless, there are some remarkable claims made for their effectiveness during the practice of traditional medicines.

1.2. Natural products

The term natural product is generally taken to mean a secondary metabolite— a small molecule that is not essential to the growth and development of the producing organism. They are at the forefront of research in the search for new therapeutic agents. Most of the natural products of interest to the pharmaceutical industry are secondary metabolites. Secondary metabolites are generally classified into five categories: terpenoids and steroids, fatty acid–derived substances and polyketides, alkaloids, nonribosomal polypeptides, and enzyme cofactors. These metabolites isolated from living organisms, plants, animals, insects, arid microorganisms have been providing novel and clinically active drugs. Plants are particularly interesting because they have the broadest spectrum of the biosynthetic capability and produce a wide variety of compounds. Plant originated natural products have played and will continue to play important role in pharmaceutical industry to discover and deliver chemicals and biological entities for the treatment of various diseases (18). As a result, the discovery of modern medicine has been concentrated mostly on folkloric herbal medicines, used in some culture or country, for natural products screening programmes. Some natural products have found direct application as drug entities, while some provide a starting point for new synthetic compounds with diverse structures (19-22). The continual search for using natural products medicines has acted as a catalyst for exploring plant materials and their constituents. Research on natural products accounts for approximately 48% of the new chemical entities reported from 1981–2002 (23). Beside that, recent approvals of several new plant-derived drugs, based on plant secondary metabolites, increased the number of chemical entities and value of natural product. Plants, which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids and polyphenols are generally superior in their anti-microbial activities (24). Among these, the important constituents like alkaloids, tannins, flavonoids and phenolic compounds are of particular interest in respect to their therapeutic effects (25). Research on the properties of herbal drugs gives the additional and scientific support in traditional system of herbal medicine for the treatment of many human diseases (26). Modern analytical tools have revealed the enormous variety of bioactive principals of medicinal plants and confirmed their potential values for use as medicines, their actions on human and animal systems. Screening of extracts of natural products has had an impressive history of identifying active agents.

1.3. Plant metabolites

The metabolic performance of plant can be distinguished into primary metabolism and secondary metabolism. Primary plant metabolites can be considered as those metabolites essential to the life of the plant. Primary metabolism refers to the synthesis and consumption of nucleic acids, α-amino acids, proteins, fats and carbohydrates that are essential for the survival. These simple molecules are used to produce polymers essential in the life of the plant. In contrast, secondary metabolism proceeds to nonessential compounds such as alkaloids, terpenes, flavonoids, certain aromatic amino acids and polyphenols for the continuity of the lifecycle or for growth and development (27). Primary metabolites provide some precursor molecules for the secondary metabolic pathway, such as acetyl-coenzyme A which would complete its metabolic pathway with the formation of isoprenoids, the largest group of secondary natural compounds (28). Enzymes are the proteins that act as organic catalysts. Such metabolic pathway ends up with products like essential oils, resins, saponins and glycosides etc.. Wide molecular diversity of secondary metabolites throughout the plant kingdom represents an extremely rich biogenic resource for the discovery of novel drugs.

The main biosynthetic pathways are outlined in fig. 1 to emphasize the basic pool of reactions which produce the major polymeric tissue materials such as polysaccharides, nucleic acid, lignins, proteins, fatty acids and fats and the secondary metabolites, which are commonly known as natural products. Among the hundreds of compounds of primary metabolism, only few important such as acetic acid, aromatic amino acids and aliphatic amino acids serve as material for the elaboration of the thousands of known natural products. Most biochemical pathways begin with the oxidative breakdown of glucose into pyruvic acid, which further oxidized to acetic acid. There are two major pathways which commence with acetyl-coenzyme A derived from acetic acid: one of which proceeds by the stepwise addition of C_2 units to a polyketide chain, and the other by condensation of C_5 units to make isoprenoids. Isoprenoids include essential metabolites such as sterols, acyclic polyphenols, carotenoids as well as a large variety of compounds with a less evident physiological role (29). Steriods are the complex compounds composed of 4 carbon rings with 17 points of attachment for other molecules thus having pronounced effects on animals.

The combination of pyruvic acid with erythrose produces shikimic acid which is converted into prephenic acid by joining with another pyruvic acid. These two cyclic compounds are building blocks for many aromatic substances. The amino acids can make both proteins and alkaloids.

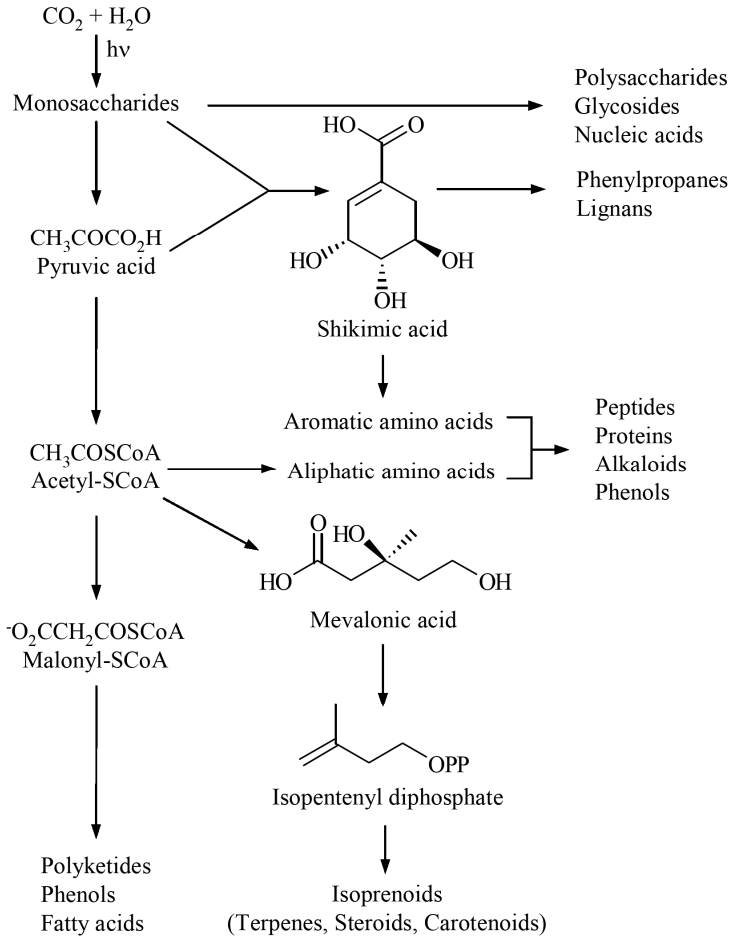

Fig. 1. An overview of primary metabolites and their links to secondary metabolites.

Alkaloids are the compounds composed of at least one nitrogen atom in a heterocyclic ring. More than 10,000 different alkaloids have been discovered in species from over 300 plant families (30). Many alkaloids, though poisons, have physiological effects that render them valuable as medicines. Their medicinal properties are diverse as they contain at least

one nitrogen atom in an amine-type structure that makes them pharmacologically active. Certain alkaloids act as cardiac stimulants in arrhythmias and respiratory diseases. Alkaloids are more common in the dicotylodons than in monocotyledons. Plant families *Liliaceae, Amaryllidaceae, Ranunculaceae, Rubiaceae, Apocynaceae, Berberidaceae, Leguminosae, Papaveraceae, Solanaceae* etc. are prominent alkaloid-containing families.

Anthocyanosides are polyphenols related to flavonoids group. Some studies have shown that anthocyanosides promote production of rhodopsin and therefore improve night vision (31) and are effective against capillary hyperfiltration (32). They also support the immune system during periods of physical and mental stress. They help to maintain the integrity of capillaries and to stabilize collagen. Anthocyanosides stabilize phospholipids of the endothelial cells and enhance synthesis of collagen and mucopolysaccharides and therefore help to maintain the structural integrity of the arterial walls and muscle damage.

Carotenoids are compounds containing 8 isoprene units. About 600 compounds naturally occurring in fruits and vegetables are known. Among these compounds, many are antioxidant. Some compounds like lutein have the potential anticarcinogenic properties (33). They can be used for the treatment or photosensitization, retinal diseases and glaucoma. In the intestine, β-carotene is converted to retinol (Vitamin A). Carotenoids are also safe colouring agents for food substances and cosmetics (34). They are responsible for the orange and yellow colors of plants.

Cardiac glycosides are glycosides of mostly C_{23}-steroidal compounds. They are composed of two structural features: the sugar (glycoside) and the non-sugar (aglycone - steroid) moieties. The sugar moiety appears to be important only for the partitioning and kinetics of action. Cardiac glycosides induce strong specific effects on the myocardium and enhance the strength of cardiac contractions, theraby control the blood pressure (35). Cardiac glycosides are well known to the treatment of congestive heart failure (36). There are no synthetic substitutes for cardiac glycosides; as medicinal plants are the sole source of these substances. Most members of the family *Asclepiadaceae* contain cardiac glycosides and most found in genus *Digitalis.*

Coumarins are shikimate-derived, benzo-α-pyrone derivatives that are present in plants both in free and as glycosides. They are used as perfumes and flavoring additives in food which gives a characteristic odour to hay. Plants containing certain amounts of coumarin, are today used as diuretics and digestives. In addition, they are also used to prevent heart diseases,

stroke and thrombosis. Coumarin and its derivatives are principal oral anticoagulants as they plays important role in the biosynthesis of prothrombin. They are frequently found in the following plant families: *Apiaceae, Rutaceae, Asteraceae and Leguminosae.*

Essential oils are complex multicomponent mixtures of volatile substances such as monoterpenes, sesquiterpenes, aromatic compounds and their derivatives. They are easily absorbed into the mucous membranes and afterwards distributed to all parts of the body and exert distinct effect. They are used in aromatherapy, perfumes, flavoring agents, natural pesticides, antioxidant agents, natural sprout inhibitors, natural preservatives and therefore they are highly valuable for their anti-spasmodic, restraining, diuretic, anti-biotical, antimicrobial, antifungal, insectisidal, and anthelmintic efficiency (37). The plant families that possesses species that yield a majority of the most economically important essential oils are *umbelliferae, compositae, pinaceae* etc.

Flavonoids are low molecular weight polyphenol substances that are widely distributed in the flora with more than 6500 different compounds described (38). Flavonoids have been shown to have anti-inflammatory, analgesic, anti-tumor, anti-HIV, anti-diarrheal, anti-fungal, anti-hepatotoxic anti-lipolytic, anti-oxidant, vasodilator, immunostimulant and anti-ulcerogenic activities (39). The chemical structures of flavonoids resemble those of nucleoside and folic acid providing a key to their biological actions (40). Multiple combinations of hydroxyl groups, sugars, oxygens, and methyl groups attached to these structures create the various classes of flavonoids: flavanols, flavanones, flavones, flavan-3-ols (catechins), anthocyanins, and isoflavones. The plants containing 0.5-3% flavonoids are characterized as flavonoid drugs.

Glycosides are compounds containing a carbohydrate and a noncarbohydrate residue in the same molecule. Depending upon the nature of their aglycones, glycosides are derived into cardiac glycosides, anthrax-glycosides, irridoids, cyanogenic glycosides, thioglycosides and isothiocyanates. If the carbohydrate portion is glucose, the resulting compound is a glucoside. They possess a variety of biological activities and have found importance in therapeutic, nutritional and clinical use. The carbohydrate residue is attached by an acetal linkage at carbon atom 1 to a noncarbohydrate residue or aglycone or nonsugar component.

Saponins are diverse group of compounds possessing an aglycon moiety linked to one or more sugar or oligosaccharides. They are known for antiarrhythmic, sedative, analgesic, anti-inflammatory, expectorant and diuretic activities (41). The steroidal

saponins are important precursors for steroid drug as well as for treating addison's disease, arthritis, blepharitis, keratitis and iritis as well as viral and fungal diseases. Plants containing terpenoidal saponin exhibit various pharmacological activities; anti-inflammatory, anti-tissuive, expectorant, analgesic etc.. The saponins make strong cytotoxic drugs, resolve the red blood cells and induce irritation on the mucous membrane, which activate the cough and sneeze reflex. Saponin inhibits the Na^+ efflux, which strengthens the concentration of heart muscles and thereby reducing congestive heart failure (36). Saponins are also used in toothpaste as well, and in gargles, shampoo or for foaming purposes in beverages.

Tannins are known as phenolic compounds of high molecular weight containing sufficient hydroxyls and other suitable groups to form effectively strong complexes with protein and other macromolecules under the particular environmental conditions. Among the plant kingdom, tannins are very abundant to protect them from herbivores. They are characterized by their ability to form complexes with other macromolecules (42). Tannin increase efficiency in nitrogen recycling to the rumen because they stimulate increased saliva production. Tannins are used against the diarrhea and as an antidote in poisoning by heavy metals. Their main characteristic is that they bind and precipitate proteins, draw the tissues closer together and improve their resistance to infection. They can have a large influence on the nutritive value of many foods.

Terpenoid family is the largest family of natural compounds, consisting of >40,000 different molecules (43). The compounds isopentenyl pyrophosphate (IIP) and 3,3-dimethylallyl pyrophosphate originated from mevalonic acid (MVA) generates the enormous diversity of carbon skeletons characteristic of the terpenoid family of natural products (Fig.1) (44,45). Many of the terpenoids including menthol, nootkatone, sclareol and linalool are commercially used as flavors and fragrances in foods and cosmetics (46). These compounds also protect against cancer by deactivating steroidal hormones and slowing cell division. Terpenoids can have medicinal properties such as anti-ulcer, hepaticidal, antimicrobial or diuretic etc (47,48). Common terpenes include limonene, pinene, chamazulene and farnesol possess remarkable anti-inflammatory, anti-bacterial, anti-fungal, anthelmintic, anti-malarial and molluscicidal activities (33). The health benefits promoted by monoterpenes, diterpenes and tetraterpenes were recently reviewed and discussed in litereature (49).

1.4. Flora of Nepal

1.4.1. Diversity of plant resources in Nepal

Nepal is located at 26° 22’ to 30° 27’ N latitude and 80° 04’ to 88° 12’ E longitude, occupying area of 147,181 square kilometer, where the altitude differs from 60 m to 8848 m above the sea level and correspondingly represents climatic zones ranging from sub tropical to alpine and shows a resultant biodiversity of 35 forest types and 75 vegetation types (50). Due to its geographic and climatic diversity, the relatively small country, Nepal, occupying just 0.1% of the world's total land mass is surprisingly ranks 27th in the world comprising 2.5% of the total global flora (51). The Himalayan region including Nepal shows the highest richness for endemic species and medicinal herbs. The floral diversity of Nepal comprises about 6,000 species of flowering plants, 380 species of pteridophytes, 1,037 species of bryophytes, 465 species of lichens, 687 species of algae and over 1,600 species of fungi (52-55). The medicinal and aromatic plants (MAP’s) database for Nepal includes 1,624 species belonging to various taxonomic groups (56).

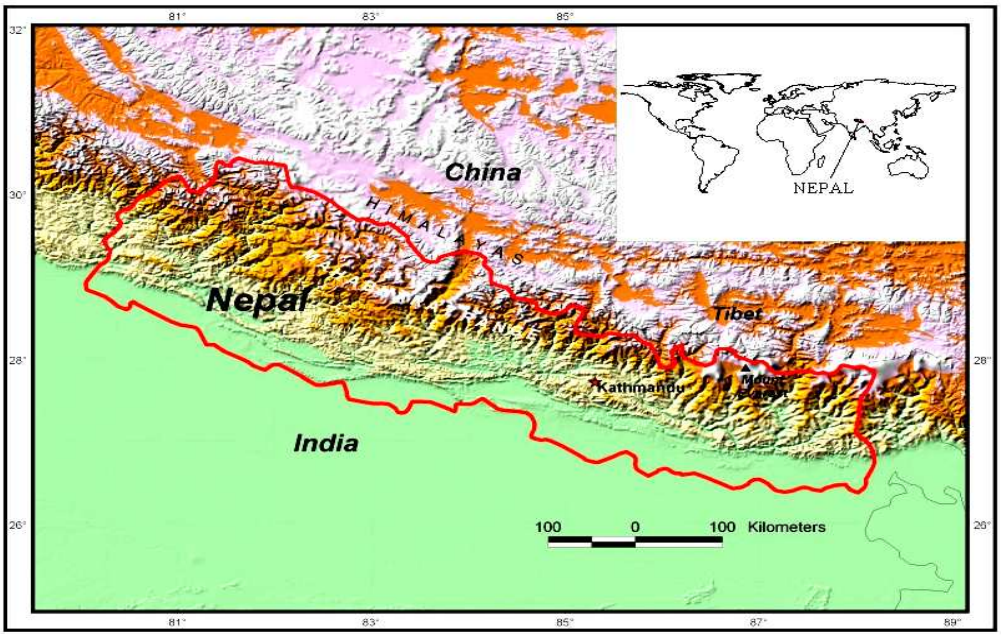

Fig. 2. Map of Nepal.

1.4.2. Traditional medicine in Nepal

Nepal is a multiethnic and multilingual country, with its 25 million people comprising 61 different ethnic groups speaking 11 languages and 71 distinct dialects. These people follow Hinduism, Buddhism, Bon religions etc. and they have strong belief on traditional herbal medicinal practice for health treatments. Throughout their long history Nepalese people have used plants as a mainstay of everyday life. The history of the medicinal plant use in the Himilayas is found in the *Rigveda.* This work was written between 4500 BC and 1600 BC, is supposed to be the oldest repository of human knowledge and describes 67 plants (57). Thus from the mythological era human being has been using plant products for cure of various ailments. After the *Rigveda, Ayurveda* describes the medicinal importance of 1200 plants. The *Charak Samhita* (900 BC) and *Susruta Samhita* (500 BC) enumerate the art of surgery, therapeutics and medicines in details on the basis of *Atharveda*. Pieces of literature written in the Nepali, Newari, and Sanskrit languages contain records of Nepali medicinal plants. The original "*Saushrut Nighantu*" written on palm leaves in Newari script and Sanskrit verses during Mandeva Era 301 (879 AD), is said to be the oldest of these books. However, the knowledge of using these systems was accessed by Nepali *Vaidhyas* as early as about 879 AD (58). In addition to Ayurvedic system, medicinal plants are also codified in other traditional medical systems, including *Chinese, Amchi, Unani, Siddha, Homeopathy* etc. (59,60). Starting from the hand-written pharmacopeia to the modern research, dealing with medicinal plants in Nepal, is widely scattered in a large number of publications. The Department of Ayurveda, Ministry of Health, HMG/Nepal in 1998 listed essential *Ayurvedic* drugs comprising of 339 preperations under 44 main heading of symptomatic diseases. Medicinal plants of Nepal were widely traded across the boarders to Tibet as early as 600 AD (61). Presently, over 90 % of the total export from Nepal is to India and mostly in the crude form. Conservative estimates of the annual Nepalese alpine and sub-alpine medicinal plants vary from 480 to 2500 tons, with a total harvest value of US$ 0.8-3.3 million (62). Presently the value is much higher already.

South Asia is home to many rich, traditional systems of medicine. Ayurvedic methods date back to 5000 B.C. Along with the Unani, Siddha and Tibetan systems, they remain an important source of everyday health and livelihood for tens of millions of

people. Medicinal and aromatic plants (MAP's), including trees, shrubs, grasses and vines, are a central resource for these traditional health systems, as well as for pharmaceutical (or allopathic) medicines. There are more than 8,000 plant species in South Asia with known medicinal uses. MAP's are widely used in Nepal as medicine, additives, beverages, cosmetics, sweeteners, bitters, spices, dying agents and insecticides. Crude-drugs are commonly given in the form of powder, decoctions, and infusions or in ointments. The powder is prepared from dried parts while infusions are extracted by boiling the plants in water. The dried plants are also prepared to smoke like cigarettes for the treatment of cough, cold and headache. The herbal medicines are applied externally on cuts, wounds, boils, pimples, ringworms, muscular swelling and dislocation of bones. Plants are also used as a hot baths for skin diseases. The single plant or plant parts such as root, rhizome, stem, leaf, bark, wood, gum, latex, ash, flower, fruit and seed, or admixture of different species of plants are recommended for treatment. The rural communities of Nepal have a long tradition of using plant resources for their various basic needs such as food, medicine, firewood, timber, fodder and agricultural tools. They collect plants from various habitats, such as forest, scrub, grassland and cultivated fields, and use them as crude drugs. Through their experience to diagnose and treat diseases they gain knowledge on the useful and harmful properties of these plants. Such knowledge forms a basis for a better and fuller utilization of the plant wealth. Since the populations of Nepal have different ethnic groups, there are disparities and commonalities in the way of employing the same plant species and preparing remedies. At many places, the knowledgeable adults assist the healers in preparation of medicine in treating patients and in collection of drug plants. It is estimated that only 12-20% of the population living in around the urban area has access to the modern medicine facility and rest has to depend on the traditional medicine (63). MAP's play a vital role in the life support systems of contemporary civilization by serving the purpose of maintaining good health and well being of mankind. But many of these herbs are undocumented and quite poorly understood.

2. Justification of This Study

The therapeutic activity of medicinal plants is due to result of synergism of certain compounds which are mainly the secondary metabolites such as alkaloids, saponins, glycosides, phenols, terpenes, coumarins etc. In recent past, much attention has been paid to record folk medicines through ethnobotanical field studies and consequently a large number of reports mentioning folk medicinal plants belonging to various tribal pockets and rural populations of Nepal have been published (51,64). The medicinal properties of few medicinal plants of Nepal have been studied (65-73) but phytochemical screenings of many of these herbs are quite poorly studied (74-76). Hence the preparation of monographs of medicinal plants that would provide a systematic account on their phytochemical profiles is in urgent need for standardization of the traditional herbal medicine system, therapeutic benefits and their possible toxic effects. This study aimed to provide the general information on bioactive secondary metabolites of MAP's of Nepal.

Quite a number of secondary metabolites are common in many species but some of them are characteristic to a particular family, genus or only to a single species. They have specific features that can be expressed in terms of ecological, taxonomic and biochemical systematics and diversity. Therefore the present work would be additionally helpful for the use of scientific community, particularly chemotaxonomic field.

3. Methods and Methodology

3.1. Collection and identification of plant materials

The herbal samples were collected from local market at Kathmandu, Nepal. All samples identified by the authors. The voucher specimens were deposited in the Department of Plant Resources, Royal Botanical Garden, Godawari, Nepal.

3.2. Phytochemical screening methods

The samples were grinded in a blender (MR 350CA, Braun, Spain) and used for the phytochemical screening test. Chemical testes were carried out on the aqueous and alcoholic extracts using standard procedures to identify the constituents as described by Sofowara (1993), Harborne (1973) Somolenski *et al.* (1974), Kapoor *et al.* (1969), Trease and Evans (1989), Rizk (1982), Salehi *et al.* (1992) (77-83).

3.2.1. Test for alkaloids

About 2.5 g of sample was extracted with methanol and evaporated to dryness and the residue was heated on a boiling water bath with 2 N HCl (5 ml). The resulting mixture was centrifuged for 10 minutes at 3000 rpm to remove filtrate. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and the second 1 ml portion was treated with equal amounts of Wagner's reagent. The samples were then observed for presence of turbity or precipitation.

3.2.2. Test for anthocyanosides

About 0.2 g of plant sample was extracted with 5 ml ethyl alcohol. The alcoholic extract (1 ml) was heated with equal volume of 10 % HCl on water bath for fifteen minutes and cooled and then extracted the solution with ether (2 ml). If the aqueous part was red in colour and did not turn to violet at neutral pH or blue in alkaline medium, it showed the presence of anthocyanosides.

3.2.3. Test for cardiac glycosides (Keller-Killani test)

Five ml of each aqueous extract corresponding to 2.5 g of plant material was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underplayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout this layer.

3.2.4. Test for carotenes (Carr-Price test)

The ether extract corresponding to 2 g of plant material was concentrated to give a residue. The residue was taken in chloroform and 2-3 drops of saturated solution of antimony trichloride ($SbCl_3$) added to it. Appearance of blue colour, which turned red later, showed the presence of carotene.

3.2.5. Test for coumarins

To the alcoholic extract, corresponding to 2 g plant material was added 1-2 drops of water and divided into two parts; test tubes -A and B. Added 10 % ammonium hydroxide to test tube –A. A second test tube was served as a standard for comparison. Presence of blue or violet florescence under UV light for alkaline solution (test tube A) deeper than that of the standard solution (test tube B) indicated the presence of coumarins. Coumarins also reacted with hydroxyalamine to give violet colour under UV light.

3.2.6. Test for flavonoids

About 2.5 g of sample was taken in each case heated with 10 ml of ethyl acetate over steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

3.2.7. Test for glycosides

About 0.2 g of powdered medicinal plant was taken in a test tube with 5 ml of water and warmed it on a water bath for two minutes. Resulting solution of plant extract was filtered and pipetted off the supernatant liquid. Added 0.1 ml of Fehling A solution and then Fehling B solution until alkaline. Warmed the resulting solution on a water bath for

two minutes and observed for ppt. The samples were observed for presence of precipitation.

3.2.8. Test for saponins

About 2.5 g of the plant material was extracted with boiling water. After cooling , the extract was shaken vigorously to froth and was then allowed to stand for 15-20 min and classified for saponin content as follows: no froth = negative; froth less than 1 cm = weakly positive; froth 1.2 cm high = positive; and froth greater than 2 cm high = strongly positive.

3.2.9. Test for tannins

About 0.5 g of sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride solution was added and observed for brownish green or blue black colouration. A blue-black precipitate was taken as evidence for the presence of tannins.

3.2.10. Test for terpenoids (Salkowski test)

Five ml of MeOH extract, corresponding to 2.5 g of plant material, was mixed in 2 ml chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

3.2.11. Test for essential oils

Plant material (1 g) and 10 ml of light petroleum were warmed on the water bath for 2-3 minutes. The resulting extract was filtered and concentrated. A drop of concentrated extract was then applied on a filter paper. The appearance of any translucent in the filter paper was considered as the presence of oil in the extract. Placed the filter paper in an oven and heated for 15 min at 105^{o}C. If the translucent spot could still be observed then that was confirmed to be fixed oil otherwise if not, then was confirmed to be volatile oil.

A (+) score was recorded in the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity, but no flocculation was observed and a (+++) score was recorded if a definite heavy precipitation or flocculation was produced (83).

4. Results and Discussion

The phytochemical screening was carried out on forty-seven MAP's of Nepal. These plants, belonging to 45 genera and 39 families, were used in Nepalese traditional medicine systems (Appendix I). Investigation revealed the presence of medicinally active secondary metabolites in all the species but their concentrations were variable (Table 1). Glycosides, tannins, terpenoids, flavonoids, alkaloids and saponins were the major secondary metabolites present in most of the plants while cardiac glycosides and carotenoids were rarely detected among these samples. Of the investigated plants, 81% plant species contained glycosides, 70% showed the presence of tannins, 66% terpenoids, 62% alkaloids, 60% flavonoids, 57% saponins, 45% volatile oils, 43% coumarins, 30% anthocyanosides, 17% cardiac glycosides and 15% carotenoids. The flowers and roots were rich in alkaloids, flavonoids, tannins and saponins.

Species *Adhatoda vasica, Dipsacus mitis* and *Sapindus mukoross* were rich in saponins. Considerable amount of saponins were also detected in *Abies spectabilis, Asparagus racemosus,, Betula utelis, Dipsacus mitis, Entada phaseoloides, Schima wallichii,* and *Woodfordia fructicisa*. The presence of saponins in *Asparagus racemosus* and *Dipsacus species* has been reported (84-86) which is in agreement with our results. It should be noted that steroidal saponin compounds are of importance and interest due to their relationship with such compounds as sex-hormones (87). This may be the reason why the above plants are of vegetable for breast-feeding mothers to ensure their hormonal balance and for others as an aphrodisiac (88,73). Saponin's hemolytic and anti-lipiemic activities and capacity to lower serum cholesterol levels can be considered to be their important characterstics (89).

Species *Asparagus racemosus*, C*entella asiatica* and X*anthoxylum armatum* were rich in terpenoids. Plants *Acorus calamus, Azadirachta indica, Aneilema scapiflorum, Nardostachys jatamansi, Podophyllum hexandrum* and *Rhododendron anthopogon* also were contained good amount of terpenoids. The present result confirmed to previous findings those reported the terpenoids of *Centella asiatica* (90-92).

Glycosides were detected in high amount in *Glycyrrhiza glabra* but they were detected by low concentrations in plants *Cassia fistula, Centella asiatica, Crataeva religiosa, Operculina turpethum, Picrorhiza scrophulariiflora, Podophyllum hexandrum, Rheum emodi, Shorea robusta* and *Swertia chirata.* Similarly an investigation on cardiac glycoside

shows that species *Acacia catechu, Aneilema scapiflorum, Centella asiatica, Glycyrrhiza glabra, Lindera nessiana* and *Tinospora cordifolia* contained glycosides in small amounts. Present findings of glycosides in *Swertia chirata Glycyrrhiza glabra* are in agreement with report of Ray et.al. (1996), Bruneton (1995) (93,94). Glycosides of *Swertia* species are known for its antimicrobial properties (95).

Species *Aegle marmelos, Berberis aristata, Glycyrrhiza glabra, Lindera nessiana, Piper longum, Tinospora cordifolia, Viola serpens, Withania somnifera* and *Woodfordia fruticosa* contained high amount of alkaloids. Leaves of *Adhatoda vasica* previously known for several alkaloids (96,97) give additional support to our result. We are first reporting the alkaloids constituents from *Crataeva religiosa, Daphne bholua, Entada phaseoloides* and *Rhododendron arboretum.*

Screening for the flavonoids of the plants *Rhododendron arboretum* and *Woodfordia fruticosa* gave the highest positive test. Similarly *Acorus calamus, Dipsacus mitis, Emblica officinalis, Myrica esculanta, Podophyllum hexandrum, Swertia chirata* and *Xanthoxylum armatum* also contained flavonoids in high amounts. Therefore these species can play the role in pharmacological activities as anti-inflammatory, analgesic, anti-oxidant, antifungal and immunostimulant providing a key role of flavonoids to their biological actions (39, 40).

Species *Myrica esculanta, Swertia chirata, Terminalia belerica, Terminalia chebula* and *Xanthoxylum armatum,* possessed very high level of tannins. *Acacia catechu, Aegle marmelos, Daphne bholua, Emblica officinalis, Entada phaseoloides, Juniperus recurva, Ocimum sanctum, Rheum emodi, Schima wallichii* and *Woodfordia fructicisa* also detected for their tannins. Literature revealed that some of these species were used to treat diarrhea in traditional medicinal practice (88). In evidence the pharmaco-chemical studies on tannins they are effective for anti-diarrheal activities (98).

Species *Cassia fistula, Rhododendron anthopogon, Rhododendron arboretum, Woodfordia fructicisa* were detected for having the considerable amount of anthocyanosides. Beside these species, *Acorus calamus, Crataeva religiosa, Myrica esculanta, Nyctanthes arbor-tristis, Ocimum sanctum, Schima wallichii, Swertia chirata* and *Xanthoxylum armatum,* also contained anthocyanosides in small amounts.

Coumarins were not detected in high amounts except in *Woodfordia fructicisa.* Species *Acacia catechu, Acorus calamus, Aegle marmelos, Asparagus racemosus, Bergenia ciliata, Cassia fistula, Daphne bholua, Dipsacus mitis, Emblica officinalis, Glycyrrhiza glabra,*

Juniperus recurva, Nardostachys jatamansi, Picrorhiza scrophulariiflora, Piper longum, Rheum emodi, Rhododendron arboretum, Shorea robusta, Terminalia belerica and *Terminalia chebula* are the plants detected as coumarin containing herbs. Remedies prepared by above few plants were taken to cure heart problem and known for blood purifier (99) that may be due to role of coumarins present in the remedies (26).

Carotenoids were detected only in few species *Azadirachta indica, Lindera nessiana, Nyctanthes arbor-tristis* and *Tinospora cordifolia.* Especially species *Petrocarpus santalinus, Swertia chirata* and *Utrica dioica* were detected with good amounts of coumarins.
Concentrations of volatile oils were high in species *Abies spectabilis, Acorus calamus Centella asiatica, Rhododendron anthopogon* and *Xanthoxylum armatum.* Similarly species *Asparagus racemosus, Betula utelis, Juniperus recurva, Nardostachys jatamansi, Ocimum sanctum* and *Woodfordia fructicisa* also contained good amount of volatile oils.

It is known that the plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids, polyphenols are generally superior in medicinal activity as well as exhibit physiological activity (41,24). Most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenolic compounds (25). The plants studied here can be seen as a potential source of useful drugs particular reference to glycosides, flavonoids, saponins, tannins, and terpenoids. Out of 47 species containing high concentrations of diverse phytochemicals, total 8 species *Asparagus racemosus, Bergenia ciliata, Daphne bholua, Rhododendron arboretum, Schima wallichii, Terminalia chebula, Tinospora cordifolia* and *Woodfordia fructiosa,* are confirmed to be potential species of medical value. There was definite co-relation between the traditional application of plants and possession of secondary metabolites, which supports the scientific basis for the traditional medicinal system. This result may serve for future workers to select a group of plants having similar chemical constituents to isolate biologically active principle or to prepare remedies for particular case.

Table 1. Phytochemical screening of selected medicinal and aromatic plants (MAP's) from Nepal

S.N.	Plant name	Alkaloids	Anthocyanosides	Cardiac glycosides	Carotenoids	Coumarin	Glycosides	Flavonoids	Saponins	Tannins	Terpenoids	Volatile oil
1	*Abies spectabilis* (D.Don) Spach	-	-	-	-	-	-	-	++	+	+	+++
2	*Acacia catechu* Willd.	-	-	+	-	+	-	+	+	++	+	-
3	*Acorus calamus* L.	-	+	-	-	+	+	++	-	-	++	+++
4	*Adhatoda vasica* Nees (L.)	+	-	-	-	-	+	-	+++	+	-	-
5	*Aegle marmelos* Corr.	++	-	-	-	+	-	+	+	++	+	-
6	*Aneilema scapiflorum* Wight.	-	-	+	-	-	+	+	-	-	++	-
7	*Asparagus racemosus* Willd	+	-	+	-	+	+	-	++	+	+++	++
8	*Azadirachta indica* A. Juss.	+	-	-	+	-	+	+	+	-	++	-
9	*Berberis aristata* DC.	++	-	-	-	-	-	+	-	+	-	-
10	*Bergenia ciliata*- (Haw.) Sternb	-	-	-	-	+	+	+	+	+	+	-
11	*Betula utelis* D.Don	-	-	-	-	-	+	-	++	+	-	++
12	*Cassia fistula* Linn.	-	++	-	-	+	++	+	-	-	-	-
13	*Centella asiatica* (L.) Urban	+	-	+	-	-	++	+	-	-	+++	+++
14	*Crataeva religiosa* auct. Non Forst.	+	+	-	-	-	++	-	-	-	-	-
15	*Daphne bholua* Buch.-Ham. ex D. Don	+	-	-	-	+	+	+	++	++	-	+
16	*Dipsacus mitis* Wall.	-	-	-	-	+	-	++	+++	-	+	-

If the PPT is slight : +, Medium : ++, Heavy :+++, Not : -

Table 1. Continued

S.N.	Plant name	Alkaloids	Anthocyanosides	Cardiac glycosides	Carotenoids	Coumarin	Glycosides	Flavonoids	Saponins	Tannins	Terpenoids	Volatile oil
17	*Emblica officinalis* Linn.	-	+	-	-	+	+	++	-	++	+	+
18	*Entada phaseoloides* (L.) Merr.	+	-	-	-	-	+	-	++	++	+	-
19	*Glycyrrhiza glabra* Linn.	+	-	+	-	+	+++	-	+	-	+	+
20	*Juglans regia* L.	-	-	-	-	-	+	+	-	-	+	-
21	*Juniperus recurva* Buch.Ham. ex D.Don	-	-	-	-	+	+	+	-	++	+	++
22	*Lindera nessiana* Benth.	++	-	+	+	-	+	-	-	+	+	-
23	*Myrica esculanta* Buch.-Ham. ex D. Don	-	+	-	-	-	+	++	-	+++	+	-
24	*Nyctanthes arbor-tristis* Linn.	-	+	-	+	-	+	-	-	+	-	-
25	*Nardostachys jatamansi* DC.	+	-	-	-	+	-	-	-	+	++	++
26	*Ocimum sanctum* Linn.	+	+	-	-	-	+	-	-	++	+	++
27	*Operculina turpethum* (Linn.)Silva Manso	+	-	-	-	+	++	-	-	-	+	+
28	*Petrocarpus santalinus* Linn.f.	-	-	-	++	+	+	-	-	+	+	-
29	*Picrorhiza scrophulariiflora* Pennel	+	-	-	-	-	++	-	-	-	+	-
30	*Piper longum* L	++	-	-	-	-	+	+	-	+	-	+
31	*Podophyllum hexandrum* Royle	-	+	-	-	-	++	++	-	+	++	+
32	*Rheum emodi* Wall	-	-	-	-	+	++	+	+	++	-	+

If the PPT is slight : +, Medium : ++, Heavy :+++, Not : -

Table 1. Continued

S.N.	Plant name	Alkaloids	Anthocyanosides	Cardiac glycosides	Carotenoids	Coumarin	Glycosides	Flavonoids	Saponins	Tannins	Terpenoids	Volatile oil
33	*Rhododendron anthopogon* D. Don.	-	++	-	-	-	+	+	+	++	++	+++
34	*Rhododendron arboretum* SM	+	++	-	-	+	+	+++	-	+	+	+
35	*Sapindus mukorossi* Gacrtn	+	-	-	-	-	+	-	+++	-	-	-
36	*Schima wallichii* (DC.) Korth.	+	+	-	-	-	+	+	++	++	+	+
37	*Semicarpus anacardium* Linn.f.	+	-	-	-	-	-	-	-	+	+	-
38	*Shorea robusta* Gaertn. f.	-	-	-	-	+	++	+	-	+	-	-
39	*Swertia chirata* Hamilt	+	+	-	++	-	++	++	+	+++	-	-
40	*Terminalia belerica* Roxb.	+	-	-	-	+	-	+	+	+++	+	-
41	*Terminalia chebula* Retz.	+	-	+	-	+	+	+	+	+++	+	+
42	*Tinospora cordifolia* (Willd.) Miers	++	-	++	+	-	+	-	+	+	-	-
43	*Utrica dioica* Linn.	+	-	-	++	-	+	-	+	-	-	-
44	*Viola serpens* Wall.	++	-	-	-	-	-	+	-	+	-	-
45	*Withania somnifera* Dunal	++	-	-	-	-	+	-	+	-	-	-
46	*Woodfordia fructicisa* (L.) Kurz	++	++	-	-	++	+	+++	++	++	+	++
47	*Xanthoxylum armatum* DC.	+	+	-	-	-	+	++	-	+++	+++	+++

If the PPT is slight : +, Medium : ++, Heavy :+++, Not : -

CHAPTER II

Volatile Organic Compounds of Medicinal and Aromatic Plants of Nepal

1. Introduction

1.1. Essential oils

Although the use of fresh fragrant flowers is still very important in South-East Asia, the most important sources of flavour and fragrance materials worldwide are essential oils: the volatile aromatic oily liquids obtained from odoriferous plant parts. Essential oils are hydrophobic liquid containing complex mixtures of naturally occurring volatile organic compounds (VOC's). These oils are the end product of secondary metabolism, and most of their components are terpenoids, generally monoterpenes and sesquiterpenes, as well as sometime diterpenes and aromatic compounds derivatives. They possess spasmolytic, antiseptic, diuretic, sedative, antiphlogistic, therapeutic, analgesic and anti-tumor properties (100-105). Oils with standarized content of components (marked FCC, for Food Chemical Codex) have to contain certain amount of certain aroma chemicals that normally occur in the oil which determine the therapeutic grade or its quality. The molecular structures of essential oils are extremely small allowing absorption into different parts of body. However, the chemistry of essential oil is complex. For this reason, single oil can help a wide variety of disorders. Each component of the essential oils contributes to the beneficial or adverse effects of these oils because the component of each essential oil has different properties and bioavailabilities (106). The essential oils are absorbed by the body either through the olfactory system via inhalation or directly through the skin via baths, compresses and massage (107). When diffused molecules of volatile oils come in contact with sensory buds of nasal mucosa, energy transfer takes place, which in turn gives rise to electrical impulses and give odour of sensation to hypothalamus, from where they enter the bloodstream (108,109). Due to the lipophilic nature of compounds, the essential oils are readily cross cell membranes and are therefore absorbed through the skin and the lung (110). There are 108 families of the higher plants known that yield over 2000 essential oils (111). The plant families possessing species that yield a majority of the economically

important essential oils are *umbelliferae, compositae, cupressaceae, geraniaceae, labiatae, lauraceae, oleaceae, pinaceae, graminae* and *rosaceae* (112).

1.2. Applications of essential oils

1.2.1. Natural pesticides

Essential oils are a potent source of environmentally and ecologically safe pesticides and could be exploited for commercialization on pest control (113). The volatility and insecticidal efficiency of the oils make them good prospective fumigants that kill insects but don't harm mammals. The effect of some constituents such as anethole, anisaldehyde, carvacrol, 1,8-cineole, limonene and myrcene against some pests and fungi has been studiesd (114-116). Essential oils obtained from cumin, anise, oregano and eucalyptus were effective as fumigants against the cotton aphid and the carmine spider mite (117). Essential oil constituents were also effective to control western corn rootworm, two-spotted spider mite and housefly (118). Certain essential oil constituents are effective against *Varroa jacobsoni*, an ectoparasite of the honey bee (119) and pathogenic nematodes (120).

1.2.2. Antioxidants

The generation of reactive oxygen species (ROS) beyond the antioxidant capacity of a biological system gives rise to oxidative stress (121). Antioxidants are obtained from various aromatic and medicinal plants (28, 122-126). It has been reported that essential oils from cinnamom, ylang-ylang, basil, lemongram, lemon, frankincense, marjoram, rosemary (127), tea tree (128), thymus (91,129) and geranium (130) showed antioxidant activity. The antioxidant activity of phenols and other compounds present in oils has been reported by several authors (131-133). It has also been reported that antioxidant property of thyme oil is due to its major component thymol. They are valuable in increasing shelf life of foodstuffs, replacing synthetic compounds such as butylated hydroxytoulene (BHT) as well as for preventing cellular damage, the cause of aging and other diseases in man.

1.2.3. Natural sprout inhibitors

In recent years, efforts have been made to replace synthetic food preservatives due to their toxicity and environment hazards. MAP derived essential oils especially the

monoterpenoids can be used as alternative sprout inhibitors (134). Oils containing menthol, 1,8-cineole, eugenol, linalool, carvone and methyl chavicol as major constituent are potent sprout inhibitors. S. carvone, a major constituent of caraway seed oil (*carum carvi*) is reported as sprout inhibitor. However, it has already been commercialized in the Netherlands. The order of activity of pure monoterpenoids is: S-carvone=linalool>methyl chavicol>anethol (135).

1.2.4. Natural preservatives

Essential oils obtained from spices have been reported as potential food preservatives (136,137). Carvacrol and thymol prevent the microbial and chemical degradation when added to food (138-140). This would be due to the presence of phenolic OH group (141). Phenolic components, present in essential oils, have been known to possess antimicrobial activity and could be used to prevent post-harvest growth of native and contaminant bacteria (142). Essential oils of spices azowain, anise, cumin, saurf, sowa and peppermint exhibited excellent antihydrolytic properties (143). Essential oils extracted from *Cymbopogon citratus, Monodora myristica, Ocimum gratissimum, Thymus vulgaris* and *Zingiber officinale* were investigated for inhibitory effect against three mycotoxin producing fungi, *Fusarium moniliforme, Aspergillus flavus and Aspergillus fumigatus.* It was concluded that the oil from *O. gratissimum* had a potential food preservative capacity (144).

1.2.5. Aromatherapy

Aromatherapy is therapeutic use of volatile constituents of plants, to calm, balance, and rejuvenate mind and body. The use of essential oils for healing purpose is common in folk medicine since ancient times (145). At present, therapeutic uses is widely practiced through various methods like diffusion, warm bath, massage, inhalation etc. (146-150). It is proved that the psychophysical aromas will have wide applications in reducing stress and depression, increase appetite, induce sleep and increase alertness (151). The benefits of aromatherapy in cancer include relaxation, stress reduction, relief from muscle pain and improved sleep patterns (152). Aromatherapy, with 1,8-cineole, increased the locomotor activity inhalation while components linalool, citronellal, α-terpineol and benzaldehyde decreased the motility (153,154).

1.2.6. Flavour and fragrances

Essential oils, as fragrance, have wide array of applications in air fresheners, candles, cosmetic, industrial cleaners, masking agents, soaps and detergents. They also are suitable for flavours in confectioneries, sauces, beverages, pharmaceuticals and dairy products. Specific green notes of pyrazines, very often, occur as the character-impact compounds in processed flavors. Rose ketone β-damascenone and violet ordrant β-ionones are potent odorants (109). Ethanol, acetaldehyde and dimethylsulfide are used as bread aroma (155). Octanal, occurring to 0.15% in lemon oil, contribute around 15% of the total aroma value to this oil. Umbellulone, the major component in Californian laurel oil has a small effect, where as 1,8-cineol (19%) contributes to 95% of the total intensity. It would be due to its lower threshold value. Apart from sensitizing compounds, essential oils may also contain potential carcinogens like safrole, methyleugenol etc (156).

1.2.7. Anti-inflammatory

Essential oils are a source of natural anti-inflammatory compounds. Traditional therapy system utilizes numerous essential oil for the treatment of inflammatory disorders (157). α-Terpinolene, p-cymene, p-cimen-8-ol, limonene and dillapiol, showed anti-inflammatory activities (158). The essential oils containing 1,8-cineole as a major constituent, reduce the histological signs of inflammation such as leukocyte infiltration, edema formation and tissue injury, as well as biochemical marker of neutrophil infiltration in the damaged tissue (153,159). It is reported that 4-terpineol suppresses production of inflammatory mediators (160). α-Pinene, β-pinene and sabinene are known to possess anti-inflammatory activity (161,162). Essential oil from Cymbopogon giganteus also possesses anti-inflammatory activity (163).

1.2.8. Antimicrobial

Essential oils have a different mode of action as compared to synthetic antibiotics, and thus may able to combat the resistant strains. Essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic, cinnamomum and onion are active against bacteria and fungi (164,165). Ranking of antimicrobial properties of some essential oils showed that many oils have the superior antimicrobial properties (166-170). Grover and Rao (1978) have studied the activity of eugenylnacetate, gerenyl acetate and menthyl heptanone, against

some pathogenic bacteria (171). Bammi *et al.* (1997) demonstrated the effect of five essential oils on Epstein-Barr virus (EBV) (172). It has been proved that antifungal activity is related to monoterpenic phenols in the oils (173-180).

1.3. Gas chromatography

Gas chromatography (GC) provides a time separation of components in a column containing a coating of stationary phase. GC makes possible to separate very complex mixtures containing up to 200 related compounds using either partition or adsorption, with very small size, but it does have inherent limitations. The sample must be able to exist in gas phase, so it may only be applied to volatile materials; although this includes substances those have an appreciable vapour pressure at temperatures up to 400 ^{o}C. The requirement for volatility of the sample means that non-polar materials are generally easier to handle than polar material, and ionic materials cannot pass through a gas chromatograph. An important facet of the GC is the use of carrier gas, such as hydrogen or helium, to transfer the sample from the injector to detector through column. The column contains a coating of stationary phase. Separation of components is determined by the distribution of each component between the mobile phase and stationary phase. Only those materials that can be vaporized without decomposition are suitable for GC analysis. Therefore, the key features of gas chromatograph are the systems that heat the injector, detector and transfer lines, and allow programmed temperature control column.

1.4. Mass spectrometry

The functions of mass spectrometers (MS) are to sort out ionized gas molecules from the substance under investigation. These ions are made to follow trajectories through application of combination of electric and magnetic field and are separated according to their mass to charge ratios (*m/z*). Electron Impact Positive-Ion (EI+) MS is the most commonly used MS method in natural product chemistry. The series of ion fragments observed for a given compound can be quite different under different modes of operation. Under Electron-Impact (EI) ionization of gases, positive ions predominate because of their stability. The first ion to appear will result from the removal of one electron from the molecule leading to the production of a parent molecular ion. Then cleavage of other bonds in the molecule gives rise to a series of fragments. Usually, it is easy to obtain the molecular weight of compound from the observation of molecular ion in the mass spectrum, provided that it can be volatilized without deposition. Sometime, softened

methods of ionization such as fast atom bombardment and chemical ionization are used if, the peak of the molecular ion so weak and fragment ion may be mistaken for parent ion.

1.5. MAP's selected for essential oil studies

1.5.1. *Acorus calamus* L

It is a perennial aromatic herb, rhizome thick, creeping, 5-5.4 cm long and 0.6-2 cm wide. Leaves distichous, nerves parallel, spathe leaf-like, spadix 4-8 cm, tapering, bi-sexual flowers. It has been used as an aromatic stimulant and mild tonic, carminative, diaphoretic, expectorant, hypotensive and sedative plant. Dried rhizomes and sometimes leaves have also been used in the formulation of alcoholic beverages and some food products. It is distributed in Himilayan region of Nepal at 1800 m.

1.5.2. *Asparagus racemosus* Willd

It is a tall climbing, excessively branched, under shrub, root 5-13 cm long, flattened branchlets 12-25 mm long, spreading; flowers bi-sexual, racemes very slender, 4 mm long and perianth petaloid. Root of this plant is refrigerant, demulcent, diuretic, aphrodisiac, antispasmodic, alternative, antidiarrhea, antidysenteric and galactagogue. Plant is used in rheumatism, diabetes and brain complaints. It is distributed in Himilayan, Madhya and Terai region of Nepal at 1200 m.

1.5.3. *Bergenia ciliata* (Haw.) Sternb

It is perennial herb, root 1 cm in diameter, outer surface brown, rough, inner, smooth; leaves ovate or round, 5-15 cm. long turning bright red, entire; flowers white, pink or purple and 3.2 cm. in diameter. Root of this plant is used as tonic, used in fever, diarrhea, and pulmonary affections, antiscorbutic, bruised and applied to boils and opthalmia. It is distributed in Mahabharat regions between 2100-3000 m in Nepal.

1.5.4. *Centella asiatica* (L.) Urb

It is a herb, leaves 1.2-6 cm diameter, usually glabrous, kidney-shaped, petiole, umbels, sometimes clustered; bracts few, flowers 3 or 4 in umbel, purple white, fruit 3-4 mm, carpeles oblong, sub-cylindric, curved, slightly compressed and seeds compressed

laterally. Plant used as an alternative for tonic, leprosy, nerves and blood purifier, as well as diuretic and for indigestion. Leaves were taken as memory improveing tonic, as well as in skin diseases, syphilis and rheumatism. It is distributed at 1800 m throughout Nepal.

1.5.5. *Dipsacus mitis* D. Don

It is an annual wild tall herb, the flowers are hermaphrodite. the root of this plant has been reported as abortifacient, and used as a traditional medicine. It is distributed in Himilayan region of Nepal, at the height of 2000-3000 m.

1.5.6. *Swertia chirata* Hamilt

An erect herb, leaves opposite, broadly lanceolate, acute, lower often much larger, sometimes petiolated, flowers green, yellow and tinged with purple. Plant is bitter, stomachic, febrifuge, laxative, anthelmintic, antidiarrhoetic and tonic to gouty person. It is distributed Himalayan region of Nepal between 1200-3000 m.

1.5.7. *Terminalia chebula* Retz.

A large deciduous tree, 24-30 m high; leaves 7.6-15.2 cm long, ovate or elliptic, acute, petioled; flowers all hermaphrodite, sessile, dull-white or yellow, fruit 1.8-3.3 cm ellipsoidal or oval from a broad base and glabrous, 5-ribbed when dry. Fruit is used as astringent, laxative, alternative, used externally as a local application to chronic ulcers and wounds as a gargle in stomatitis; finely powdered used as a dentifrice and considered useful in carious teeth and bleeding and ulcerations of the gums. The plant is distributed in inner Madesh, Terai and Himalayan regions upto 1500 m in Nepal.

1.5.8. *Woodfordia fruticosa* (L.) Kurz

A pubescent shrub, leaves opposite, sometimes whorls of three, sessile, lanceolate, 5-10 cm long, entire, under surface white, and with blank glandular dots, flowers clustered, numerous, shortly stalked and red corolla. Dried flowers are used in dysentery, menorrhagia, in derangements of the liver, disorders of the mucous membrane and in haemorrhoids, considered safe stimulatant in pregnancy. It is distributed in Himalayan, inner Madesh and Terai region of Nepal upto 1500 m.

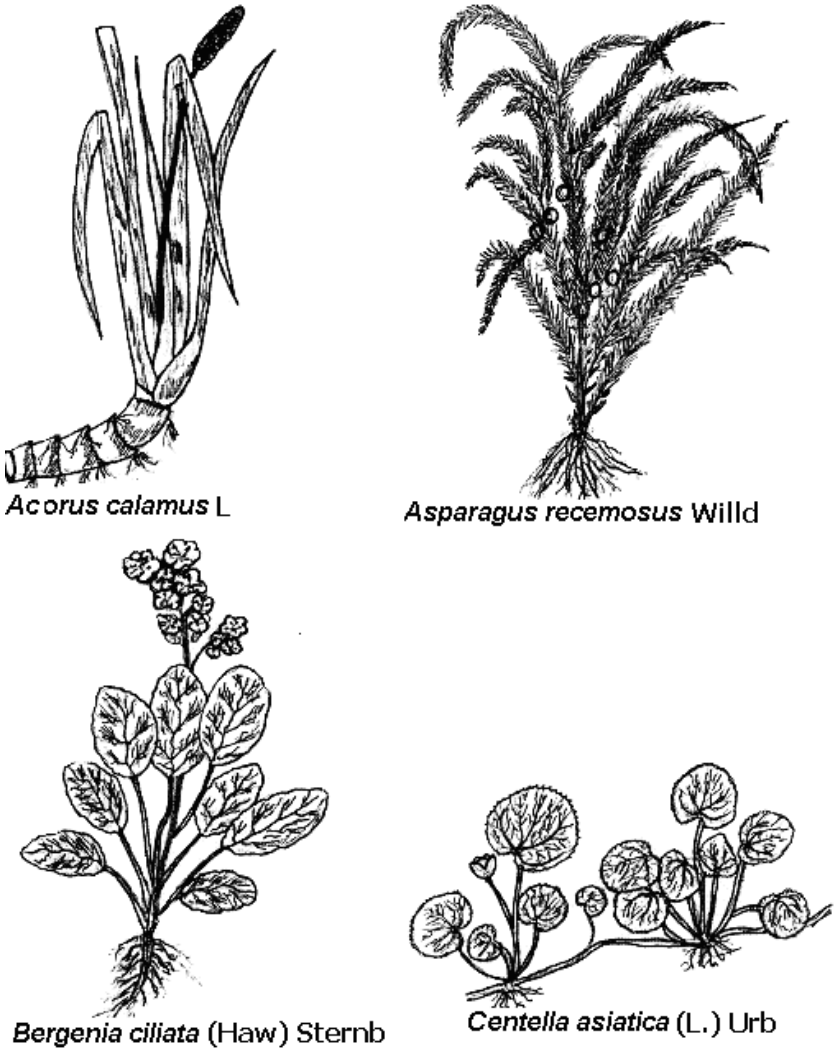

Fig. 3. MAP's selected for essential oil studies.

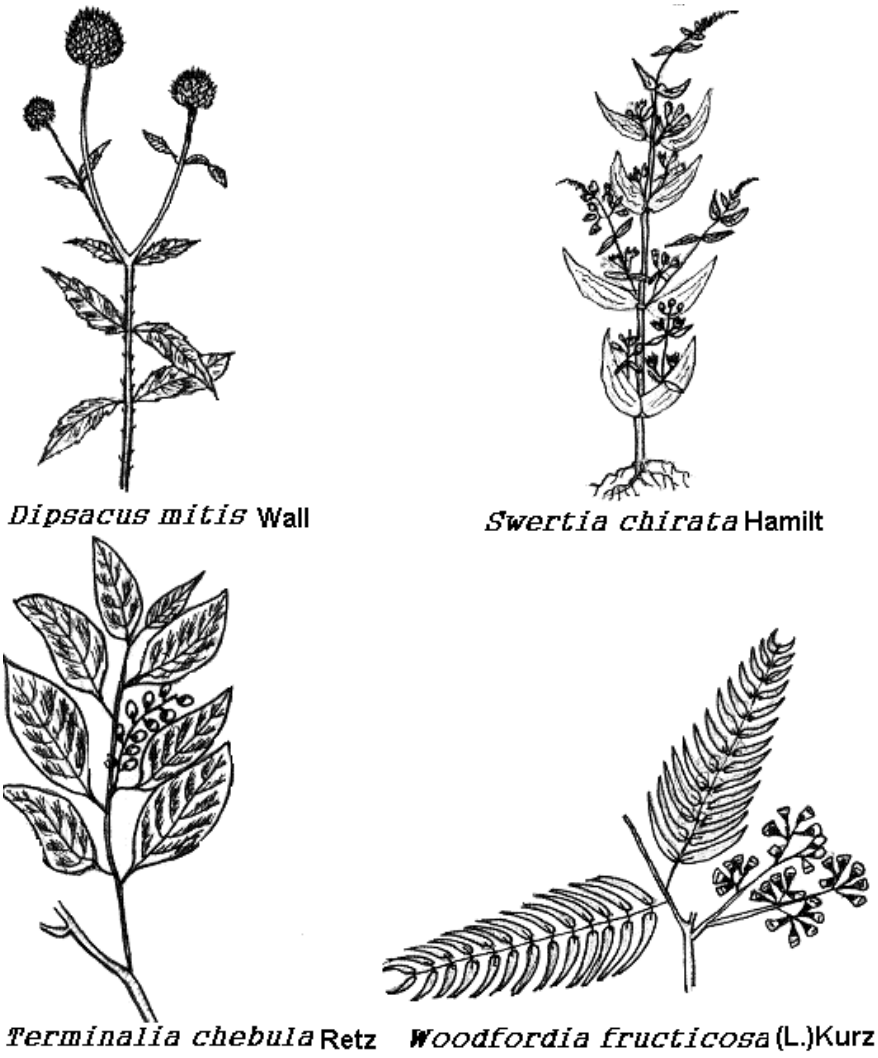

Fig. 4. MAP's selected for essential oil studies.

2. Justification of This Study

Knowledge of the chemical constituents of the plants would be valuable in discovering the actual value of folkloric remedies and extremely important in the standardization of the traditional herbal medicine system for therapeutic benefits and their possible toxic effects. Knowledge of essential oils of Nepalese medicinal plants appears to be very limited. The major constituents of the essential oils of a few of these plants were earlier assigned from the existing data in the literature on identical species from other parts of the world. Preparation of monographs of aromatic and non-aromatic medicinal plants that would provide a systematic account on their VOC's is important. Hopefully, this research will lead to new information on plant applications and use of these herbs. This work has a significant importance because neither governments nor private sectors have paid much attention to this subject.

In the other hand, the Himalayan region shows the highest richness for endemic species and some of the plants found in the Himalayas can not be found elsewhere. The variety of herbs and plants are unique for their potential in discovering, combining, manipulating and synthesizing new medicine. Therefore the number of people and institutions seeking information on Himalayan medicinal plant is increasing very rapidly. If the active fraction of synthetic drugs can be found in any plants and herbs, we can get them cheaply and easily from medicinal herbs rather than from synthesizing process. So this study aimed to find out the active principles present in VOC's of MAP's.

3. Materials and Methods

3.1. Plant samples

Medicinal herbs were collected from local markets and traditional healers, in Kathmandu, Nepal. Voucher specimens were deposited at the Department of Plant Resources, Royal Botanical Garden, Godawari, Nepal. Following plants have been selected for essential oil studies (Table 2).

Table 2. MAP's selected for the study of essential oil components

Name of Plants	Parts collected	Local name	Family
Acorus calamus L	Rhizomes	Bojho	Araceae
Asparagus racemosus Willd	Roots	Kurilo	Liliaceae
Bergenia ciliata (Haw) Sternb	Rhizomes	Pashanved	Saxifragaceae
Centella asiatica (L) Urb	Whole	Ghodetapre	Apiaceae
Dipsacus mitis D. Don	Roots	Banmula	Dipsaceae
Swertia chirata Hamilt	Whole	Chirato	Gentianaceae
Terminalia chebula Retz	Fruits	Harro	Combretaceae
Woodfordia fruticosa (L) Kurz	Flowers	Dhairo	Lythraceae

3.2. Reagents

All the reagents used in the experiments were purchased from Sigma Co. (USA) and Fisher Scientific (USA). The organic solvents used for the extraction and the chromatography were redistilled using a wire spiral packed double distilling apparatus (Normschliff Geratebau, Wertheim, Germany) and Milli-Q water that was generated through a water purification system (Millepore Corporation, Bedford, USA).

3.3. Analytic apparatus

a. Distilling apparatus: Wire spiral packed double distilling apparatus
(Normschliff Geratebau, Germany)

b. Blender: Multi mixer (Braun MR 550 CA, Braun, Spain)

c. pH meter: pH/ION meter (DMS, Korea)

d. Extraction apparatus: Simultaneous steam distillation and extraction (SDE), Likens & Nickerson type simultaneous steam distillation & extraction apparatus, (Normschliff, Wertheim, Germany)

e. Concentration column: Vigreux column (250 ml, Normschliff, Wertheim, Germany)

f. Gas chromatography: Hewlett Packard 5890 II Plus GC
equipped with FID and HP Chemstation 1050 Data system

g. Gas chromatography/mass spectrometery: Shimadzu GC/MS QP-5000
equipped with mass spectrum library WILEY 139,
NIST 62, NIST 12 (Shimadzu, Japan)

h. Capillary column: DB-WAX (60 m × 0.25 mm i.d., 0.25 μm film thickness, J&W, USA)

3.4. Extraction of volatile organic compounds

Fifty grams of samples were homogenized in a blender (MR 350CA, Braun, Spain) and mixed with 1 L of distilled water. After adjusting the pH at 6.5 by 1% NaOH solution, 1 ml *n*-butylbenzene was added as an internal standard. The resultant slurry was used for extraction of volatile flavor compounds with 200 ml redistilled n-pentane:diethylether (1:1, v/v) .The extraction experiment was carried out for 2 h using simultaneous steam distillation (SDE) apparatus of Nikerson and Likens (1966) type as modified under atmospheric pressure by Schultz *et al.* (1977) (181,182). The solvent containing extracted volatile compounds was dehydrated for 12 h using 10 g anhydrous Na_2SO_4 and then concentrated to approximately 1.5 ml using the vigreux column. This final sample was used for gas chromatography-mass spectrometry (GC/MS) analysis.

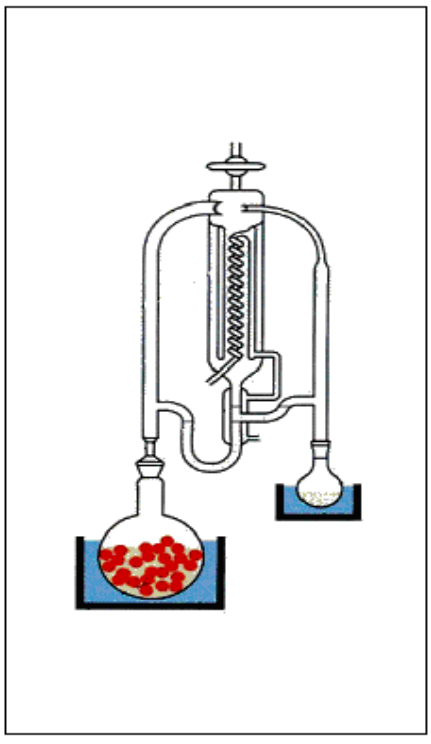

Fig. 5. Diagram of simultaneous steam distillation extraction (SDE) apparatus according to Likens-Nickerson.

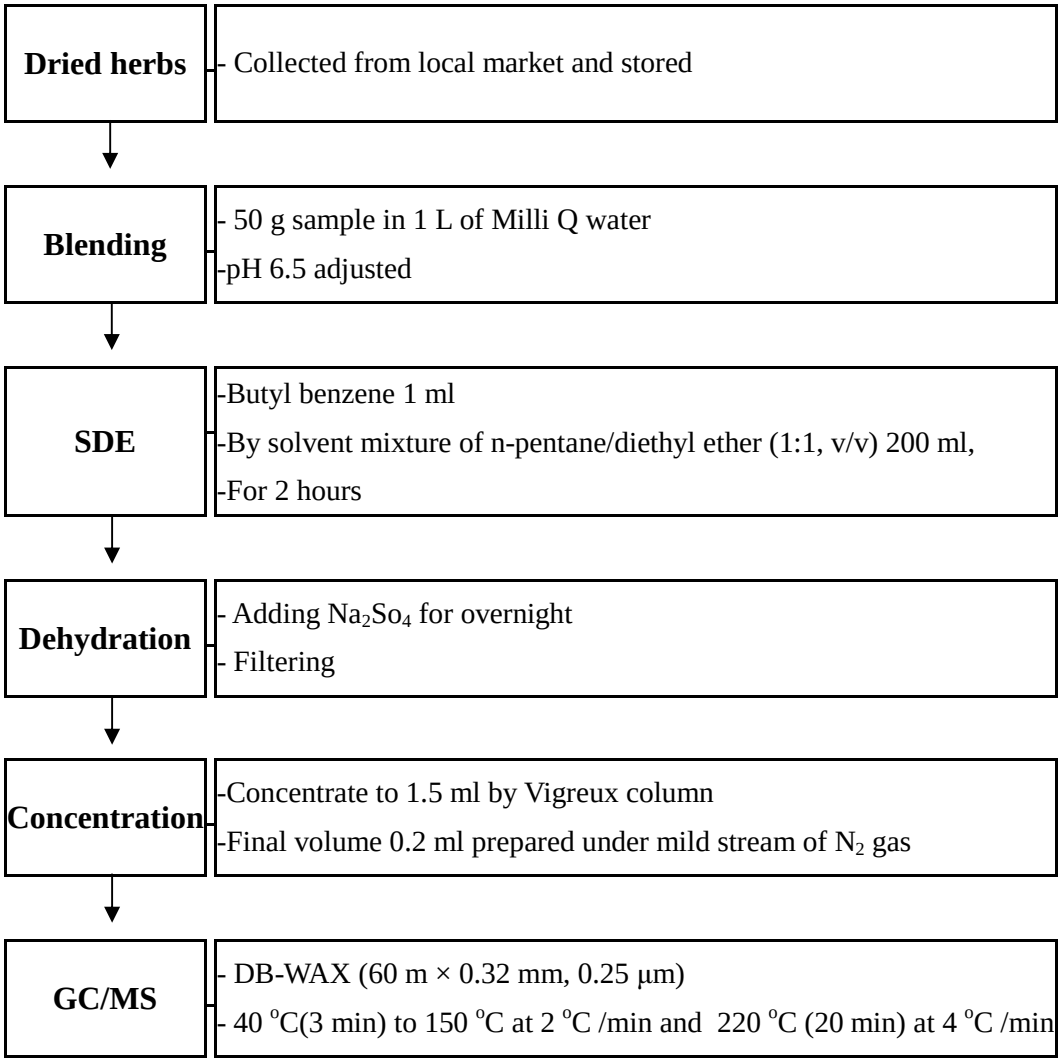

Fig. 6. Scheme for analysis of volatile organic compounds of herbs.

3.5. Establishment of retention index

Kovats (1958) suggested RI (retention index or Kovats index), as a suitable indication tool for retention indication which was indicated by the same compound to retention time for standard alkane (183). Retention index as a parameter used for checking a solute from chromatogram by comparing the retention time of both alkane that appeared the above and below of the solute.

$$RI_i = 100\,Z + 100\left\{\frac{\text{Log } V_{R(i)} - \text{Log } V_{R(Z)}}{\text{Log } V_{R(Z+1)} - \text{Log } V_{R(Z)}}\right\}$$

RI_i : Retention index of compound i

$V_{R(i)}$, $V_{R(Z)}$, $V_{R(Z+1)}$: Retention time of standard alkanes (alkanes eluted before and after the substance of interest) which bracket the substance of interest.

Factor Z: Factor Z contains the number of carbon eluted eg. Z+1, Z=2…….etc.

According to definition, retention time of alkane has the value as multiplying carbon number that the compound has to be unrelated with column solid phase, the temperature of separation and requirements of other chromatography. Therefore, n-alkane was indicated as a standard index for CH4 (RI=100), C2H6 (RI=200) … CnH2n+2 (RI=100n), and even anything in analysis column.

In order to obtain a scaled Retention Time (RT) of standard sample of known hydrocarbon, diluted mixture of n-alkane; mixture I (C_7 ~ C_{17}) and mixture II (C_{13} ~ C_{23}), was used as an internal standard. 1 μL mixture was analyzed to determine the RT of the internal standard by GC-FID under the condition of Table 3. Retention index (RI) of each peak was established by a basic program that substituted the RT of each peak of *n*-alkane confirmed at GC chromatogram.

3.6. Analysis and Identification of Volatile Organic Compounds

3.6.1. Analysis of compounds by gas chromatography-mass spectrometery (GC/MS)

Chromatographic analysis was carried out using a Shimadzu GC–MS (Model QP-5000, Shimadzu Co., Kyoto, Japan) in EI (Electron Impact) mode. The ionization voltage was 70 eV and temperatures of ion source and injector were 230 and 250^{o}C, respectively. The capillary column used was a DB-WAX (60 m, 0.2 mm i.d. and 0.25 mm, film thickness; J & W, USA). The oven temperature programmed at 40^{o}C (Isothermal for 3 min) was ramped to 150^{o}C at 2^{o}C /min and to 220^{o}C at 4^{o}C /min (Isothermal for 20 min) followed to 230^{o}C at 5^{o}C /min. Helium was used as the carrier gas at a flow rate of 1 ml/min, with an injector volume of 1 ml using 1:20 split ratio (Table 4).

3.6.2. Identification and quantitative analysis of volatile compounds

Mass spectra of each compound obtained from GC/MS were identified with the aid of our own mass spectral data and those contained within the WILEY 139, NIST 62 and NIST 12 libraries and mass spectral data books (Robert 1995, Stehagen *et.al.* 1974) as well as by the comparison of retention indices to reference data (Davies 1990, SRL 1986) (184-187). The following formula was used for quantitative analysis of volatile compounds.

$$\text{Compounds Content (mg/kg)} = \frac{C \times 1000}{A \times B}$$

A : Peak area of internal standard

B : Amount of sample (g)

C : Peak area of each compounds in sample

Table 3. GC conditions for identification of volatile compounds of herbs

GC	Hewlett-Packard 5890 series II Plus
Column	DB-Wax (60 m × 0.25 mm I.D., 0.25 μm film thickness, J&W, USA)
Detector	FID
Carrier gas	He (1.0 ml/min)
Make up gas	N_2 (20 ml/min)
Temp. program	40°C (3 min), to 2°C /min-150°C, to 4°C /min-220°C (20 min)
Detector temp.	300°C
Injector temp.	250°C
Injection volume	1 μl

Table 4. GC/MS conditions for identification of volatile flavor compounds of herbs

GC/MS	Shimadzu GC/MS QP-5000
Column	DB-Wax (60 m × 0.25 mm id, 0.25 μm film thickness, J&W, USA)
Carrier gas	Helium (1.0 ml/min)
Tempterature program	40 °C (3 min), to 2 °C /min-150 °C, to 4 °C /min-220 °C (20 min)
Injector	250 °C
Ion source temp.	230 °C
Ionization	Electron Impact (EI)
Ionization voltage	70 eV
Mass range (m/z)	40~350
Injection volume	1 μl

4. Results and Discussion

4.1. Establishment of retention index of *n*-alkane

The standard value of retention index (RI) was determined by two different mixture of *n*-alkane, mixture I (C_7 ~ C_{17}) and mixture II (C_{13} ~ C_{23}) considering as an standard. 1 μL mixture of alkane was analyzed to find out the retention time (RT) of internal standard by GC-FID (Fig. 7). RI of each peak was established by a basic program (as described in 3.5) that substituted the RT of each peak of *n*-alkane confirmed at GC chromatogram (Table 5).

Table 5. Retention time of *n*-alkane mixture for gas chromatographic retention index

Alkanes	Retention time	Alkanes	Retention time
$C_{7:0}$	4.957	$C_{16:0}$	49.467
$C_{8:0}$	6.119	$C_{17:0}$	55.541
$C_{9:0}$	8.289	$C_{18:0}$	61.108
$C_{10:0}$	11.320	$C_{19:0}$	65.568
$C_{11:0}$	16.500	$C_{20:0}$	69.260
$C_{12:0}$	22.836	$C_{21:0}$	72.772
$C_{13:0}$	29.638	$C_{22:0}$	78.403
$C_{14:0}$	36.462	$C_{23:0}$	81.94
$C_{15:0}$	42.950		

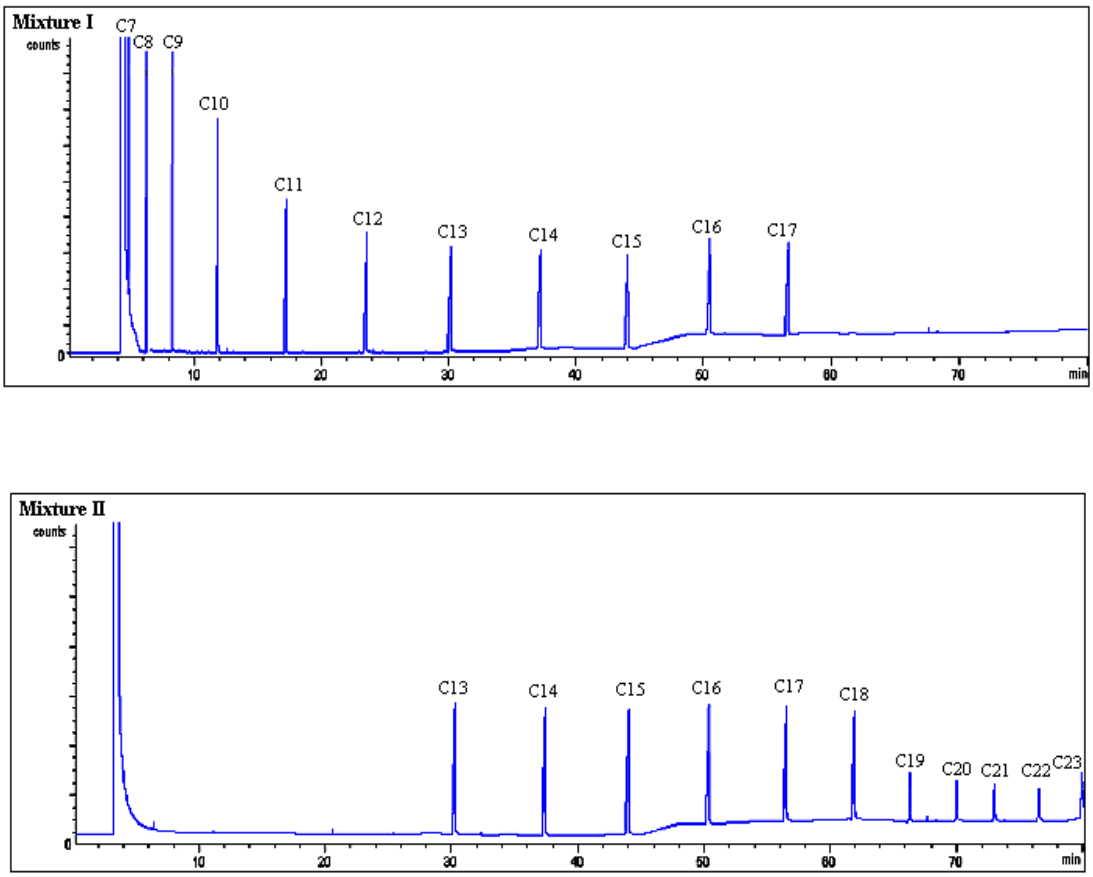

Fig. 7. GC chromatograms of n-alkane standard mixture I (C_7~C_{17}) and II (C_{13}~C_{23}).

4.2. Analysis of volatile organic compounds of MAP's

The composition of VOC's of selected medicinal plants of Nepal has been investigated and quantified. Mass spectra of each compound obtained from GC/MS were identified with the aid of our own mass spectral data and those contained within the libraries. Mass spectra of some important compounds are presented in Appendix II and profile of VOC's present in MAP's has been discussed below.

4.2.1. Volatile organic compounds of *Acorus calamus* L

The essential oil of *A. calamus* was extracted by solvent extraction (P:E, 1:1) method for 2 h using SDE apparatus and analysed by GC/MS (Fig. 8). Investigation confirmed that the yield of essential oil obtained from Nepal originated *A. calams* rhizome was 7493.59 mg/kg. The identified VOC's are listed together according to their elution order on DB-WAX column with their amounts (Table 7). A total of fifty three VOC's so far belonging to chemical classes of alcohol (11), aldehyde (14), ester (3), furan (1), hydrocarbon (19), ketone (4), N-containing, miscellaneous (1) were tentatively identified and quantified (Table 6). Ketones were dominant with highest proportion (55.40%). The major ketone compounds were α-asarone (8.71%) and *β*-asarone (46.78%). Alcohol accounting 19.29% was also characterized as major chemical group. Farnesol (11.09%) and methyleugenol (6.10%) were detected as the main components of alcohol group while remaining 9 alcohols were quantified at levels lower than 1%. Similarly, aldehyde was the third major group accounting 17.87%. Except myrtenal (3.07%) and [*E,Z*]-2,4-decadienal (14.15%) almost all aldehyde compounds were detected at levels lower than 0.2%. All of the compounds related to hydrocarbon group were terpene hydrocarbons. The major hydrocarbons were patchulane (0.81%), δ-cadinene (0.69%) and [*Z*]-ocimene (0.68%) while remaining 16 hydrocarbons were detected at levels lower than 0.5%. Beside these hydrocarbon terpenes some other terpenoids such as alcohol terpenoids and aldehyde terpenoids were also detected. Oxygenated sesquiterpene, farnesol (11.09%) and *α*-bisabolol (0.96%) occupied the major position in terpenoids (12.05%). Similarly hydrocarbon monoterpenes accounted 0.94%, oxygenated monoterpenes accounted 3.51% and hydrocarbon sesquiterpene accounted 3.31%. This result indicates that *β*-asarone was the dominant compound and some major compounds ranged in content order as follows:

[*E,Z*]-2,4-decadienal, farnesol, α-asarone and methyleugenol.

Qualitative studies of chemical constituents of essential oils provide an idea to evaluate the quality of such oils. The percentage composition of the essential oil provides probably the most important parameter for the characterization of the plant (188). Therefore we discussing the individual components and their pharmacological properties those based on previous studies. In previous investigation, Indian *A. calamus* yields oil containing 5~75% β-asarone (189) while the European variety yields oil with approximately 5% β-asarone have been reported. We identified the essential oil of Nepal originated *A. calamus* containing high amount of β-asarone. Keller and Stahl (1983) determined that β-asarone was absent in diploid varieties (190). According to Rsst and Bos (1979), β-asarone constituted 96 % in the oil of the triploid variety (191). European triploid type has been found to contain average 5% β-asarone (192). β-Asarone is useful against insects, acting as repellent (193) and as sleeping time enhancer (194). This is also used in production of alcoholic beverages and foods at lower level (195). But FDA prohibited the utilization of this herb owing to the potential carcinogenic effects of its essential oil, with particular reference to β-asarone (196). Annex II of Directive 88/388/ ECC on flavorings fixed the maximum levels of β-asarone to 0.1 mg/kg in foodstuffs and beverages, with the exception of 1 mg/kg in alcoholic beverages and seasonings used in snack foods (197). Compound [*E,Z*]-2,4-decadienal, a major aldehyde compound of this oil, possess dioxygenase and fatty acid lyase activities (198). It strongly inhibits cell growth and affects cell viability (199) and produces negative effects on marine invertebrates (200). Another aldehyde, myrtenal is terpene-derived aldehydes considered to be produced by tropospheric oxidation of α-pinene (201). Linalool is very important substances used in foodstuffs as food additives (202,203) and pharmacology as sedative effect inducer (204), glutamatergic neurons inhibitor (205) and also exhibits anti-inflammatory (206), anticarcinogenic (207) and antiseptic (208,209) activities. The medicinal uses of linalool are based on some of its known antibacterial, antifungal, acaricidal, anticonvulsant and sedative activities (210,211). However the concentration of linalool was very low in this oil. Compound farnesol was detected as a dominant alcohol compound among the 11 compounds of alcohol group. Anti-cancer effects of farnesol have been demonstrated in a number of studies that showed suppression of tumor cell proliferation (212) and induction of tumor cell apoptosis in vitro (213), anticarcinogenic (214) and antibacterial activity

(215). Another major alcohol compound was methyleugenol, which is used as a fragrance in cosmetics, soaps and shampoos and as flavouring agent in jellies, baked goods, nonalcoholic beverages, chewing gum and icecream (216). Many biological actions of methyleugenol have been previously reported to induce hypothermic, myorelaxant, antispasmodic, anticonvulsant and anesthetic effects (217-221). Camphor is well-known chemical with its pronounced antimicrobial potentials (222,223). Similarly, α-pinene and its structural isomers have strong inhibition of AChE and prevent the audiogenic seizures in susceptible rats and antifungal properties (224-226). Camphor and α-pinene were detected by small concentration in essential oil of *A. calamus*. Some of the important bioactive hydrocarbon compounds such as limonene, β-caryophyllene, β-elemene, [*E*]-β-ocimene, myrcene were also detected in this species. Limonene has been shown to be an anti-cancer (207) and also has been reported to have antiseptic (208) activities. Limonene is also used as fragrances in household products. β-Caryophyllene has been commonly used as a fragrance chemical since the 1930s (227). The odour of β-caryophyllene, is described as woody and spicy (228). β-Elemene, has been proved for anti-tumor activity including brain tumors (229-231). Carene is a cyclopropane containing mono terpene, derivatives of which have shown anesthetic property (232). It has also shown strong inhibition of AChE (224) and anti-inflammatory activities (161). [*E*]-β-Ocimene is a component of floral scents and has flavor and fragrance values (233). β-Myrcene, has been used as flavoring additives in foods and beverages, as fragrances in cosmetics, and as scent in household products (234). The compounds [*E,Z*]-2,4-decadienal, farnesol, aromadendrene, α- and β-pinene, and [*E*]-farnesene have flavor characteristics as follows: seaweed, flower, wood, terpentine and sweet (235). It possesses a peculiar but pleasant, slightly sweetish and fatty odour reminiscent of stale milk.

Hence it is verified that the gentle curative action of essential oil of *A. calamus* rhizome is due to its various constituents acting together synergistically. It also cleared that the volatile compounds of *A. calamus* could be useful for flavor, fragrance and cosmetics. It would be advantageous to use one or two characteristic compounds instead of the whole oil. Moreover, though there are a number of bioactive components in the essential oil of calamus, it seems use of this oil could be riskable due to particular reference with β-asarone in high amount. However systematic fractionalization of this oil could give a number of bioactive compounds of medicinal and commercial values.

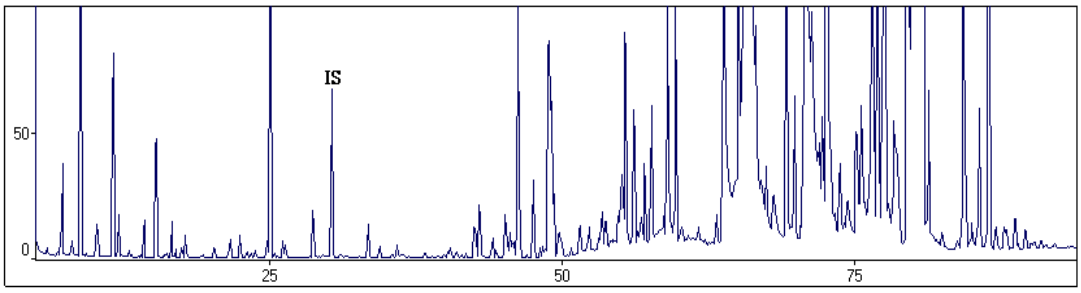

Fig. 8. GC/MS chromatogram of volatile organic compounds obtained from *Acorus calamus* L.

Table 6. Relative content of functional groups of volatile organic compounds identified in *Acorus calamus* L

No.	Functional groups	Relative peak area (%)	Number of compounds
1	Alcohol	19.29	11
2	Aldehyde	17.87	14
3	Ester	0.77	3
4	Furan	0.15	1
5	Hydrocarbon	4.27	19
6	Ketone	55.40	4
7	Miscellaneous	1.75	1
8	Unknown	0.50	6
	Total	**100**	**59**

Table 7. Volatile organic compounds of *Acorus calamus* L

No.	RT[a)]	RI [b)]	Compound name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
1	7.36	861	Ethyl acetate	$C_4H_8O_2$	88	8.98	0.12
2	8.17	895	3-Methylbutanal	$C_5H_{10}O$	86	1.55	0.02
3	8.89	923	Ethanol	C_2H_6O	46	42.79	0.57
4	10.29	969	2-Pentanone	$C_5H_{10}O$	86	5.58	0.08
5	11.67	1008	Methyl 2-methylbutyrate	$C_6H_{12}O_2$	116	19.89	0.27
6	12.15	1019	α-Pinene	$C_{10}H_{16}$	136	4.62	0.06
7	14.34	1063	Camphene	$C_{10}H_{16}$	136	3.61	0.05
8	15.33	1080	Hexanal	$C_6H_{12}O$	100	11.89	0.17
9	16.07	1093	Isobutanol	$C_4H_{10}O$	74	0.43	0.02
10	16.70	1104	β-Pinene	$C_{10}H_{16}$	136	4.11	0.06
11	17.03	1110	3-Pentanol	$C_5H_{12}O$	88	0.89	0.02
12	17.55	1119	Sabinene	$C_{10}H_{16}$	136	0.82	0.02
13	17.84	1124	2-Pentanol	$C_5H_{12}O$	88	2.31	0.03
14	20.32	1164	β-Myrcene	$C_{10}H_{16}$	136	1.23	0.02
15	21.68	1184	Heptanal	$C_7H_{14}O$	114	1.90	0.03
16	22.51	1196	Limonene	$C_{10}H_{16}$	136	2.35	0.03
17	23.12	1205	β-Phellandrene	$C_{10}H_{16}$	136	0.48	0.02
18	23.50	1211	3-Methylbutanol	$C_5H_{12}O$	88	0.43	0.02
19	23.83	1216	2-Hexenal	$C_6H_{10}O$	98	0.84	0.02
20	24.82	1232	2-Pentylfuran	$C_9H_{14}O$	138	1.62	0.15
21	25.11	1236	[*Z*]-Ocimene	$C_{10}H_{16}$	136	45.87	0.68
22	26.18	1252	[*E*]-Ocimene	$C_{10}H_{16}$	136	1.58	0.02
23	26.41	1256	Pentanol	$C_5H_{12}O$	88	1.35	0.02
24	28.75	1288	Octanal	$C_8H_{16}O$	128	5.28	0.08
IS	30.37	1312	*Butylbenzene*	*C10H14*	*134*	-	0.00
25	33.48	1359	Hexanol	$C_6H_{14}O$	102	3.87	0.05
26	35.94	1393	Nonanal	$C_9H_{18}O$	142	1.45	0.02
27	40.50	1464	Furfural	$C_5H_4O_2$	96	1.63	0.02
28	42.54	1494	α-Copaene	$C_{15}H_{24}$	204	3.77	0.05
29	42.71	1497	Unknown	-	-	3.22	0.05
30	42.97	1500	Decanal	$C_{10}H_{20}O$	156	6.91	0.09

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 7. Continued

No.	RT[a)]	RI [b)]	Compound name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
31	44.13	1519	Camphor	$C_{10}H_{16}O$	152	2.57	0.03
32	44.43	1524	Benzaldehyde	C_7H_6O	106	1.14	0.02
33	45.19	1536	[*Z*]-6-Nonenal	$C_9H_{16}O$	140	5.86	0.08
34	45.61	1542	[*Z*]-4-Decenal	$C_{10}H_{18}O$	154	3.95	0.05
35	46.29	1553	Linalool	$C_{10}H_{18}O$	154	31.59	0.41
36	47.60	1573	Unknown	-	-	10.16	0.14
37	48.89	1592	β-Elemene	$C_{15}H_{24}$	204	29.66	0.39
38	49.12	1595	Junipene	$C_{15}H_{24}$	204	22.95	0.38
39	49.35	1598	[*E*]-Caryophyllene	$C_{15}H_{24}$	204	8.29	0.11
40	49.78	1605	Unknown	-	-	4.55	0.06
41	52.36	1649	α-Humulene	$C_{15}H_{24}$	204	3.69	0.05
42	53.47	1667	Unknown	-	-	4.21	0.06
43	54.82	1689	Dodecanal	$C_{12}H_{24}O$	184	3.40	0.05
44	55.15	1694	Unknown	-	-	10.01	0.14
45	55.43	1698	Geramerene B	$C_{15}H_{24}$	204	31.37	0.42
46	56.20	1712	Aromadendrene	$C_{15}H_{24}$	204	18.90	0.26
47	56.81	1724	Unknown	-	-	3.14	0.05
48	57.09	1729	[*E*]-Farnesene	$C_{15}H_{24}$	204	10.92	0.15
49	57.68	1740	Geranyl acetate	$C_{12}H_{20}O_2$	196	28.09	0.38
50	59.07	1764	δ-Cadinene	$C_{15}H_{24}$	204	52.04	0.69
51	63.92	1864	Myrtenal	$C_{10}H_{16}O$	152	230.92	3.07
52	65.09	1890	Patchulane	$C_{15}H_{26}$	206	61.27	0.81
53	65.92	1910	[*E,Z*]-2,4-Decadienal	$C_{10}H_{16}O$	152	1063.93	14.15
54	70.88	2047	Farnesol	$C_{15}H_{26}O$	222	833.84	11.09
55	72.73	2099	Methyleugenol	$C_{11}H_{14}O_2$	178	458.33	6.10
56	76.52	2167	Elemicin	$C_{12}H_{16}O_3$	208	131.43	1.75
57	76.97	2175	α-Bisabolol	$C_{15}H_{26}O$	222	72.88	0.96
58	77.59	2186	α-Asarone	$C_{12}H_{16}O_3$	208	654.97	8.71
59	80.22	2252	β-Asarone	$C_{12}H_{16}O_3$	208	3508.28	46.78
					Total	**7493.59**	**100.00**

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

4.2.2. Volatile organic compounds of *Asparagus racemosus* Willd

The essential oil of *A. racemosus* was extracted by solvent extraction (P:E, 1:1) method for 2 h using SDE apparatus and analysed by GC/MS. Investigation confirmed that Nepal originated *A. racemosus* contained small amount (59.61 mg/kg) of essential oil. Identification of VOC's of *A. racemosus* is presented in Table 9 and GC/MS chromatogram is presented in Fig. 9. Total 49 volatile organic compounds, belonging to chemical classes of acid (5), alcohol (15), aldehyde (12), ester (1), hydrocarbon (8), ketone (5), N-containing compounds (1), miscellaneous (1) were tentatively identified (Table 8). Alcohol was the dominant family with the highest proportion accounting by 49.82 % of total content. Five alcohols, out of 15, were monoterpene alcohols. The major alcohol compounds were borneol (26.40%), myrtanol (13.72%), pinocarveol (2.37%) and 2-ethylhexanol (1.76%). Aldehyde was characterized as second largest chemical group containing 16.70%. Perillaldehyde (8.97%) was abundant aldehyde compound and 4-[1-hydroxyethyl]benzaldehyde (1.55%), hexanal (1.34%) and furfural (1.17%) were also detected in considerable amount. Acid and ketone containing 8.97% and 6.98% respectively were also characterized as major chemical groups present in essential oil of *A. racemosus*. Decanoic (4.19%) and undecanoic (2.72%) acids were important acid components while camphor (3.33%) and 6,10,14-trimethyl pentadecanone (1.71%) were characterized as important ketone components. The percentage of total hydrocarbons was 5.27%. All the hydrocarbons except [*E*]-4-hexadecen-6-yne were monoterpenes. Remaining chemical classes i.e. ester, S-containing compound and N-containing compounds were detected at levels lower than 3%. Only three compounds; borneol, myrtanaol and paraldehyde could occupy 45.09% of the whole content. The analysis of terepenoids in this result shows that the oil dominated by terpenes (mainly monoterpene and its derivatives) accounting more than fifty percent of the oil. This result indicated the presence of a high percentage of oxygenated monoterpenes (49.73%) in essential oil of *A. racemosus*.

The present study shows that *A. racemosus* oil exclusively is composed of terpenes, mainly oxygenated monoterpenes dominated by two compounds, borneol and myrtanol. Such essential oils, containing monoterpene as their major constituents are known highly effective for pharmacological activities (236,237). Compounds myrtanal, α-pinene, perillaldehyde, 2-carene and butyrophenones are well known for their biological activities

but unfortunately concentrations were detected by very small amounts. Oxygen-containing monoterpes have apparent antispasmodic, sedative and tranquilizing action and beneficial to various systems and metabolic processes in human organism (98). Compounds borneol, myrtanol and camphor were detected by high amounts. Borneol, a major constituent of *Asparagus* oil, is a very important ingredient in many Japanese incense formulas, used for analgesia and anesthesia in traditional Chinese and Japanese medicine as well as known for antimicrobial activities (238-240). Myrtanol, a second major constituent of this oil, exhibits activity as an insect repellent for lice (241). Camphor, a major constituent of this oil is well-known chemical with its pronounced antimicrobial potentials (222,223). Some of the hydrocarbon compounds such as structural isomers of pinene and 2-carene, detected in this sample are very important bioactive compounds as mentioned in litereatures (225,226,232). Similarly aldehyde compounds such as myrtenal and perillaldehyde were also identified in this study. Myrtenal is terpene-derived aldehydes considered to be produced by tropospheric oxidation of α-pinene (201). Perillaldehyde inhibits the vasoconstriction as well as therapeutic agents against infections caused by fungus (242-244). Beside a flavouring use of furfural, it has a wide variety of uses such as a weed killer, fungicide, affects yeast survival and also affects biochemical enzyme activities (245,246). Butyrophenones are widely used drugs for treatment of psychoses and are frequently encountered in forensic chemistry and clinical toxicology (247). But it is remarkable that compounds β-pinene, myrtenal, 2-carene, butyrophenone were detected in very small amounts i.e. below 1% of this oil. Although they usually occur as complex mixtures, their activity can generally be accounted for in terms of their major components.

Conclusively the prime volatile composition of *A. racemosus* was borneol and some major compounds ranged in content order as follows: myrtanol, perillaldehyde, decanoic acid, camphor, α-pinene oxide and pinocarveol. This species could be also utilized as a new source for isolation of borneol and other bioactive constituents. Profile of VOC's shows that this oil could be used for pharmacological activities and natural pesticides. But due to the low concentrations of VOC's, it is not feasible to commercial production of such oil in large volume.

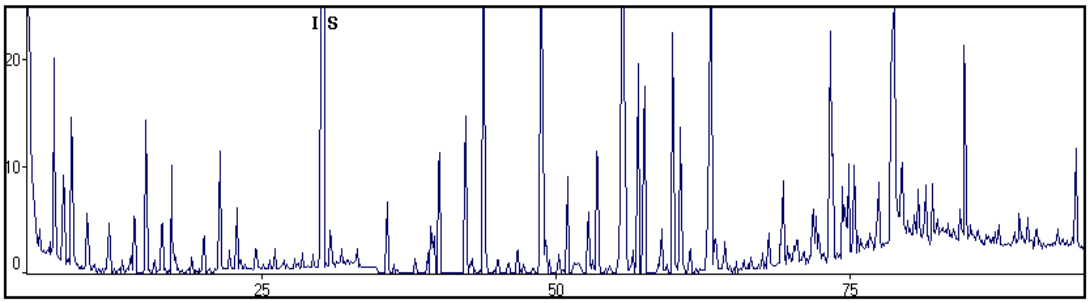

Fig. 9. GC/MS chromatogram of volatile organic compounds obtained from *Asparagus racemosus* Willd.

Table 8. Relative content of functional groups of volatile organic compounds identified in *Asparagus racemosus* Willd

No.	Functional groups	Relative peak area (%)	Number of compounds
1	Acid	8.97	5
2	Alcohol	49.82	15
3	Aldehyde	16.7	12
4	Ester	2.3	1
5	Hydrocarbon	5.27	8
6	Ketone	6.98	5
7	N-Compound	1.22	1
8	S-Compound	0.02	1
9	Miscellaneous	2.57	1
10	Unknown	6.15	6
	Total	**100**	**55**

Table 9. Volatile organic compounds of *Asparagus racemosus* Willd

No.	RT[a)]	RI [b)]	Compound name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
1	7.40	862	Ethyl acetate	$C_4H_8O_2$	88	1.35	2.30
2	8.07	891	2-Methyl butanal	$C_5H_{10}O$	86	0.18	0.30
3	8.20	896	3-Methyl butanal	$C_5H_{10}O$	86	0.55	0.94
4	8.86	921	2-Propanol	C_3H_8O	60	0.91	1.55
5	10.18	966	2-Pentanone	$C_5H_{10}O$	86	0.33	0.56
6	10.31	970	Pentanal	$C_5H_{10}O$	86	0.19	0.32
7	12.10	1018	Thujene	$C_{10}H_{16}$	136	0.24	0.40
8	14.21	1060	Camphene	$C_{10}H_{16}$	136	0.22	0.37
9	15.19	1078	Hexanal	$C_6H_{12}O$	100	0.79	1.34
10	15.90	1090	2-Methyl-1-propanol	$C_4H_{10}O$	74	0.02	0.03
11	16.55	1101	β-Pinene	$C_{10}H_{16}$	136	0.22	0.38
12	17.40	1116	Sabinene	$C_{10}H_{16}$	136	0.53	0.90
13	19.11	1145	Butanol	$C_4H_{10}O$	74	0.07	0.13
14	20.13	1161	α-Phellandrene	$C_{10}H_{16}$	136	0.14	0.22
15	21.47	1181	Pyridine	C_5H_5N	79	0.71	1.22
16	22.91	1201	β-Phellandrene	$C_{10}H_{16}$	136	0.31	0.46
IS	*30.24*	*1310*	*Butylbenzene*	*$C_{10}H_{14}$*	*136*	-	-
17	30.87	1320	3-Methyl-1-pentanol	$C_6H_{14}O$	102	0.06	0.10
18	33.20	1355	Hexanol	$C_6H_{14}O$	102	0.03	0.05
19	34.79	1377	Dipropyl disulfide	$C_6H_{14}S_2$	150	0.02	0.02
20	35.70	1390	Nonanal	$C_9H_{18}O$	142	0.36	0.61
21	39.41	1448	Acetic acid	$C_2H_4O_2$	60	0.34	0.58
22	39.67	1452	2,2-Dimethyl hexanal	$C_8H_{16}O$	128	0.21	0.37
23	40.13	1458	Furfural	$C_5H_4O_2$	96	0.68	1.17
24	42.36	1491	2-Ethyl hexanol	$C_8H_{18}O$	130	1.03	1.76
25	43.88	1515	Camphor	$C_{10}H_{16}O$	152	1.94	3.33
26	44.06	1518	Benzaldehyde	C_7H_6O	106	0.08	0.14
27	48.83	1591	Perillaldehyde	$C_{10}H_{14}O$	150	5.24	8.97
28	49.21	1596	Unknown	-	-	0.11	0.16
29	51.02	1627	Myrtenal	$C_{10}H_{14}O$	150	0.56	0.96
30	52.79	1656	Nonanol	$C_9H_{20}O$	144	0.42	0.72

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 9. Continued

No.	RT[a)]	RI [b)]	Compound name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
31	53.55	1669	Isoboroneol	$C_{10}H_{18}O$	154	0.79	1.34
32	55.37	1697	Unknown	-	-	0.13	0.19
33	55.71	1703	Borneol	$C_{10}H_{18}O$	154	15.42	26.40
34	55.95	1708	Unknown	-	-	0.86	1.30
35	57.01	1727	Pinocarveol	$C_{10}H_{16}O$	152	1.38	2.37
36	57.57	1737	[*E*]-4-Hexadecen-6-yne	$C_{16}H_{28}$	220	1.31	2.25
37	59.04	1764	2-Carene	$C_{10}H_{16}$	136	0.19	0.29
38	59.97	1780	α-Pinene oxide	$C_{10}H_{16}O$	152	1.50	2.57
39	60.62	1792	4-[1-Hydroxyethyl]benzaldehyde	$C_9H_{10}O_2$	150	0.91	1.55
40	60.72	1793	Butyrophenone	$C_{10}H_{12}O$	148	0.32	0.48
41	61.41	1807	[*E*,*Z*]-2,4-Decadienal	$C_{10}H_{16}O$	152	0.02	0.03
42	62.91	1841	Unknown	-	-	0.24	0.37
43	63.20	1848	Myrtanol	$C_{10}H_{18}O$	154	8.02	13.72
44	63.65	1858	Guaiacol	$C_7H_8O_2$	124	0.11	0.19
45	68.14	1970	α-Methylbenzyl alcohol	$C_8H_{10}O$	122	0.15	0.22
46	69.34	2002	Unknown	-	-	0.64	0.96
47	72.15	2083	Benzyl alcohol	$C_7H_8O_2$	108	0.28	0.48
48	73.33	2110	6,10,14-Trimethyl pentadecanone	$C_{18}H_{36}O$	268	1.00	1.71
49	74.42	2130	Octanoic acid	$C_8H_{16}O_2$	144	0.52	0.78
50	74.91	2139	*p*-Cymen-3-ol	$C_{10}H_{14}O$	150	0.50	0.75
51	75.41	2148	3-Methoxyacetophenone	$C_9H_{10}O_2$	150	0.59	0.90
52	77.47	2184	Nonanoic acid	$C_9H_{18}O_2$	158	0.41	0.70
53	78.59	2205	Decanoic acid	$C_{10}H_{20}O_2$	172	2.79	4.19
54	78.78	2211	Unknown	-	-	2.10	3.17
55	84.76	2373	Undecanoic acid	$C_{11}H_{22}O_2$	186	1.59	2.72
					Total	**59.61**	**100.00**

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

4.2.3. Volatile organic compounds of *Bergenia ciliata*- (Haw) Sternb

The essential oil of *B. ciliata* was extracted by solvent extraction (P:E,1:1) method for 2 h using SDE apparatus and analysed by GC/MS. Investigation confirmed that Nepal originated *B. ciliata* contained small amount (67.12 mg/kg) of essential oil. The VOC's were identified by GC/MS. The GC/MS chromatogram of VOC's obtained from this plant is presented in Fig. 10. Identified compounds, their retention times and area percentages are summarized in Table 11. This study enabled the identification of the 43 constituents of *B. ciliata* oil. Identified compounds belonged to chemical classes of acid (7), alcohol (13), aldehyde (5), ester (4), hydrocarbon (3), ketone (8), N-containing compounds (2) and miscellaneous (1) (Table. 10).

Acid was the family present in *B. ciliata* with the highest proportion accounting for 34.06% of the total content. The major acid compounds were capric (decanoic) (24.27%), caproic (hexanoic) (2.48%), and pelargonic (nonanoic) (2.31%) acids. Fatty acids such as valeric (pentanoic) acid, enanthoic (heptanoic) acid and caprylic (octanoic) acid, were also detected. Ketone group of chemical class (33.01%) was characterized as a second major chemical group containing 5,6-dihydro-2-pyranone (29.74%) as a dominant compound. Some of the N-containing compounds such as hexanenitril (1.49%) and 2-nitropropane (0.03%) were also detected. Alcohols and hydrocarbons containing 13.77% and 5.16% respectively were also detected at high levels. Aliphatic alcohols were dominant among this group while linalool (7.51%) contributed the major portion of alcohol. Remaining alcohol compounds were detected lower than 2%. Hydrocarbon group was also detected in this oil including some aliphatic and aromatic constituents. Compounds limonene (1.89%), β-phellandrene (0.34%) and β-caryophyllene (2.71%) were the major hydrocarbons related to terpene group. Only six terpenoids containing 11.8% were detected.

The characteristic of some VOC's is also discussed after identification of compounds. Linalool is important substance used in foodstuffs as a food additive (202,203) and bioactive compound (204-211). Litereatures confirm that limonene is an antiseptic chemotherapeutic agent beside its fragrance value (207,208). α-Terpineol has myorelaxant and antispasmodic effects (248). β-Caryophyllene has been commonly used as a fragrance chemical since the 1930s and recent litereature described as woody and spicy odour (227, 228). Camphor is well-known chemical with its pronounced antimicrobial potentials (222,223). But, 2-nitropropane has been found to cause hepatotoxicity in occupationally

exposed humans (249). Compounds α-terpineol, camphor and 2-nitropropane contained by less than 1% concentration while compounds β-caryophyllene contained by 2.71% concentration of this oil.

In conclusion, the prime volatile compound of *B. ciliata* was 5,6-dihydro-2-pyranone and major compounds can be ranged in content the following order: decanoic acid, linalool, nonanoic acid, β-caryophyllene and hexanal. The rhizome and root of this species can be utilized as a new source for isolation of 5,6-dihydro-2-pyranone and some other bioactive components. But due to the low concentrations of VOC's, it is not feasible to commercial production of such oil in large volume.

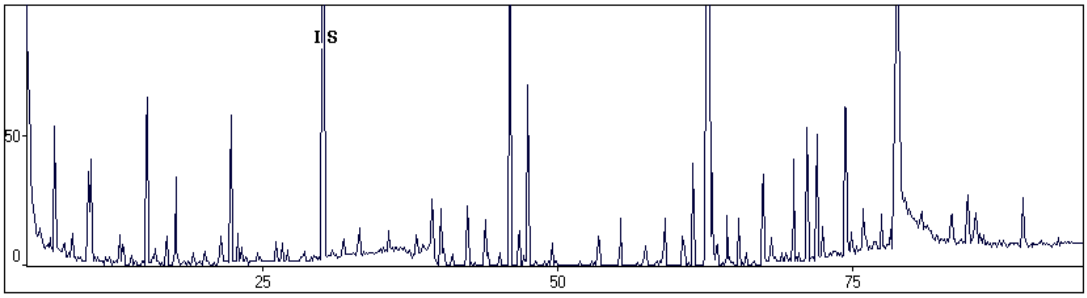

Fig. 10. GC/MS chromatogram of volatile organic compounds obtained from *Bergenia ciliata*- (Haw) Sternb.

Table 10. Relative content of functional groups of volatile organic compounds identified in *Bergenia ciliata*- (Haw) Sternb

No.	Functional groups	Relative peak area (%)	Number of compounds
1	Acid	34.06	7
2	Alcohol	13.77	13
3	Aldehyde	4.56	5
4	Ester	4.93	4
5	Hydrocarbon	5.16	3
6	Ketone	33.01	8
7	N-Compound	1.74	2
8	Miscellaneous	0.36	1
9	Unknown	2.41	5
	Total	**100**	**48**

Table 11. Volatile organic compounds of *Bergenia ciliata*- (Haw) Sternb

No.	RT[a)]	RI [b)]	Compound name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
1	7.42	864	Ethyl acetate	$C_4H_8O_2$	88	1.24	1.84
2	8.90	923	Ethanol	C_2H_6O	46	0.19	0.29
3	10.28	969	2-Pentanone	$C_5H_{10}O$	86	1.04	1.55
4	10.51	976	Unknown	-	-	0.76	1.14
5	13.21	1041	2,4-Dimethyl-3-pentanone	$C_7H_{14}O$	114	0.17	0.26
6	15.23	1079	Hexanal	$C_6H_{12}O$	100	1.48	2.21
7	15.91	1090	2-Methyl propanol	$C_4H_{10}O$	74	0.07	0.10
8	16.91	1108	3-Pentanol	$C_5H_{12}O$	88	0.21	0.30
9	17.71	1122	2-Pentanol	$C_5H_{12}O$	88	0.87	1.30
10	21.50	1182	Heptanal	$C_7H_{14}O$	114	0.13	0.19
11	22.35	1193	Limonene	$C_{10}H_{16}$	136	1.42	2.11
12	22.94	1202	*β*-Phellandrene	$C_{10}H_{16}$	136	0.23	0.34
13	23.27	1207	3-Methyl butanol	$C_5H_{12}O$	88	0.09	0.14
14	26.17	1252	Pentanol	$C_5H_{12}O$	88	0.25	0.37
15	26.68	1260	3-Methyl-4-hexen-2-one	$C_7H_{12}O$	112	0.07	0.11
16	28.41	1284	2-Nitropropane	$C_3H_7NO_2$	89	0.02	0.03
IS	*30.23*	*1310*	*Butylbenzene*	*$C_{10}H_{14}$*	*134*	*0.00*	*0.00*
17	31.89	1335	[*E*]-4-Hepten-2-one	$C_7H_{12}O$	112	0.19	0.24
18	33.22	1355	Hexanol	$C_6H_{14}O$	102	0.19	0.29
19	35.74	1390	Unknown	-	-	0.20	0.30
20	39.41	1448	Acetic acid	$C_2H_4O_2$	60	0.88	1.31
21	40.14	1459	Heptanol	$C_7H_{16}O$	116	0.67	0.99
22	40.41	1463	2,4-Hexadienal	C_6H_8O	96	0.16	0.24
23	42.41	1492	2-Ethyl hexanol	$C_8H_{18}O$	130	0.88	1.31
24	43.91	1516	Camphor	$C_{10}H_{16}O$	152	0.43	0.64
25	46.03	1549	Linalool	$C_{10}H_{18}O$	154	5.03	7.51
26	46.79	1561	Unknown	-	-	0.29	0.43
27	47.52	1572	*β*-Caryophyllene	$C_{15}H_{24}$	204	1.82	2.71
28	53.49	1668	2-Methyl butanoic acid	$C_5H_{10}O_2$	102	0.41	0.62
29	55.37	1697	*α*-Terpineol	$C_{10}H_{18}O$	154	0.47	0.70
30	57.53	1737	Pentanoic acid	$C_5H_{10}O_2$	102	0.10	0.14

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 11. Continued

No.	RT[a)]	RI [b)]	Compound name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
31	59.13	1766	2,4-Nonadienal	$C_9H_{14}O$	138	0.37	0.56
32	60.64	1792	δ-Hexalactone	$C_6H_{10}O_2$	114	0.12	0.18
33	60.80	1795	Isobutyrophenone	$C_{10}H_{12}O$	148	0.19	0.29
34	61.51	1809	[*E,Z*]-2,4-Decadienal	$C_{10}H_{16}O$	152	0.91	1.36
35	62.77	1838	5,6-Dihydro-2-pyranone	$C_5H_6O_2$	98	19.94	29.74
36	63.04	1844	Hexanoic acid	$C_6H_{12}O_2$	116	1.67	2.48
37	63.56	1856	Unknown	-	-	0.15	0.19
38	64.37	1874	5-[2-Propenyl]-1,3-benzodioxole	$C_{10}H_{10}O_2$	162	0.39	0.58
39	65.42	1897	Unknown	-	-	0.28	0.35
40	67.46	1952	Heptanoic acid	$C_7H_{14}O_2$	130	1.02	1.52
41	68.21	1972	α-Phenylethyl alcohol	C_8H_8O	122	0.19	0.29
42	70.03	2023	Hexanenitril	$C_6H_{11}N$	97	1.00	1.49
43	71.18	2055	Octanoic acid	$C_8H_{16}O_2$	144	1.36	2.03
44	71.99	2078	Methyl cinnamate	$C_{10}H_{10}O_2$	162	1.26	1.89
45	74.44	2130	Nonanoic acid	$C_9H_{18}O_2$	158	1.55	2.31
46	75.97	2158	Methyl nonanoate	$C_{10}H_{20}O_2$	172	0.38	0.58
47	76.16	2161	2-Phenylisopropanol	$C_9H_{12}O$	136	0.12	0.18
48	78.79	2211	Decanoic acid	$C_{10}H_{20}O_2$	172	16.26	24.27
					Total	**67.12**	**100.00**

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

4.2.4. Volatile organic compounds of *Centella asiatica* (L) Urb

The essential oil of *C. asiatica* was extracted by solvent extraction (P:E, 1:1) method for 2 h using SDE apparatus and analysed by GC/MS. Investigation confirmed that the yield of essential oil obtained from Nepal originated *C. asiatica* rhizome was 1075.04 mg/kg. VOC's were identified by GC/MS (Fig. 11). A total of 53 volatile organic compounds were tentatively identified and quantified from the essential oil of *C. asiatica* so far belonging to chemical classes of acid (1), alcohol (12), aldehyde (11), ester (2), hydrocarbon (19), ketone (7), miscellaneous (1). The result obtained by qualitative and quantitative analysis of VOC's of essential oil is listed according to their elution order on DB-WAX column and their amounts (Table 13).

Hydrocarbon group (67.35%) was detected as the main functional group in this oil. Total 18 hydrocarbons, out of 19, were terpenoids. Alkane hydrocarbons were minor among them. The analysis of terpenoids showed that essential oil of *C. asiatica* is highly composed of terpene accounting 72.89% of the total content. Mainly sesquiterpenes contributed the large portion (65.30%) while oxygenated monoterpenes (3.78%), oxygenated sesquiterpenoid (2.1%) and monoterpene hydrocarbon (1.71%) achieved for low amounts. Major sesquiterpene were [*Z*]-*β*-farnesene (24.74%), *β*-selinene (12.66%), *β*-bisabolene (8.85%), [*E*]-caryophyllene (7.76%), *β*-elemene (5.05%). Alcohol group (7.8%) was the second major chemical group. Compounds linalool (0.61%), α-terpineol (0.24%), [*E*]-geraniol (1.16%), farnesol (0.94%) and nerolidol (1.16%) were the most abundant terpene alcohols. Aliphatic alcohols such as ethanol, 2-methyl pentanol, hexanol, heptanol and decanol were detected at low amounts. Similarly ketone and aldehyde, containing 4.47% and 2.37% respectively were characterized as major chemical groups. Compounds 3-nonen-2-one (2.42%), camphor (1.36%), 5-methyl-5-hexen-2-one (0.42%) and 2-butanone (0.11%) were major ketones while remaining ketone compounds were detected at levels lower than 0.1%. Most of all the aldehydes were detected at very low amounts. Altogether total 15 constituents, in an amount higher than 1%, were identified in this oil. The prime constituent was [*Z*]-*β*-farnesene and major compounds ranged in content order as follows: *β*-selinene, *β*-bisabolene, [*E*]-caryophyllene and *β*-elemene.

Investigatioin revealed that some of the alcohols compounds such as α-terpineol and linalool, were detected by very small amounts i.e. below 1% but other alcohol compounds such as nerolidol and geraniol detected by high concentratin. Among these components,

α-terpineol is known for myorelaxant and antispasmodic effects (248). Linalool, a dominant compound of this oil, is very important substance used in foodstuffs as a food additives (202,203) and pharmacology (204-211) for different activities. Anti-cancer effects and antibacterial activity of farnesol have been demonstrated in a number of studies (212-215). Nerolidol and geraniol have high relative ovicidal activity, against human lice (250). The compounds 3-carene, [*E*]-β-ocimene, myrcene, *p*-cymene and limonene are detected by less than 1% concentration. Among the hydrocarbon compounds, some compounds such as β-elemene, limonene, [*E*]-β-ocimene, β-myrcene, *p*-cymene and 3-carene are important compounds finding application in fragrance, pharmaceutical and agrochemical fields (161,208, 224, 229-233,). Another hydrocarbon compound *p*-cymene smells citrusy flavor and known as anti-microbial activity against *Escherichia coli* (251). Limonene has been shown to be an anti-cancer (207) activity. Camphor, important ketone detected in this sample, is well-known constituent with its pronounced antimicrobial potentials (222,223). VOC's such as *trans*-caryophyllene and α-humulene, are likely to be the precursors of the complex menthols or resins which have been claimed to also contain the antibacterial, antifungal or antioxidant properties (252,253).

It is concluded that *C. asiatica* can yield an essential oil useful for the pharmaceutical and flavor and fragrance industries for its high content of compounds especially [*Z*]-β-farnesene, β-selinene, β-bisabolene, [*E*]-caryophyllene and β-elemene.

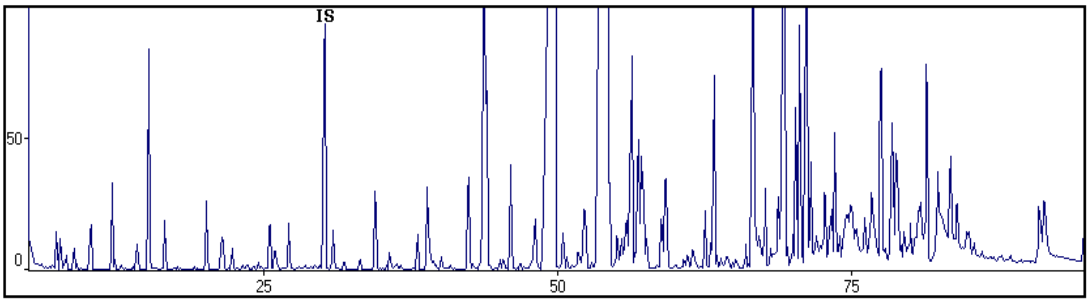

Fig. 11. GC/MS chromatogram of volatile organic compounds obtained from *Centella asiatica* (L) Urb.

Table 12. Relative content of functional groups of volatile organic compounds identified in *Centella asiatica* (L) Urb

No.	Functional groups	Relative peak area (%)	Number of compounds
1	Acid	0.13	1
2	Alcohol	7.8	12
3	Aldehyde	2.37	11
4	Ester	0.7	2
5	Hydrocarbon	67.35	19
6	Ketone	4.47	7
7	Miscellaneous	0.41	1
8	Unknown	16.77	5
	Total	**100**	**58**

Table 13. Volatile organic compounds of *Centella asiatica* (L) Urb

No.	RT[a)]	RI[b)]	Compound Name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
1	7.43	864	Ethyl acetate	$C_4H_8O_2$	88	1.59	0.15
2	7.77	878	2-Butanone	$C_4H_8O_2$	72	1.24	0.11
3	8.13	893	2-Methylbutanal	$C_6H_{10}O$	86	0.29	0.03
4	8.24	898	3-Methylbutanal	$C_6H_{10}O$	86	0.59	0.03
5	8.91	923	Ethanol	C_2H_6O	46	1.13	0.10
6	9.13	931	3-Buten-2-one	$C_4H_6O_2$	70	0.26	0.03
7	10.22	967	2,3-Butanedione	$C_4H_6O_2$	86	0.43	0.04
8	10.33	971	Pentanal	$C_5H_{10}O$	86	2.04	0.19
9	12.16	1019	3-Carene	$C_{10}H_{16}$	136	4.31	0.41
10	14.30	1062	Camphene	$C_{10}H_{16}$	136	1.14	0.11
11	15.27	1079	Hexanal	$C_6H_{12}O$	100	11.14	1.06
12	16.62	1102	*β*-Pinene	$C_{10}H_{16}$	136	2.54	0.24
13	20.18	1162	*β*-Myrcene	$C_{10}H_{16}$	136	3.44	0.33
14	21.39	1180	2-Heptanone	$C_7H_{14}O$	114	1.00	0.09
15	21.54	1182	Heptanal	$C_7H_{14}O$	114	1.66	0.15
16	22.36	1194	Limonene	$C_{10}H_{16}$	136	1.07	0.10
17	25.55	1243	γ-Terpinene	$C_{10}H_{16}$	136	2.31	0.22
18	25.99	1250	[*E*]-*β*-Ocimene	$C_{10}H_{16}$	136	0.79	0.08
19	27.18	1267	*p*-Cymene	$C_{10}H_{14}$	134	2.30	0.22
IS	*30.24*	*1310*	*Butylbenzene*	*$C_{10}H_{14}$*	*134*	*20.00*	*0.00*
20	30.95	1321	2-Methyl pentanol	$C_6H_{14}O$	102	2.28	0.22
21	33.24	1355	Hexanol	$C_6H_{14}O$	102	0.66	0.06
22	34.53	1374	5-Methyl-5-hexen-2-one	$C_7H_{12}O$	112	4.45	0.42
23	34.98	1380	Nonanal	$C_9H_{18}O$	142	0.02	0.00
24	38.14	1427	[*E*]-2-Octenal	$C_8H_{14}O$	126	2.04	0.19
25	40.16	1459	Furfural	$C_5H_4O_2$	96	0.87	0.08
26	42.48	1493	*α*-Copaene	$C_{15}H_{24}$	204	8.04	0.76
27	43.80	1514	3-Nonen-2-one	$C_9H_{16}O$	140	25.56	2.42
28	44.01	1517	Camphor	$C_{10}H_{16}O$	152	14.31	1.36
29	45.17	1536	Heptanol	$C_7H_{16}O$	116	0.56	0.05
30	46.06	1549	Linalool	$C_{10}H_{18}O$	154	6.38	0.61

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 13. Continued

No.	RT[a)]	RI[b)]	Compound Name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
31	48.14	1581	Limonene oxide	$C_{10}H_{16}O$	152	4.24	0.41
32	49.23	1597	*β*-Elemene	$C_{15}H_{24}$	204	53.30	5.05
33	49.60	1602	[*E*]-Caryophyllene	$C_{15}H_{24}$	204	81.85	7.76
34	49.81	1606	*β*-Bisabolene	$C_{15}H_{24}$	204	93.33	8.85
35	52.32	1648	*α*-Humulene	$C_{15}H_{24}$	204	8.97	0.85
36	53.91	1674	[*Z*]-β-Farnesene	$C_{15}H_{24}$	204	260.87	24.74
37	54.30	1680	*β*-Selinene	$C_{15}H_{24}$	204	133.53	12.66
38	54.44	1683	Decyl acetate	$C_{12}H_{24}O_2$	200	5.82	0.55
39	55.47	1699	*α*-Terpineol	$C_{10}H_{18}O$	154	2.53	0.24
40	56.37	1715	Calarene	$C_{15}H_{24}$	204	26.00	2.47
41	56.93	1726	Junipene	$C_{15}H_{24}$	204	12.94	1.22
42	57.20	1731	Valencen	$C_{15}H_{24}$	204	9.99	0.94
43	57.38	1734	Unknown	-	-	6.22	0.59
44	59.22	1767	Decanol	$C_{10}H_{22}O$	158	6.68	0.64
45	61.53	1810	[*E*,*E*]-2,4-Decadienal	$C_{10}H_{16}O$	152	1.39	0.13
46	62.60	1834	Patchulane	$C_{15}H_{26}$	206	3.62	0.34
47	63.08	1845	Hexanoic acid	$C_6H_{12}O_2$	116	1.37	0.13
48	63.33	1851	[*E*]-Geraniol	$C_{10}H_{18}O$	154	12.15	1.16
49	66.04	1913	Phenyl ethyl alcohol	$C_8H_{10}O$	122	1.49	0.14
50	66.66	1930	Dodecanol	$C_{12}H_{26}O$	186	26.21	2.48
51	66.74	1932	Unknown	-	-	3.99	0.38
52	67.69	1958	Tetradecanal	$C_{14}H_{28}O$	212	3.97	0.38
53	68.69	1985	Hexadecanal	$C_{16}H_{32}O$	240	1.30	0.13
54	69.32	2002	Unknown	-	-	82.00	7.78
55	70.30	2030	Farnesol	$C_{15}H_{26}O$	222	9.90	0.94
56	70.64	2040	Nerolidol	$C_{15}H_{26}O$	222	12.24	1.16
57	71.26	2058	Unknown	-	-	67.82	6.43
58	81.43	2286	Unknown	-	-	16.85	1.60
						1075.04	**100.00**

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

4.2.5. Volatile organic compounds of *Dipsacus mitis* D.Don

The essential oil of *D. mitis* was extracted by solvent extraction (P:E, 1:1) method for 2 h using SDE apparatus and analysed by GC/MS. Investigation confirmed that the yield of essential oil obtained from Nepal originated *D. mitis* was 64.11 mg/kg. VOC's were identified by GC/MS (Fig. 12). The percentage content of the individual components, retention indices, and retention times are summarized in Table 15. Fifty three volatile organic compounds of the essential oil so far belonging to chemical classes of acid (4), alcohol (21), aldehyde (14), ester (2), furan (3), ketone (6), N-containing compounds (3), miscellaneous (1) were tentatively identified (Table 14). Alcohol was the chemical family with the highest concentration accounting 44.43% of the total content. The major alcohol compounds were α-terpineol (6.30%), hexanol (5.72%), 2-heptanol (4.73%), linalool (4.29%), 2-pentanol (2.52%). Aliphatic alcohols were the dominant among the alcohol compounds. Aldehyde (28.66%) is characterized as second major chemical group. Compounds hexanal (6.24 %), 2-butenal (8.47%), 3-methylbutanal (3.03%) and [*E,E*]-2,4-decadienal (1.61%) were the dominant aldehydes. Similarly ketone and acid containing 8.34% and 5.89% respectively were characterized as major chemical groups. 2-Pentanone (2.95%), 3-methoxyacetophenone (2.51%) and [*E*]-geranyl acetone (1.92%) were the dominant ketones. Compounds acetic acid (2.61%), hexanoic acid (1.28%), octanoic acid (0.52%) and nonanoic acid (1.48%) were the major acid compounds. Terpene components [*E*]-geraniol, α-terpineol, linalool and 1,8-cineole detected in this oil, were oxygenated monoterpenes. Four major terpenoids 1-8-cineole, linalool, α-terpeniol and [*E*]-geraniol accounted 16.99%.

The VOC's obtained from the *D. mitis* have great variety of phytochemicals with possibilities of wide range of bioactivities. Therefore the characteristics of few important VOC's of this herb are discussed here. Among the major components, α-terpineol has myorelaxant and antispasmodic effects and it is probably the most important of the monocyclic monoterpene alcohols. It is a colorless, crystalline solid with a lilac odor. Major use are various flavor or compositions, such as berry, lemon, lime, nutmeg, orange, ginger, anise, peach, etc. and has myorelaxant and antispasmodic effects (248). Another compound linalool is also important compound detected in this species. Linalool is important compound used in foodstuffs as a food additive (202,203) and pharmacology (204-211). Compound 1,8-cineole is well-known chemical with its pronounced antimicrobial potentials

(222,223). 1,8-Cineole has several functions such as inhibition of 5-lipooxygenase, the formation of LTB4, LCT4, LTD4 and LET4, inhibition of COX enzymes etc (254). Some water-soluble components such as α-terpineol and 1,8-cineole indicated that it has anti-inflammatory properties (255). Beside a flavouring use of furfural, it has a wide variety of uses such as a weed killer, fungicide, affects yeast survival and also affect biochemical enzyme activities (245,246). Geraniol has high relative ovicidal activity, against human lice (250). It has been known that the oils containing 1,8-cineole and linalool, as major constituents are potent sprout inhibitors and can be extended for commercialization (135).

On the basis of above result we concluded that compound 2-butenal was the prime compound and major compounds ranged in content order as follows: α-terpineol, hexanal, hexanol, 1,8-cineole, 2-heptanol, linalool. Systematic fractionization of this oil could give a number of terpenoids such as α-terpineol, 1,8-cineole, and linalool. But due to their small quantity of yield, it is not feasible to commercial production of such oil/compounds in large volume.

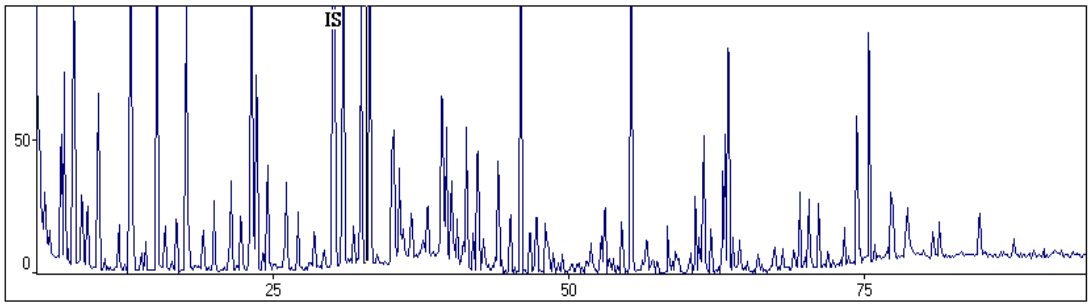

Fig. 12. GC/MS chromatogram of volatile organic compounds obtained from *Dipsacus mitis* D.Don.

Table 14. Relative content of functional groups of volatile organic compounds identified in *Dipsacus mitis* D.Don

No.	Functional groups	Relative peak area (%)	Number of compounds
1	Acid	5.89	4
2	Alcohol	44.43	21
3	Aldehyde	28.66	14
4	Ester	2.85	2
5	Furan	2.47	3
6	Ketone	8.34	6
7	N-Compound	4.07	3
8	Unknown	3.42	6
	Total	**100**	**59**

Table 15. Volatile organic compounds of *Dipsacus mitis* D.Don

No.	RT[a)]	RI[b)]	Compound Name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
1	7.12	850	Butanal	C_4H_8O	72	0.80	1.26
2	7.39	862	Ethyl acetate	$C_4H_8O_2$	88	1.54	2.40
3	7.59	871	2-Methylbutanal	$C_5H_{10}O$	86	0.02	0.03
4	8.20	896	3-Methylbutanal	$C_5H_{10}O$	86	1.94	3.03
5	8.87	922	Ethanol	C_2H_6O	46	0.62	0.95
6	9.33	938	2-Ethylfuran	C_6H_8O	96	0.37	0.58
7	10.28	969	2-Pentanone	$C_5H_{10}O$	86	1.90	2.95
8	12.03	1016	Unknown	-	-	0.26	0.42
9	12.96	1036	2-Butenal	C_4H_6O	70	5.43	8.47
10	13.18	1040	2,3-Dihydrofuran	C_4H_6O	70	0.56	0.86
11	15.23	1079	Hexanal	$C_6H_{12}O$	100	4.00	6.24
12	15.90	1090	2-Methylpropanol	$C_4H_{10}O$	74	0.32	0.51
13	16.88	1107	3-Pentanol	$C_5H_{12}O$	88	0.31	0.48
14	17.69	1121	2-Pentanol	$C_5H_{12}O$	88	1.65	2.57
15	17.86	1124	[*E*]-2-Pentenal	C_5H_8O	84	0.30	0.48
16	19.14	1146	Butanol	$C_4H_{10}O$	74	0.17	0.26
17	20.03	1160	Unknown	-	-	0.43	0.68
18	21.35	1179	2-Heptanone	$C_7H_{14}O$	114	0.10	0.15
19	21.50	1181	Pyridine	C_5H_5N	79	0.65	1.01
20	22.31	1193	[*Z*]-4-Heptenal	$C_7H_{12}O$	112	0.35	0.54
21	23.18	1206	1,8-Cineole	$C_{10}H_{18}O$	154	3.30	5.15
22	23.27	1207	3-Methylbutanol	$C_5H_{12}O$	88	0.98	1.54
23	23.64	1213	2-Hexenal	$C_6H_{12}O$	88	1.31	2.04
24	24.57	1228	2-Pentylfuran	$C_9H_{14}O$	138	0.66	1.03
25	26.16	1252	Pentanol	$C_5H_{12}O$	88	0.69	1.08
26	28.54	1285	Octanal	$C_8H_{16}O$	128	0.26	0.40
IS	*30.28*	*1310*	*Butylbenzene*	*$C_{10}H_{14}$*	*134*	-	-
27	31.00	1322	2-Heptanol	$C_7H_{16}O$	116	3.04	4.73
28	31.85	1335	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	126	0.23	0.35
29	32.50	1344	2-Octanol	$C_8H_{18}O$	130	1.91	2.98
30	33.25	1356	Hexanol	$C_6H_{14}O$	102	3.67	5.72

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 15. Continued

No.	RT[a)]	RI[b)]	Compound Name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
31	35.13	1382	4-Ethylpyridine	C_7H_9N	107	0.93	1.46
32	35.27	1384	3-Hexenol	$C_6H_{12}O$	100	1.03	1.61
33	35.73	1390	Nonanal	$C_9H_{18}O$	142	0.65	1.01
34	36.74	1405	2-Hexenol	$C_6H_{12}O$	100	0.25	0.38
35	38.11	1427	Unknown	-	-	0.38	0.60
36	39.32	1446	Acetic acid	$C_2H_4O_2$	60	1.68	2.61
37	39.70	1452	1-Octen-3-ol	C_8H_6O	128	0.87	1.35
38	40.14	1459	Furfural	$C_5H_4O_2$	96	0.62	0.97
39	41.40	1477	4-Ethyenylpyridine	C_7H_7N	105	1.02	1.60
40	42.31	1491	[*E*,*E*]-2,4-Heptadienal	$C_7H_{10}O$	110	0.77	1.20
41	42.39	1492	2-Ethylhexanol	$C_8H_{18}O$	130	0.97	1.52
42	44.07	1518	Benzaldehyde	C_7H_6O	106	0.89	1.40
43	45.11	1535	Octanol	$C_8H_{18}O$	130	0.34	0.52
44	46.01	1549	Linalool	$C_{10}H_{18}O$	154	2.74	4.29
45	47.34	1569	3,5-Octadien-2-one	$C_8H_{12}O$	124	0.29	0.46
46	48.08	1580	Linalool acetate	$C_{12}H_{20}O_2$	196	0.29	0.45
47	53.12	1662	Nonanol	$C_9H_{20}O$	144	0.47	0.74
48	54.53	1684	Unknown	-	-	0.35	0.54
49	55.37	1697	α-Terpineol	$C_{10}H_{18}O$	154	4.04	6.30
50	58.41	1753	Unknown	-	-	0.29	0.46
51	61.49	1809	[*E*,*E*]-2,4-Decadienal	$C_{10}H_{16}O$	152	1.04	1.61
52	63.04	1844	Hexanoic acid	$C_6H_{12}O_2$	116	0.82	1.28
53	63.26	1849	[*E*]-Geraniol	$C_{10}H_{18}O$	154	0.79	1.25
54	63.55	1856	[*E*]-Gerayl acetone	$C_{13}H_{22}O$	194	1.23	1.92
55	64.48	1876	Benzyl alcohol	C_7H_8O	108	0.23	0.35
56	69.59	2010	Unknown	-	-	0.47	0.72
57	71.18	2055	Octanoic acid	$C_8H_{16}O_2$	144	0.33	0.52
58	74.42	2130	Nonanoic acid	$C_9H_{18}O_2$	158	0.95	1.48
59	75.43	2148	3-Methoxyacetophenone	$C_9H_{10}O_2$	150	1.61	2.51
						64.11	**100.00**

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

4.2.6. Volatile organic compounds of *Swertia chirayita* Hamilt

The essential oil of *S. chirayita* was extracted by solvent extraction (P:E,1:1) method for 2 h using SDE apparatus and analysed by GC/MS. Investigation confirmed that the yield of essential oil obtained from Nepal originated *S. chirayita* was 249.73 mg/kg. The VOC's were identified and listed in Table 17 and chromatogram is shown in Fig. 13. Seventy seven compounds so far belonging to chemical classes of acid (4), alcohol (21), aldehyde (15), ester (3), furan (7), ketone (17), N-containing compounds (1), miscellaneous (6) were tentatively identified. Ketone was the chemical class with the highest proportion 27.16%. The major ketone compounds were 3-buten-2-one (8.18%), camphor (7.36%), 2-heptadecanone (5.90%), 3-ethnyl cyclohexenone (1.84%) and [Z]-geranylacetone (1.00%). Most of the compounds related to ketone group were aliphatic compounds and most of them were found in amounts lower than 1%. Similarly, alcohols containing 25.61% were characterized as second major chemical group. Cedrol was the most abundant compound while patchoulol (3.32%), β-eudesmol (1.85%), isothujol (1.74%), *p*-cymen-3-ol (1.62%), linalool (1.39%) and farnesol (1.11%) were also detected by high amounts. All of these compounds are terpene alcohols. Similarly acids and aldehydes containing 16.73% and 10.53% respectively were characterized as major chemical groups. All the acids compounds were fatty acids viz: undecanoic acid (11.46%), nonanoic acid (2.57%), decanoic acid (2.12%) and octanoic acid (0.58%). 2-Butenal (3.48%), tetradecanal (1.80%) and hexanal (1.07%) were the important aldehydes while remaining aldehydes were quantified below 1%. Hydrocarbons (2.54%) constituted the small part of total content. However, the hydrocarbons were related to terpene group. The complete profile of the terpenoids showed, 8 oxygenated monoterpenes (13.99%). Eight compounds of sesquiterpenes included 4 hydrocarbon (1.39%) and 4 oxygenated (11.51%). Only one compound belonging to hydrocarbon monoterpene was also detected.

The essential oil obtained from the *S. chirayita* found a great variety of phytochemicals having wide range of bioactivities. The characteristics of some important compounds those detected in this sample are discussed here. Among the identified terpenoids, compounds linalool, α-terpineol and geraniol were major oxygenated monoterpenes while farnesol and β-eudesmol were the major oxygenated sesquiterpene. Although monoterpenes are generally regarded as safe substances, some monoterpenoids of plant essential oils have been found to possess genotoxic and carcinogenic properties (e.g. safrole). The compounds linalool and *p*-

cymene, detected with good amounts in this species, have anti-microbial activities as described in literature (256). Linalool, a dominant compound of this oil is important substance used in foodstuffs as a food additives (202,203) and pharmacology (204-211). Terpenoidal alcohols such as α-terpineol, geraniol, farnesol, β-eudesmol have a number of biological activities including myorelaxant, antispasmodic, anti-cancer, anti-tumer and antioxidant activities (212-215,248,250). The potential of β-eudesmol to serve as an antiepileptic and antagonistic agent with very low toxicity was suggested previously (257,258). Oxygenated monoterpenes were dominant terpene in this oil. Consequently monoterpene phenols were previously reported to be active against fungi (166,173,178, 180) and can be used as alternative sprout inhibitors (134,135). Carvone was found to be potentially good therapeutic agents against infections caused by fungus and bacteria (259,244) and also used as flavoring agent in food items (260). To preserve the agricultural products, carvone can be used as a good potato sprouting inhibitor (261) and an insecticide (262,118). Furfural has a wide variety of uses such as a solvent, an ingredient of phenolic resins, chemical intermediate, weed killer, fungicide and also as a flavouring agent (245,246). Some of the hydrocarbon sesquiterpenes were below 0.6% concentrations. But they are well known for their pharmacological activities. The isomer of farnesene is known as electrophysiologically active component for pheromonal activities (263). Compounds, such as camphor, *trans*-caryophyllene and α-humulene, are likely to be the precursors of the complex menthols or resins which have been claimed to also contain the antibacterial, antifungal or antioxidant properties (252,253). Major uses are in various flavor compositions, such as berry, lemon, lime, nutmeg, orange, ginger, anise, peach, etc. with myorelaxant and antispasmodic effects (248). Camphor, a third major compound of this oil, is well known with its pronounced antimicrobial potentials (222,223). Compound cedrol, major constituent of this oil, has parasympathetic activity and decrease blood pressure (264).

On the basis of the above results it concludes that undecanoic acid was the prime component in volatile oil of *S. chirayita* and major compounds ranged in content order as follows: 3-buten-2-one, camphor, 2-heptadecanone and cedrol. This species can yield an essential oil useful for the pharmaceutical industry. Additionally, the oil could be used as sprout inhibitor as it contains such components.

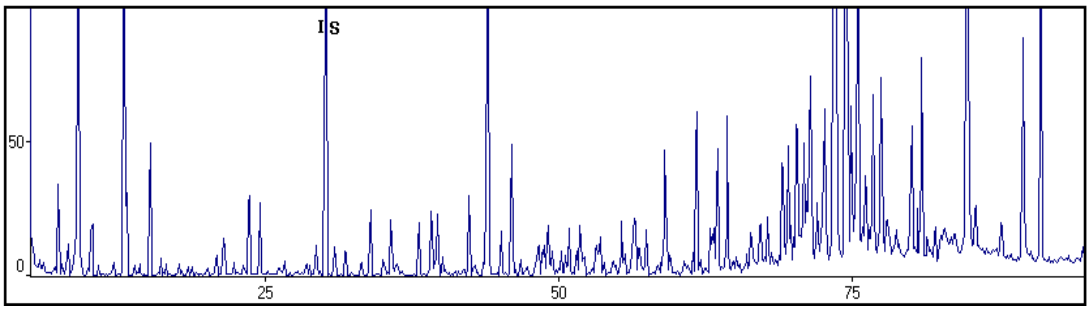

Fig. 13. GC/MS chromatogram of volatile organic compounds of *Swertia chirayita* Hamilt.

Table 16. Relative content of functional groups of volatile organic compounds identified in *Swertia chirayita* Hamilt

No.	Functional groups	Relative peak area (%)	Number of compounds
1	Acid	16.73	4
2	Alcohol	25.61	21
3	Aldehyde	10.53	15
4	Ester	3.13	3
5	Furan	1.31	3
5	Hydrocarbon	2.54	7
6	Ketone	27.16	17
7	N-Compound	0.22	1
8	Miscellaneous	7.46	6
9	Unknown	5.31	4
	Total	**100**	**81**

Table 17. Volatile organic compounds of *Swertia chirayita* Hamilt

No.	RT[a)]	RI[b)]	Compound Name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
1	6.18	803	Ethyl formate	$C_3H_6O_2$	74	0.19	0.07
2	7.43	864	Ethyl acetate	$C_4H_8O_2$	88	1.58	0.63
3	7.77	878	2-Butanone	$C_4H_8O_2$	72	0.12	0.04
4	8.11	893	2-Methylbutanal	$C_5H_{10}O$	86	0.27	0.11
5	8.24	898	3-Methylbutanal	$C_5H_{10}O$	86	0.59	0.24
6	8.74	917	2-Methyl-1-propen-1-one	C_4H_6O	70	0.20	0.08
7	8.91	923	Ethanol	C_2H_6O	46	1.02	0.41
8	9.15	932	3-Buten-2-one	C_4H_6O	70	20.42	8.18
9	9.37	939	2-Ethyl furan	C_6H_8O	96	0.23	0.10
10	10.22	967	3-Methyl-2-butanone	$C_5H_{10}O$	86	0.87	0.35
11	10.33	971	Pentanal	$C_5H_{10}O$	86	1.25	0.51
12	13.00	1037	2-Butenal	C_4H_6O	70	8.70	3.48
13	13.23	1041	Methyl butenol	$C_5H_{10}O$	86	1.81	0.72
14	15.24	1079	Hexanal	$C_6H_{12}O$	100	2.68	1.07
15	16.60	1102	*β*-Pinene	$C_{10}H_{16}$	136	0.18	0.07
16	17.71	1122	3-Penten-2-one	C_5H_8O	84	0.24	0.10
17	21.38	1180	2-Heptanone	$C_7H_{14}O$	114	0.25	0.10
18	21.52	1182	Heptanal	$C_7H_{14}O$	114	0.74	0.30
19	23.67	1214	2-Hexenal	$C_6H_{10}O$	98	1.74	0.70
20	24.60	1228	2-Pentylfuran	$C_9H_{14}O$	138	1.48	0.59
21	26.19	1253	Pentanol	$C_5H_{12}O$	88	0.16	0.07
22	28.56	1286	Octanal	$C_8H_{16}O$	128	0.23	0.10
IS	*30.23*	*1310*	*Butylbenzene*	*$C_{10}H_{14}$*	*134*	*0.00*	*0.00*
23	30.95	1321	2,3-Octanedione	$C_8H_{14}O_2$	142	0.88	0.35
24	31.88	1335	6-Methyl-5-methyl ideneheptane-2-one	$C_9H_{16}O$	140	0.50	0.20
25	33.24	1355	Hexanol	$C_6H_{14}O$	102	0.30	0.13
26	34.02	1367	2,4-Dimethylfuran	C_6H_8O	96	1.56	0.62
27	35.10	1382	4-Methylhexanol	$C_7H_{16}O$	116	0.26	0.10
28	35.74	1390	Nonanal	$C_9H_{18}O$	142	1.46	0.59
29	38.14	1427	[*E*]-2-Octenal	$C_8H_{14}O$	126	1.09	0.44

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 17. Continued

No.	RT[a)]	RI[b)]	Compound Name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
30	39.20	1444	Limonene oxide	$C_{10}H_{16}O$	152	1.65	0.66
31	39.74	1453	Hexen-3-ol	$C_6H_{12}O$	100	1.42	0.56
32	40.15	1459	Furfural	$C_5H_4O_2$	96	0.25	0.10
33	42.41	1492	2-Ethylhexanol	$C_8H_{18}O$	130	2.10	0.84
34	43.64	1511	Pyrrole	C_4H_5N	67	0.54	0.22
35	44.02	1517	Camphor	$C_{10}H_{16}O$	152	18.40	7.36
36	45.15	1535	Octanol	$C_8H_{18}O$	130	0.91	0.37
37	46.03	1549	Linalool	$C_{10}H_{18}O$	154	3.46	1.39
38	48.35	1584	2,6-Nonadienal	$C_9H_{14}O$	138	0.68	0.27
39	49.13	1595	[*E*]-Caryophyllene	$C_{15}H_{24}$	204	1.41	0.56
40	49.51	1601	6-Undecanone	$C_{11}H_{22}O$	170	0.64	0.25
41	51.86	1641	*α*-Humulene	$C_{15}H_{24}$	204	1.37	0.55
42	51.97	1643	Decanal	$C_{10}H_{20}O$	156	0.77	0.31
43	52.25	1647	Acetophenone	C_8H_8O	120	0.33	0.13
44	55.39	1698	α-Terpineol	$C_{10}H_{18}O$	154	1.01	0.41
45	56.35	1715	Unknown	-	-	1.00	0.41
46	56.55	1719	[*Z*,*E*]-*α*-Farnesene	$C_{15}H_{24}$	204	1.84	0.73
47	56.88	1725	[*Z*]-*β*-Farnesene	$C_{15}H_{24}$	204	0.26	0.11
48	57.50	1736	Carvone	$C_{10}H_{14}O_3$	150	1.15	0.46
49	59.06	1764	[*E*]-3-Nonen-2-ol	$C_9H_{18}O$	142	4.32	1.73
50	59.54	1773	α-Curcumene	$C_{15}H_{22}$	202	0.35	0.14
51	60.76	1794	Butyrophenone	$C_{10}H_{12}O$	148	0.27	0.11
52	61.80	1816	3-Ethnyl cyclohexenone	$C_8H_{12}O$	124	4.59	1.84
53	62.97	1843	2-Methylbutyl cyclohexane	$C_{11}H_{22}$	154	0.96	0.38
54	63.30	1850	[*E*]-Geraniol	$C_{10}H_{18}O$	154	0.89	0.35
55	63.57	1856	[*Z*]-Geranylacetone	$C_{13}H_{22}O$	194	2.49	1.00
56	64.39	1874	Safrole	$C_{10}H_{10}O_2$	162	3.78	1.52
57	66.04	1913	*β*-Phenylethanol	$C_8H_{10}O$	122	0.21	0.08
58	66.45	1924	Dodecanol	$C_{12}H_{26}O$	186	1.17	0.46
59	67.87	1963	1,2,3-Trimethoxybenzene	$C_9H_{12}O_3$	168	1.49	0.59

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 17. Continued

No.	RT[a)]	RI[b)]	Compound Name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
60	69.23	1999	Unknown	-	-	1.33	0.53
61	70.32	2031	Tetradecanal	$C_{14}H_{28}O$	212	4.50	1.80
62	70.92	2048	3,4,5-Trimethoxytoluene	$C_{10}H_{14}O_3$	182	2.53	1.01
63	71.20	2056	Octanoic acid	$C_8H_{16}O_2$	144	1.45	0.58
64	71.52	2065	Isothujol	$C_{10}H_{18}O$	154	4.35	1.74
65	72.02	2079	Hexadecanal	$C_{16}H_{32}O$	240	1.28	0.51
66	72.67	2097	3,4,5-Trimethoxybenzaldehyde	$C_{10}H_{12}O_4$	196	4.97	1.99
67	73.43	2112	2-Heptadecanone	$C_{17}H_{34}O$	254	14.72	5.90
68	73.64	2116	Cedrol	$C_{15}H_{26}O$	222	13.07	5.23
69	74.44	2130	Nonanoic acid	$C_9H_{18}O_2$	158	6.43	2.57
70	74.59	2133	Unknown	-	-	4.12	1.64
71	74.65	2134	Eugenol acetate	$C_{12}H_{14}O_3$	206	6.05	2.43
72	74.96	2140	*p*-Cymen-3-ol	$C_{10}H_{14}O$	150	4.04	1.62
73	75.57	2151	Patchoulol	$C_{15}H_{26}O$	222	8.29	3.32
74	76.17	2161	Farnesol	$C_{15}H_{26}O$	222	2.77	1.11
75	76.84	2173	*β*-Eudesmol	$C_{15}H_{26}O$	222	4.64	1.85
76	77.50	2184	Decanoic acid	$C_{10}H_{20}O_2$	172	5.30	2.12
77	80.14	2250	[*E*]-Propenyl guaiacol	$C_{10}H_{12}O_2$	164	4.24	1.70
78	80.95	2272	Unknown	-	-	6.81	2.73
79	84.83	2375	Undecanoic acid	$C_{11}H_{22}O_2$	186	28.63	11.46
80	85.56	2393	Hexadecanol	$C_{16}H_{34}O$	242	1.29	0.52
81	89.60	2549	Octadecanol	$C_{18}H_{38}O$	270	8.26	3.32
					Total	**249.73**	**100.00**

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

4.2.7. Volatile organic compounds of *Terminalia chebula* Retz

The essential oil of *T. chebula* was extracted by solvent extraction (P:E, 1:1) method for 2 h using SDE apparatus and analysed by GC/MS. Investigation confirmed that the yield of essential oil was 44.17 mg/kg. GC/MS chromatogram obtained from *T. chebula* oil is shown in Fig. 14. The result is listed together according to their elution order on DB-WAX column with ranges of their amounts in Table 19. Seventy seven compounds of the essential oil so far belonging to chemical classes of acid (7), alcohol (16), aldehyde (11), ester (5), furan (2), hydrocarbon (2), ketone (6), N-containing compounds (3), miscellaneous (1) were tentatively identified. Identified compounds represent above 80% of the total peak area. Aldehyde group contained the highest proportion (29.36%) of the total volatile content. Furfural (12.59%), α-tolualdehyde (7.64%) and 5-methylfurfural (2.98%) were detected as major aldehyde compounds. Alcohol (25.61%) was characterized as second major chemical group. Aliphatic compounds were the main compounds among the alcohols. Acid and aldehyde containing 16.73% and 10.53% respectively were characterized as major chemical groups. The prime composition was furfural (12.59%) and other ranged in content order as follows: *α*-tolualdehyde (7.64%), camphor (6.13%), 2-heptadecanone (5.77%), nonanoic acid (4.38%) and 5-methyl furfural (2.98%). The analysis shows that fatty acids; butyric (butanic) acid, valeric (pentanoic) acid, caproic (hexanoic) acid, enanthoic (heptanoic) acid, caprylic (octanoic) acid and pelargonic (nonanoic) acid contained 8.65% of total oil. The analysis of terpenoid showed terpenes achieved 10.27% of the oil.

The result demonstrates a few VOC's that are important for their pharmacological applications. Furfural has a wide variety of uses including weed killer and fungicide, affects yeast survival and also affect biochemical enzyme activities (245,246). Tolualdehyde is used as an additive in non-alcoholic beverages, ice cream, candy, baked goods, gelatins/puddings and chewing gum, (265). Camphor is well-known chemical with its pronounced antimicrobial potentials, antiseptic, stimulant and antispasmodic properties (222,223). On the basis of the above investigation, it may be concluded that the *T. chebula* can yield small quantity of essential oil with a few important VOC's. But due to the low concentrations of VOC's, it is not feasible to commercial production of such oil in large volume.

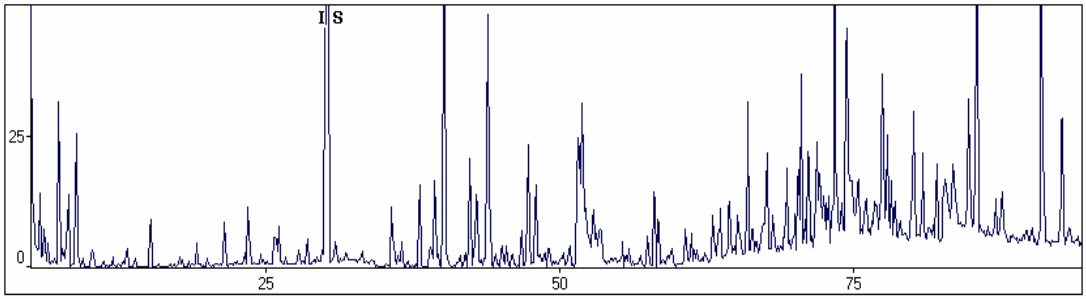

Fig. 14. GC/MS chromatogram of volatile organic compounds obtained from *Terminalia chebula* Retz.

Table 18. Relative content of functional groups of volatile organic compounds identified in *Terminalia chebula* Retz

No.	Functional groups	Relative peak area (%)	Number of compounds
1	Acid	10.99	7
2	Alcohol	23.64	16
3	Aldehyde	29.36	11
4	Ester	6.57	5
5	Furan	2.06	2
5	Hydrocarbon	1.66	2
6	Ketone	14.79	6
7	N-Compound	3.44	3
8	Miscellaneous	0.83	1
9	Unknown	6.67	3
	Total	**100**	**56**

Table 19. Volatile organic compounds of *Terminalia chebula* Retz

No.	RT[a)]	RI[b)]	Compound Name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
1	6.19	804	Ethyl formate	$C_3H_6O_2$	74	0.24	0.54
2	7.43	864	Ethyl acetate	$C_4H_8O_2$	88	1.20	2.72
3	8.11	893	2-Methyl butanal	$C_5H_{10}O$	86	0.20	0.45
4	8.23	898	3-Methyl butanal	$C_5H_{10}O$	86	0.49	1.11
5	8.91	923	2-Propanol	C_3H_8O	60	1.07	2.41
6	15.22	1079	Hexanal	$C_6H_{12}O$	100	0.32	0.71
7	19.16	1146	Butanol	$C_4H_{10}O$	74	0.17	0.38
8	21.52	1182	Pyridine	C_5H_5N	79	0.37	0.83
9	23.52	1211	2-Pentynal	C_5H_6O	82	0.51	1.16
10	23.67	1214	[*E*]-2-Hexenal	$C_6H_{10}O$	98	0.08	0.17
11	25.79	1247	Unknown	-	-	0.20	0.45
12	25.97	1249	α-Ocimene	$C_{10}H_{16}$	136	0.16	0.38
13	26.17	1252	2-Pyridyl nitrile	$C_6H_4N_2$	104	0.26	0.57
14	28.56	1286	Octanal	$C_8H_{16}O$	128	0.14	0.31
IS	*30.30*	*1311*	*Butylbenzene*	*$C_{10}H_{14}$*	*134*	*0.00*	*0.00*
15	35.74	1390	Nonanal	$C_9H_{18}O$	142	0.44	0.99
16	35.87	1392	2,3-Dihydro-3-methyl furan	C_5H_8O	84	0.27	0.62
17	36.62	1403	3-Methyl-4-heptanone	$C_8H_{16}O$	128	0.09	0.21
18	38.12	1427	3-Methyl-3-penten-2-one	$C_6H_{10}O$	98	0.73	1.66
19	39.39	1447	Acetic acid	$C_2H_4O_2$	60	1.03	2.34
20	40.22	1460	Furfural	$C_5H_4O_2$	96	5.56	12.59
21	42.41	1492	2-Ethylhexanol	$C_8H_{18}O$	130	1.16	2.63
22	42.98	1500	2-Acetylfuran	$C_6H_6O_2$	110	0.64	1.44
23	43.93	1516	Camphor	$C_{10}H_{16}O$	152	2.71	6.13
24	44.10	1519	Benzaldehyde	C_7H_6O	106	0.56	1.25
25	45.51	1541	2,3-Dimethyl-2-cyclopentenone	$C_7H_{10}O$	110	0.05	0.12
26	46.81	1561	Octanol	$C_8H_{18}O$	130	0.20	0.45
27	47.37	1569	5-Methylfurfural	$C_6H_6O_2$	110	1.31	2.98
28	48.02	1579	Cynopyrrolidine	C_5H_8N	96	0.90	2.04
29	50.90	1625	Butanoic acid	$C_4H_8O_2$	88	0.19	0.43

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 19. Continued

No.	RT[a)]	RI[b)]	Compound Name	MF[c)]	MW[d)]	Amount (mg/kg)	Content (%)
30	51.60	1637	α-Tolualdehyde	C_8H_8O	120	3.37	7.64
31	51.96	1642	Unknown	-	-	1.99	4.52
32	52.91	1658	Furfuryl alcohol	$C_5H_6O_2$	98	0.17	0.38
33	53.45	1667	3-Methyl butanoic acid	$C_5H_{10}O_2$	102	0.35	0.80
34	57.51	1737	Pentanoic acid	$C_5H_{10}O_2$	102	0.17	0.38
35	58.09	1747	α-Farnesene	$C_{15}H_{24}$	204	0.56	1.28
36	58.42	1753	Decanol	$C_{10}H_{22}O$	158	0.41	0.92
37	60.73	1794	Butyrophenone	$C_{10}H_{12}O$	148	0.39	0.90
38	63.08	1845	Hexanoic acid	$C_6H_{12}O_2$	116	0.36	0.83
39	63.70	1859	Guaiacol	$C_7H_8O_2$	124	0.52	1.16
40	64.38	1874	Methyl-3-phenylpropenoate	$C_{10}H_{10}O_2$	162	0.34	0.76
41	64.50	1877	Benzylalcohol	$C_7H_8O_2$	108	0.51	1.16
42	65.40	1896	Dodecanol	$C_{12}H_{26}O$	186	0.10	0.24
43	66.02	1913	Benzeneethanol	$C_8H_{10}O$	122	1.30	2.96
44	67.47	1952	Heptanoic acid	$C_7H_{14}O_2$	130	0.31	0.71
45	67.66	1957	*p*-Creosol	$C_8H_{10}O_2$	138	0.87	1.96
46	68.18	1971	Phenylethyl alcohol	$C_8H_{10}O$	122	0.40	0.90
47	70.33	2031	Unknown	-	-	0.76	1.70
48	70.59	2039	Nerolidol	$C_{15}H_{26}O$	222	1.27	2.86
49	71.21	2056	Octanoic acid	$C_8H_{16}O_2$	144	0.84	1.92
50	71.93	2077	2,6-Dimethylphenol	$C_8H_{10}O$	122	1.11	2.51
51	72.19	2084	O-Cresol	C_7H_8O	108	0.66	1.49
52	72.91	2103	2-Methoxy-4-propyl phenol	$C_{10}H_{14}O_2$	166	0.37	0.83
53	73.38	2111	2-Heptadecanone	$C_{17}H_{34}O$	254	2.54	5.77
54	74.45	2131	Nonanoic acid	$C_9H_{18}O_2$	158	1.93	4.38
55	74.63	2134	Eugenol acetate	$C_{12}H_{14}O_3$	206	0.78	1.75
56	74.77	2136	3,4-Dimethylphenol	$C_8H_{10}O$	122	0.54	1.23
					Total	**44.17**	**100**

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

4.2.8. Volatile organic compounds of *Woodfordia fruticosa* (L) Kurz

The essential oil of *W. fruticosa* was extracted by solvent extraction (P:E, 1:1) for 2 h using SDE apparatus and analysed by GC/MS. Investigation confirmed that the yield of essential oil obtained from Nepal originated *W. fruticosa* was 211.55 mg/kg. VOC's of this oil were identified and quantified by GC/MS and presented in Table 21. GC/MS chromatogram obtained from oil of *W. fruticosa* flower is shown in Fig. 15. Eighty one compounds of the essential oil so far belonging to chemical classes of acid (8), alcohol (23), aldehyde (16), ester (4), furan (4), hydrocarbon (9), ketone (7), N-containing compounds (7), miscellaneous (3) were tentatively identified and summarized in Table 20. Identified compounds represents above 80% of the total peak aera. From quantitative point of view, aldehyde group was the dominant family with the highest proportion accounting 30% of the total volatile content. Furfural (10.52%), 3-methyl butanal (6.43%), 5- methyl furfural (2.03%), 2-methyl butanal (2.71%) were the main aldehydes. Alcohol group (28.12%) was characterized as second major chemical group. α-Terpineol (4.72%), [*Z*]-linalool oxide (3.17%), geraniol (2.22%), [*Z*]-nerolidol (2.12%) were detected as major alcohol compounds. All of these alcohol compounds belong to oxygenated monoterpens. Similarly, acid and ketone containing 14.72% and 8.26% respectively were characterized as major chemical groups. All of the acid compounds were related to fatty acid i.e. from butanoic acid to undecanoic acid accounting from 0.1 ~ 3.70%. Compounds junipene (1.63%), caryophyllene (1.09%), α-humulene (0.46%), [*Z,E*]-α-farnesene (0.27%), β-myrcene (0.14%) and α-terpinene (0.10%) were the hydrocarbons related to terpene group. Two compounds tetradecane and docosane, related to aliphatic hydrocarbon were detected in this study. Methyl ester of pyrazine compounds such as methyl pyrazine (0.18%), 2,3-dimethyl pyrazine (0.12%), trimethyl pyrazine (0.30%), tetramethyl pyrazine (0.14%) were also detected. A profile of terpenoid constitutents showed, 8 oxygenated mono-terpenes (20.40%), 4 hydrocarbon sesquiterpenes (3.45%) and 2 of each hydrocarbon monoterpene (0.24%) and oxygenated sesquiterpenes (2.53%).

Numerous bioactive compounds were detected among the identified compounds and their characteristics are described here. The most abundant compound furfural has a wide variety of uses such as a weed killer, fungicide, affects yeast survival and also affects biochemical enzyme activities (245,246). The compounds linalool and *p*-cymene have an anti-microbial activity which has been studied previously (256). Linalool, a dominant

compound of this oil, is important substance used in foodstuffs as a food additives (202,203) and pharmacology (204-211). α-Terpineol is probably the most important of the monocyclic monoterpene alcohol possessing various biological activities and flavor compositions, (211,248). 4-Terpinenol, which occurs in appreciable amounts in this oil, is also reported to show activity against the microorganisms (211). β-Myrcene, as well as plant oils containing these hydrocarbon monoterpenes used as flavoring additives in foods and beverages, as fragrances in cosmetics, and as scent in household products (234). α-Terpinene is one of the putative active ingredients of essential oil and an antibacterial and antifungal remedy employed in both veterinary and human medicine (266). Geraniol, exerts anti-tumor activity against various cancer cells both in vitro and in vivo (135, 248, 267,268,). In another report, it was reported that nerolidol and geraniol have high relative ovicidal activity, against human lice (250). Safrole has been used as a topical antiseptic and it is carcinogenic to the liver so it is no longer used as a flavoring agent in foods. Farnesol is a precursor of vitamin E and K1, a precursor of pentalenene used for antibiotic synthesis and a modulator of G protein activity (269), modulates cholesterol synthesis (270) is metabolized to steroids in retina (271) and inhibits arterial vasoconstriction (272, 212). Although monoterpenes are generally regarded as safe, some monoterpenoid constituents of plant essential oils have been found to possess genotoxic and carcinogenic properties (e.g. safrole). The presence of pyrazine compounds in many plant species results from Maillardtype non-enzymetic reactions between reducing sugars and free amino acid or amide. The pyrazine compounds impart a reportedly nut-like aroma.

The compound furfural was detected as the dominant compound and other ranged in content order as follows: linalool, 3-methyl butanal, heptadecanone, α-terpineol, undecanoic acid. Result shows that the VOC's of *W. fruticosa* could be useful in flavor, fragrance and cosmetics. Systematic fractionalization of this oil may give the number of bioactive compounds of medicinal and commercial values.

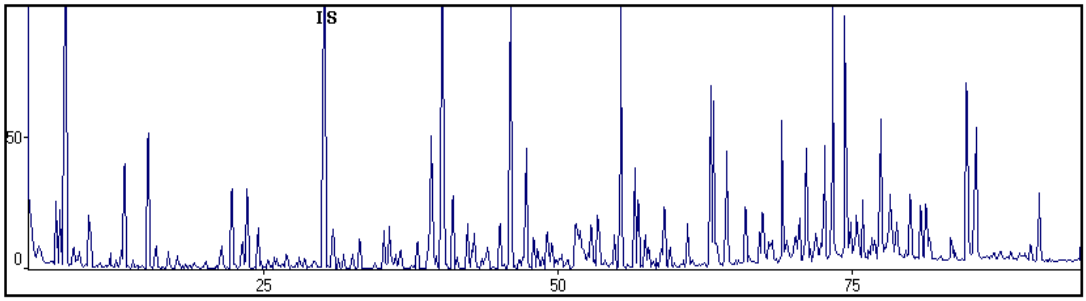

Fig. 15. GC/MS chromatogram of volatile organic compounds obtained from *Woodfordia fruticosa* (L) Kurz.

Table 20. Relative content of functional groups of volatile organic compounds identified in *Woodfordia fruticosa* (L) Kurz

No.	Functional groups	Relative peak area (%)	Number of compounds
1	Acid	14.72	8
2	Alcohol	28.12	23
3	Aldehyde	30.00	16
4	Ester	3.95	4
5	Furan	2.01	4
6.	Hydrocarbon	5.14	9
7	Ketone	8.26	7
8	N-Compound	1.73	7
9	Miscellaneous	2.81	3
10	Unknown	3.26	4
	Total	**100**	**85**

Table 21. Volatile organic compounds obtained from *Woodfordia fruticosa* (L) Kurz

No.	RT[a)]	RI [b)]	Compound name	MF [c)]	MW [d)]	Amount (mg/kg)	Content (%)
1	7.42	864	Ethyl acetate	$C_4H_8O_2$	88	1.48	0.77
2	7.75	878	2-Butanone	$C_4H_8O_2$	72	1.27	0.67
3	8.11	893	2-Methyl butanal	$C_5H_{10}O$	86	5.19	2.71
4	8.24	898	3-Methyl butanal	$C_5H_{10}O$	86	12.33	6.43
5	8.89	923	Ethanol	C_2H_6O	46	0.47	0.24
6	9.35	939	2-Ethylfuran	C_6H_8O	96	0.38	0.20
7	10.19	967	2,3-Butanedione	$C_4H_6O_2$	86	1.18	0.61
8	10.30	970	Pentanal	$C_5H_{10}O$	86	0.95	0.50
9	12.50	1026	2-Butanol	$C_4H_{10}O$	74	0.12	0.07
10	12.95	1036	2-Butenal	$C_4H_6O_2$	70	0.53	0.27
11	13.22	1041	4-Heptanone	$C_7H_{14}O_2$	114	2.44	1.28
12	15.22	1079	Hexanal	$C_6H_{12}O$	100	3.14	1.64
13	15.91	1090	2-Methylpropanol	$C_4H_{10}O$	74	0.46	0.24
14	19.14	1146	Butanol	$C_4H_{10}O$	74	0.15	0.08
15	20.14	1161	*β*-Myrcene	$C_{10}H_{16}$	136	0.26	0.14
16	21.35	1179	2-Heptanone	$C_7H_{14}O_2$	114	0.33	0.18
17	21.50	1182	Heptanal	$C_7H_{14}O_2$	114	0.49	0.26
18	22.32	1193	[*Z*]-4-Heptenal	$C_7H_{12}O$	112	1.96	1.02
19	23.15	1205	2-Methylbutanol	$C_5H_{12}O$	88	0.61	0.31
20	23.26	1207	Pentanol	$C_5H_{12}O$	88	0.59	0.31
21	23.64	1213	[*E*]-2-Hexenal	$C_6H_{10}O$	98	2.01	1.05
22	24.56	1228	2-Pentyl furan	$C_9H_{14}O$	138	0.85	0.45
23	26.98	1264	Methylpyrazine	$C_5H_6N_2$	94	0.33	0.18
24	28.04	1279	α-Terpinene	$C_{10}H_{16}$	136	0.19	0.10
25	28.53	1286	Octanal	$C_8H_{16}O$	128	0.19	0.10
IS	*30.23*	*1310*	*Butyl benzene*	*$C_{10}H_{14}$*	*134*	*0..00*	0.00
26	30.95	1321	2-Methyltetrahydrofuran	$C_5H_{10}O$	86	1.47	0.76
27	31.39	1328	2,3-Dimethylpyrazine	$C_6H_8N_2$	108	0.22	0.12
28	31.85	1335	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	126	0.33	0.18
29	32.61	1346	Unknown	-	-	0.43	0.22
30	33.21	1355	Hexanol	$C_6H_{14}O$	102	0.77	0.41

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 21. Continued

No.	RT[a)]	RI [b)]	Compound name	MF [c)]	MW [d)]	Amount (mg/kg)	Content (%)
31	35.26	1384	3-Hexen-1-ol	$C_6H_{12}O$	100	0.97	0.50
32	35.73	1390	Nonanal	$C_9H_{18}O$	142	1.23	0.64
33	36.28	1398	Tetradecane	$C_{14}H_{30}$	198	0.25	0.14
34	36.67	1403	Trimethylpyrazine	$C_7H_{10}N_2$	122	0.56	0.30
35	38.11	1427	5-Methylhexanol	$C_7H_{16}O$	116	0.65	0.34
36	39.28	1446	[*Z*]-Linalool oxide	$C_{10}H_{18}O_2$	170	6.07	3.17
37	39.69	1452	3-Decanone	$C_{10}H_{20}O$	156	0.38	0.20
38	40.28	1461	Furfural	$C_5H_4O_2$	96	20.17	10.52
39	41.12	1473	[*E*]-Linalool oxide	$C_{10}H_{18}O_2$	170	1.88	0.98
40	41.46	1478	Tetramethylpyrazine	$C_8H_{12}N_2$	136	0.22	0.12
41	42.38	1492	2-Ethylhexanol	$C_8H_{18}O$	130	1.63	0.86
42	42.93	1500	2-Acetylfuran	$C_6H_6O_2$	110	1.14	0.60
43	43.59	1510	Pyrrole	C_4H_5N	67	0.35	0.18
44	44.09	1519	Benzaldehyde	C_7H_6O	106	0.65	0.34
45	45.12	1535	2-Nonenal	$C_9H_{16}O$	140	1.23	0.64
46	46.05	1549	Linalool	$C_{10}H_{18}O$	154	14.66	7.65
47	47.35	1569	5-Methylfurfural	$C_6H_6O_2$	110	3.89	2.03
48	47.98	1579	Methyl-3-pyrrolin-2-one	C_5H_7NO	97	0.92	0.48
49	48.31	1583	[*E,Z*]-2,6-Nonadienal	$C_9H_{14}O$	138	0.41	0.22
50	49.12	1595	β-Caryophyllene	$C_{15}H_{24}$	204	0.94	0.49
51	49.56	1602	4-Terpineol	$C_{10}H_{18}O$	154	0.59	0.31
52	50.89	1625	Butanoic acid	$C_4H_8O_2$	88	0.19	0.10
53	51.57	1636	Benzeneacetaldehyde	C_8H_8O	120	3.16	1.64
54	52.87	1657	Furfuryl alcohol	$C_5H_6O_2$	98	1.33	0.69
55	53.41	1666	3-Methylbutyrate	$C_5H_{10}O_2$	102	2.85	1.48
56	54.83	1689	α-Humulene	$C_{15}H_{24}$	204	0.87	0.46
57	55.39	1698	α-Terpineol	$C_{10}H_{18}O$	154	9.04	4.72
58	56.60	1720	Junipene	$C_{15}H_{24}$	204	3.12	1.63

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

Table 21. Continued

No.	RT[a)]	RI [b)]	Compound name	MF [c)]	MW [d)]	Amount (mg/kg)	Content (%)
59	56.88	1725	Caryophyllene	$C_{15}H_{24}$	204	2.08	1.09
60	57.47	1736	Pentanoic acid	$C_5H_{10}O_2$	102	1.16	0.60
61	58.86	1761	[*Z*,*E*]-α-Farnesene	$C_{15}H_{24}$	204	0.52	0.27
62	59.10	1765	Epoxylinalol	$C_{10}H_{18}O_2$	170	1.60	0.84
63	59.57	1773	Methyl salicylate	$C_8H_8O_3$	152	0.53	0.27
64	61.06	1799	Nerol	$C_{10}H_{18}O$	154	1.17	0.61
65	63.01	1844	Hexanoic acid	$C_6H_{12}O_2$	116	5.23	2.73
66	63.27	1849	Geraniol	$C_{10}H_{18}O$	154	4.25	2.22
67	64.36	1874	Safrole	$C_{10}H_{10}O_2$	162	3.24	1.70
68	64.48	1876	Benzyl alcohol	C_7H_8O	108	1.05	0.54
69	65.99	1912	Benzeneethanol	$C_8H_{10}O$	122	1.56	0.82
70	67.43	1951	Heptanoic acid	$C_7H_{14}O_2$	130	1.46	0.76
71	68.26	1974	2-Acetylpyrrole	C_6H_7NO	109	0.69	0.35
72	69.07	1995	[*Z*]-Nerolidol	$C_{15}H_{26}O$	222	4.07	2.12
73	70.56	2038	Farnesol	$C_{15}H_{26}O$	222	0.78	0.41
74	71.04	2051	Unknown	-	-	1.60	0.83
75	71.16	2055	Octanoic acid	$C_8H_{16}O_2$	144	2.68	1.40
76	72.69	2098	Unknown	-	-	3.08	1.60
77	73.39	2111	Heptadecanone	$C_{17}H_{34}O$	254	9.84	5.14
78	74.40	2130	Nonanoic acid	$C_9H_{18}O_2$	158	6.65	3.47
79	74.61	2134	Eugenol acetate	$C_{12}H_{14}O_3$	206	1.91	0.99
80	74.92	2139	Unknown	-	-	1.18	0.61
81	75.53	2150	Tetradecanol	$C_{14}H_{30}O$	214	1.01	0.53
82	75.94	2157	Methyl nonanoate	$C_{10}H_{20}O_2$	172	1.36	0.71
83	77.47	2184	Decanoic acid	$C_9H_{18}O_2$	158	3.75	1.96
84	78.24	2197	Docosane	$C_{22}H_{46}$	310	1.55	0.82
85	84.76	2373	Undecanoic acid	$C_{11}H_{22}O_2$	186	7.08	3.70
					Total	**191.55**	**100.00**

[a)]retention time, [b)]retention index, [c)]molecular formula, [d)]molecular weight

4.3. Comparision of VOC's of MAP's

The study on the essential oil of 8 MAP's of Nepal revealed that all the plants have the existence of essential oils but their quantities were varied (Fig 16). The yield of essential oil obtained from *Acorus calamus*, *Asparagus racemosus*, *Bergenia ciliata*, *Centella asiatica*, *Dipsacus mitis*, *Swertia chirata*, *Terminalia chebula*, and *Woodfordia fruticosa* was 0.749, 0.006, 0.007, 0.108, 0.006, 0.025, 0.004 and 0.021 % respectively. Similarly the numbers of VOC's tentatively identified are 53, 49, 44, 53, 53, 77, 53 and 81 respectively.

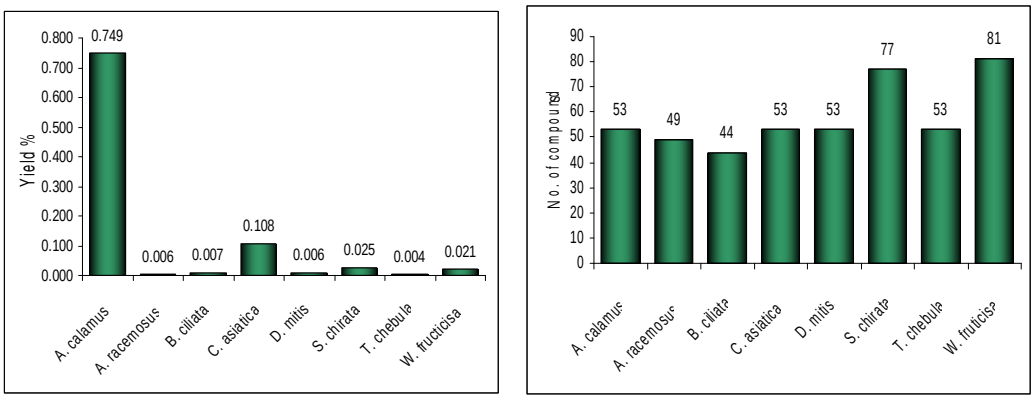

Fig. 16. Number of compounds and yield of essential oil obtained from MAP's.

Aldehyde and alcohol groups are detected in all the species. Aldehyde group is dominant in *T. chebula* and *W. fruticosa*. Similarly ketone and alcohols group are dominant in *A. calamus, S. chirata A. racemosus,* and *D. mitis* respectively. Hydrocarbon group is dominant in *C. asiatica*. Aldehyde dominating species show anti-inflammatory, sedative, hypotensive, vasodilatory and antipyretic properties. This would be due to electronegativity and neutral polarity of aldehydes. A particular property of the aldehyde volatile oils is their insect repellent activity due to very strong scent. This would be the reason that the above plant *A. calamus* is used as food preservative (57,99). They are often irritating to the skin and thus show general characteristics of being cooling and drying. They also tend to promote tissue growth and healing of injuries. Ketone dominating species show lipolytic, mucolytic, sedative, analgesic, anti-coagulant, anti-inflammatory,

digestive, expectorant and stimulatant properties. This would be due to moderate electronegativity and strong polarity of ketones. Some ketones are known to be neurotoxic. Ester and related compounds were also found in many species in small amounts. They enhance the essence of oils. Generally esters are mildly electro-negative and have neutral polarity. They exhibit spasmolytic, soothing effects and anti-inflammatory and antifungal agents. Some of the chemical classes such as furan, hydrocarbon, N-containing compound, and acid were absent in the some of the species. S-containing compound was presnt only in *A. racemosus* representing only one compound dipropyl disulfide.

VOC's β-asarone, borneol, 5,6-dihydro-2-pyranone, [*Z*]-β-farnesene, 2-butenal and undecanoic acid, were detected as major compounds in *A. calamus, A. racemosus, B. ciliata, C. asiatica, D. mitis, S. chirata,* respectively. Furfural was dominant in *T. chebula* and *W. fruticosa*. Linalool, camphor and α-terpineol present in most of the oils show that all species have antinociceptive, antispasmodic, anti-inflammatory and antimicrobial activities. It was identified that only components would be responsible for the biological activity of those oils. It would be also possible that their constituents have synergistic effect on the activity. The relationship between identified compounds and previous studies on their activity suggest good biological activity of essential oils from the MAP's possesses of both major components (phenolic, terpenic, or ketonic compounds) and the minor ones.

The percentage composition of the essential oil and characteristics of VOC's provide an important parameter for the characterization of the plant (188). Careful identification of VOC's for fragrance and pharmacologically active ingredients show the presence of numerous useful compounds. Concerning our interest on the bioactive organic VOC's, the study focoused in the evaluation of individual component's. Some of the bioactive compounds such as β-asrone, camphor, and carvone of ketone group were found in the plants *A. calamus, A. racemosus,* and *D. mitis* respectively. Camphor was common in *A. racemosus, B. ciliata* and *C. asiatica, S. chirata* and *T. chebula.* The bioactive compounds such as [*E,Z*]-2,4-decadienal and tolualdehyde related to aldehydes were detected in *A. calamus* and *T. chebula* respectively. Furfural was common in *A. racemosus, D. mitis, S. chirata, T. chebula* and *W. fruticosa* which was ranged from 0.08 ~ 12.59%. The compound myrtanal was in *A. calamus* and *A. racemosus* while perillaldehyde was found only in *A. racemosus.* Similarly, linalool, farnesol, methyleugenol, borneol, myrtanol, α-

terpiniol, neralidol, geraniol, β-eudesmol and 4-terpeniol, related to alcohol group were frequently detected. Linalool and farnesol were common in *A. calamus, C. asiatica, S. chirayita* and *W. fruticosa.* Linalool and α-terpeniol were common in *B. ciliata.* Similarly, α-terpeniol and geraniol were common *C. asiatica, D. mitis, S. chirayita and W. fruticosa.* Neralidol was found in *C. asiatica and W. fruticosa.* β-Eudesmol and 4-terpeniol were found only in *S. chirayita and W. fruticosa.* The compounds such as α-pinene, [*E*]-farnesene, caren, β-caryophyllene, β-elemene, [*E*]-β-ocimene, β-myrcene, *p*-cymene and α-humulene, related to hydrocarbon group were detected as important hydrocarbon components of many species. Evaluation of terpenoid showed that majority of compounds was monoterpenes. More than 9 monoterpene hydrocarbons, such as [*Z*]-ocimene, β-phellandrene, β-myrcene,, β-pinene, α-pinene, camphene, thujene, limonene and 3-carene were prevalent constituents in: *A. calamus, A. racemosus* and *C. asiatica;* some of the compounds were common in all three species and some were not. As monoterpenoids show electropositivity and are non-polar, they are associated with the therapeutic properties such as, external antisepsis, anti-viral, mucus membrane irritants and possibily some immuno-stimulatory actions. Sesquiterpene hydrocarbons such as α-copaene, β-elemene, junipene, [*E*]-caryophyllene, α-humulene and β-farnesene were distributed in *A. calamus,* and *C. asiatica* with high concentrations. Some of them were also detected in *S. chirata* and less quantity in *T. chebula* and *A. racemosus*. Sesquiterpene hydrocarbons are non-polar and have anti-inflammatory, sedatives, anti-spasmodic, anti-allergenics and decongestants properties. Oxygenated terpenes such as camphor, linalool, α-terpineol, geraniol and farnesol were common in most of the samples particularly higher in *S. chirata* and *W. fructicisa.* Oxygenated terpenes are mildly electro-positive and have antiseptic, stimulating and emerging properties. Usually, they are non-irritating to and non-toxic. Generally sesquiterpene alcohols are less electropositive than monoterpene alcohols and show liver and glandular stimulation, anti-inflammatory and anti-allergic action and decongestant properties. In addition, most of all plants contained common compounds such as ethyl acetate, ethanol, linalool etc.

Essential oils from *C. asiatica. A. calamus, A. racemosus* and *S. chirata* are seen as a source of terpenoids. These are much wanted aromatic chemicals in perfume, flavour and pharmaceutical industries. Due to the low concentrations, essential oils of plants *D. mitis, B. ciliate, A. racemosus* and *T. chebula,* can not be recommend to further studies in course

of extraction and separation. It is believed that research institutes and universities continue their efforts to discover new bioactive compounds derived from few of these MAP's. Bioactivity test of these essential oils of *S. chirata, W. fructicisa, A. calamus* and *C. asiatica* is recommended. Little variation in content and constituents in essential oil of *A. calamus* were found with previous study (189). The variations are important, as the value of an essential oil in aromatherapy is related to its chemical composition (273). The reasons for this variaiton of quality of essential oil could be due to the factors influencing the composition of the oils, namely, climatic, seasonal and geographic conditions, harvest period and distillation technique and genetic characteristics (274,190-192). The effect of plant maturity at the time of oil production and the existence of chemotypic differences can also drastically affect composition (275).

Species *W. fruticosa,* and *S. chirata,* offer new interest whereas essential oil content of *C. asiatica* and *A. calamus* were verified. These plants are important source of fragrance and pharmacologically active constituents. Further study with larger random samples is recommended.

CHAPTER III

γ-Irradiation Effect on Volatile Organic Compounds of *Glycyrrhiza uralensis* F

1. Introduction

1.1. Radiation treatment of food and agricultural products

Treatment of food and agricultural products by ionizing radiation has been used for many years for a number of purposes such as reduction of number of microorganisms, improve shelf life, and delay of certain natural processes such as ripening, sprouting and germination maturation (276). Interest in the irradiation process is widely increasing because of persistently high food losses from infestation and spoilage, concerns over food-borne diseases, and growing international trade in food products. Selection of appropriate treatment conditions can minimize or prevent objectionable changes in food quality. The use of this process has been established as safe upto an overall average level of absorbed dose of 10 kGy but this level is not an upper limit above which irradiated foods become unsafe (277). Following the successful results of safety studies on food irradiated with high dose, the irradiation of culinary herbs, seeds, spices, vegetable, seasonings and blends of aromatic vegetable substance has been permitted by FDA upto 30 kGy (278). The various safety issues have been addressed in many expert reviews over many years, and a conclusion is made that food irradiatioin, properly carried out, is safe process (279,280). A review published covers the scientific litereature on technological objective and safety issues of irradiated foods (281). Consumer acceptance has been demonstrated that consumers preferred the irradiated product over a comparable non-irradiated items (282). Therefore, use of ionizing radiation for food processing is currently permitted in 52 countries for the treatment of approximately 250 food products and ever-increasing number of countries have approved lot of irradiated foods (283,284)(Appendix III). Cobalt-60 γ-ray and electron accelerator are currently the most widely used radiation sources for commercial application. All types of ionizing radiation produce similar chemical changes in an irradiated material. Ions and molecules are the first reactive

species formed when ionizing radiation and matter interact thereby causing some chemical changes in the irradiated material (285). Different dose of radiation will therefore not only produce different amount of new molecules, but also different kinds as well.

Contaminations of spices, medicinal herbs and additives with microorganisms pose a widespread threat to human health and cause to reduce economic productivity. Therefore, before they can be safely incorporated into other food products, the microbial load should be reduced. Until recently, most spices and herbs were fumigated using number of chemical fumigants. Under the Montreal Protocol and Clean Air Act, the developing countries have to implement the phase out of methyl bromide upto year 2015 and to be phased out by the year 2010 in developed countries (286). Similarly, the use of ethylene oxide was prohibited by European Union (EU) directive in 1991 and has been banned in a number of other countries because it is a carcinogen. On other hand, heat treatment is not feasible causing significant loss of flavor and aroma. Hence, irradiation has been accepted as an effective alternative method to protect food and as a quarantine treatment of fresh product due to increasingly restricted regulations on the use of a number of chemical fumigants and some technical disadvantages of other methods. The predominant useful effects of irradiation rely on reaction of newly formed free radicals. The Council of Agricultural Science and Technology estimated that a dose of 1 kGy would break fewer than 10 chemical bonds for every 10 million bonds present (287). Even though an extremely small percentage of chemical bonds are broken when food is irradiated, the effect can be enough to breaking bonds in DNA. This results in the loss of cell's ability to replicate and can destroy the cell of the microorganisms as well as disrupt the genetic material in living cell on food, consequently delay to ripening and prevent to sprouting thereby preserving the food. Irradiation has since emerged as a viable alternative and its use results in cleaner, better quality herbs and spices compared to those other chemical and physical processes. Although the gamma irradiation is one of the currently used methods for the decontamination, the flavor quality may be affected during the process. Chemical analysis of VOC's in irradiated medicinal herbs is very important for the basis of safety evaluation of irradiated herbs (288). Consequently, there is an increasing demand from consumers for more information about the quality and safety of irradiated species and herbs (289). Accordingly, the objective of this study was to examine the effect in the flavor precursors of dried licorice roots when exposed to γ-irradiation.

1.2. Irradiation of medicinal herbs

Medicinal herbs are valued for their distinctive flavours, taste, aromas as well as their pharmacological properties. However, they are often heavily contaminated with microorganisms because of the environmental and processing conditions under which they are produced. Current practices of harvesting, handling, storage and production may cause additional contamination that makes them inadequate for commercial applications (290). Irradiation is an effective control measure for eliminating pathogenic bacteria and parasites from solid herbal commodities, especially those eaten in raw or without causing any significant changes. Herbs and spices are examples of commodities for which irradiation can gurantee safety for microbial contamination and insect pests. Therefore irradiation of medicinal dried herbs has been permitted upto 15 kGy (283). Several studies on the effect of irradiation on pharmacological characteristics, microbial status and physiochemical properties of irradiated medicinal herbs have reported positive results (288, 291-297). Medicinal herbs irradiated at doses of 10~30 kGy showed the identical pharmacological activities with similar content of essential biologically active plant secondary metabolites as non-irradiated preparations (298, 299). Moreover many studies have been concluded that, exposure to ionizing radiation such as γ-rays offers an effective alternative means of reducing microbial contamination of medicinal herbs without adversely affecting their biologically active components (301) and flavor attributes (302). It is due to fact, dry commodities are known to be less affected chemically by irradiation than high moisture containing foods (303, 304). But chromatographic analysis of some herbal extracts indicated that changes in total yield and constituents of volatile oil following gamma irradiation were ranged from none to slight (305-308) depending upon dose-based irradiation in the variety of herbs. Recently we reported that high-dose γ-irradiation of dried Welsh onion is feasible as ionizing radiation enhanced the total concentration of volatile organic compounds by 31.60% and 24.85% at 10 and 20 kGy, respectively (309). It can be assumed, therefore, that the dose which can be applied and hence extent to the microbial kill, may be limited by undesirable changes in volatile constituents, their yield and flavor quality.

Table 22. A database of herbs cleared for irradiation processing by country

Country	Max dose (kGy)			
	Herbs	Dried herbs	Frozen herbs	Herbal infusions
Australia	30			10
Austria		10		
Belgium		10		
Brazil	Unstated			
Canada	10			
Denmark		15		
Egypt	10			
Finland		10		
France		10	10	
Germany		10		
Ghana	10			
Greece		10		
Ireland		10		
Italy		10		
Luxembourg		10		
Mexico	10			
Netherlands		3		
New Zealand	30			10
Norway	10			
Pakistan	10			
Portugal		10		
South Africa	10			
Spain		10		
Sweden		10		
United Kingdom		10		
USA	30			

Source: http://www.iaea.org/icgfi/data.htm

1.3. Licorice (*Glycyrrhiza uralensis* F)

Licorice is a native medicinal herb in the Mediterranean region, central to southern Russia, and Asia minor to Iran, now widely cultivated throughout Europe, the Middle East, and Asia (3,5). It belongs to genus *Glycyrrhiza* (Family Leguminosae) that consists of about 30 species. Therapeutically it has been used for several thousand years as a tonic, antiphlogistic, mucolytic, expectorant, inflammation, muscle spasms, undigestion, sore throat, asthma, rheumatism and all pectoral diseases in both western and eastern systems of oriental medicine (310). Its use is first documented in Assyrian clay tablets (ca. 2500 B.C.E.) and Egyptian papyri (311). It was used in ancient Arabia to treat coughs and to relieve the unwanted effects of laxatives (94). Greek natural scientist, Theophrastus (ca. 372–287 B.C.E.) reported its use for dry cough, asthma, and all pectoral diseases in Greece (312). Pliny the Elder (ca. 23–79 C.E.) reported licorice cleared the voice and had expectorant and carminative actions (313). In China, licorice is first mentioned in the *Shen Nong Ben Cao Jing* (ca. 25 C.E.), reconstructed "materia medica" from lost text attributed to Shen Nong Shi (ca. 3000 B.C.E.) (314). In India, licorice is used in traditional ayurvedic, siddha, and unani medicines (6). The present-day extracted yield of the licorice root is commercially used in pharmaceuticals, cosmetics, tobacco and food industries (315-318). The most important industrial use of this herb is in the production of food additives as flavor and sweetening agents (319,320). In Nepal, licorice is used as tonic, laxative, demulcent, emollient, used in urinary diseases, coughs and sore throat and in scorpion-sting (88).

Fig. 17. Licorice (*Glycyrrhiza uralensis* F).

Chemistry and Pharmacological records of licorice showed that licorice root contains glycyrrhizin (also knows as glycyrrhizic or glycyrrhizinic acid, about 5–9% by weight), a compound that is about 50 times sweeter than sucrose. Beside that, others are triterpenoid saponins (4–24%); flavonoids (1%) mainly the flavanones, liquiritin and liquiritigenin, chalcones isoliquiritin, isoliquiritigenin and isoflavonoids (formononetin); amines (1–2%) asparagine, betaine, and choline; glucose and sucrose (3–15%); starch (2–30%); polysaccharides (arabinogalactans); sterols (*b*-sitosterol); coumarins (glycerin); and volatile oils (0.047%) (5,94,315,321-323). The *British Herbal Compendium* reported its actions as anti-inflammatory, expectorant, demulcent, and adrenocorticotropic (324). New research suggests that the glycyrrhetenic acid, the hydrolytic metabolite of glycyrrhizic acid, is the primary active component that causes inhibition of peripheral metabolism of corticol, which binds to mineralocorticoid receptors in the same way as aldosterone (325).

Research suggests two hypotheses for licorice's mechanism of action: binding of glycyrretinic acid to mineralocorticoid receptors and blocking the action of 11-beta-hydroxysteroid dehydrogenase. Recent publications suggest that both may be involved, especially with the confirmation that the blocking of the 11-beta-hydroxysteroid dehydrogenase is temporary and that after this occurs, the pseudoaldesteronism is directly related to increased plasma concentration of licorice metabolites and their binding to mineralocorticoid receptors. Glucocorticoids are usually rapidly metabolized into inactive compounds by 11-beta-hydroxysteroid dehydrogenase, thus controlling glucocorticoid access to mineralocorticoid and glucocorticoid receptors. When licorice prevents the inactivation of hydrocortisone, the result is increased glucocorticoid concentration in mineralocorticoid-responsive tissues, thus resulting in glucocorticoids' occupying mineralocorticoid receptors and producing a mineralocorticoid response, as shown by increased sodium retention and hypertension (326).

2. Justification of This Study

Licorice is one of the most extensively researched medicinal and food plant (326). However, little study is performed on volatile flavor components of licorice. Kameoka and Nakai (1987) reported 0.047% yield of essential oils from licorice (*G. glabra* Linne) roots (327) but Miyazawa and Kameoka (1990) reported the 0.03% (328). Hatsuko Sakagami *et al.* (1992) reported 0.040~0.059% yield of essential oil of licorice *(G. glabra* Linne) on samples that collected from different countries. The composition of volatile organic compounds obtained from Spanish licorice was rather different from that of oil produced from Turkeys licorice, as it did not contain few compounds such as, 1-hexanol, 4-terpineol, nerol, 2-butyl-2-octanal and heptadecane (329). A recent study on γ-irradiated licorice has shown that doses upto 15 kGy reduce the microbial load without changing flavor and texture whereas the high dose (20kGy) significantly decreases the flavor and texture (330).

No report was found about the effect of γ-irradiation on volatile organic compounds of *Glycyrrhiza uralensis* (F). Further studies to better understand the effect of irradiation on its volatile constituents is in urgent need to ascertain their chemical safety and acceptability. Therefore we interested to investigate the changes, if any, in the major volatile organic compounds of the licorice when exposed to γ-irradiation at doses of 1, 3, 5, 10 and 20 kGy.

3. Materials and Methods

3.1. Plant samples

3.1.1 *Glycyrrhiza uralensis* F

Commercially available roots of *G. uralensis* collected in April 2005 and identified by author. Vacuum packing of the samples was carried out by removing air from the packages and stored at -18 ^{0}C before irradiation. The non-irradiated samples were used as control.

3.1.2. Irradiation treatment

The γ-irradiation treatment in a Co-60 irradiator (Point source, AECL, IR-79, Nordion International Co. Ltd., Ottawa, ON, Canada) was carried out at Korea Atomic Energy Research Institute, Korea. The irradiation doses were 1, 3, 5, 10 and 20 kGy. The source strength was about 100 kCi and the dose rate was 2.5 kGy/h at 12±0.5^{0}C. Immediately after irradiation treatment all the samples were stored at -18 ^{0}C for further experimental use.

3.2. Reagents

All the reagents used in experiments were same as mentioned in Chapter II unit 3.2.

3.3. Analytic apparatus

All the analytical apparatus were same as mentioned in Chapter II unit 3.3.

3.4. Extraction of volatile flavor compounds

Same conditions as mentioned in Chapter II unit 3.4

3.5. Establishment of retention index

Same conditions were implemented as described in Chapter II unit 3.5

3.6. Analysis and identification of volatile flavor compounds

3.6.1. Analysis by gas chromatography/mass spectrometery (GC/MS)

Same conditions of GC/MS were implemented as mentioned in Chapter II unit 3.6.1.

3.6.2. Identification and quantification of volatile organic compounds

Same methodology was followed as mentioned in Chapter II unit 3.6.2

4. Results and Discussion

VOC's were extracted from non-irradiated and irradiated licorice separately and identified by comparing their spectral data and retention indices. The result obtained by qualitative and quantitative analysis of volatile organic compounds from experimental samples is listed together according to their elution order on DB-WAX column with ranges of their amounts corresponding to irradiation doses (Table 24). Essential oil extracted from rhizomes of *G. uralensis* contained 102.44 mg/kg yield.

4.1. Volatile organic compounds identified in licorice

Sixty-one volatile organic compounds of the essential oil so far belonging to chemical classes of acid (2), alcohol (16), aldehyde (8), ester (6), furan (2), hydrocarbon (14), ketone (10) and N-containing compounds (3) were tentatively identified in licorice. Alcohols group contained the highest proportion of VOC's in licorice oil (46.40%). Similarly aldehyde, ester and ketone containing 14.48%, 11.65% and 9.71% respectively were characterized as major chemical groups present in volatile oil of licorice. The prime composition of the licorice oil was 2-ethoxy-1-propanol which makes up 22.82% and other can be ranged in content order as follows: 4-terpineol (7.58%), ethyl acetate (7.47%), hexanal (5.69%), hexanol (4.78%), [*E*]-2-tetradecenal (4.01%) and *p*-cymen-8-ol (2.39%). Some important volatile flavor compounds such as; tetramethylpyraziane (1.74%), α-terpineol (1.67%), γ-nonalactone (2.51%) and pulegone (1.29%) were also identified. Investigations on licorice flavor have been reported that the compound tetramethylpyraziane has a pungent sweet odour, γ-nonalactone has a creamy and coconutty odour, while pulegone has a sharp-minty odour (328). Compound 4-terpineol has warm-peppery and musky wood odour (331) whereas α-terpineol posses a lilac-type of odour (224). Three nitrogenous compounds tetramethylpyrazine, indole (1.81%) and benzyl isocyanide (0.58%) were found in considerable amount. Such type of result has indeed been documented previously on unheated licorice juice with remarkable number of alcohol compounds but trace amount of nitrogenous compounds (332). Total extract, instead showing a typical licorice aroma, indicated that this might have caused due to an integrated response to the proper mixture of the proper volatiles, rather than to the odour

of one or two components. Qualitative and quantitative analysis of volatile organic compounds from present finding showed that the composition of volatile oil of licorice was significantly agreed with literature values reported by Miyazawa and Kameoka (1990) in terms of containing common volatile organic compounds (328). Factors such as the sample maturity, agro-climatic origin, commercial status and ploidy difference are known to influence the oil content of tissues in a great deal.

Table 23. Relative content of functional groups of volatile organic compounds identified in non-irradiated and irradiated licorice

No.	Functional Group	Control		1 kGy		3 kGy		5 kGy		10 kGy		20 kGy	
		Area %	No.	Area %	No.	Area %	No.	Area %	No.	Area %	No.	Area %	No.
1	Acid	3.59	2	3.35	2	3.22	2	3.13	2	3.15	2	3.37	2
2	Alcohol	46.40	16	50.60	16	48.94	16	51.71	16	50.01	16	44.12	15
3	Aldehyde	14.48	8	13.00	8	14.57	9	13.98	9	15.98	9	18.08	9
4	Ester	11.65	6	11.48	6	10.98	6	11.95	6	10.02	6	13.75	6
5	Furan	2.23	2	2.00	2	2.02	2	2.08	2	2.07	2	2.46	2
6.	Hydrocarbon	7.82	14	7.00	14	6.97	14	6.09	14	6.56	14	5.70	13
6	Ketone	9.71	10	9.62	10	10.48	10	8.06	10	8.70	10	9.13	10
7	N-Compound	4.12	3	2.95	3	2.82	3	3.00	3	3.51	3	3.39	3
	Total	**100**	**61**	**100**	**61**	**100**	**62**	**100**	**62**	**100**	**62**	**100**	**60**

The result shows that licorice composed of 10.42% terpenoids. Among them monoterpene alcohols were dominant. Numerous bioactive compounds were detected in licorice. Furfural, myrtenal, linalool, 4-terpeniol has been described for their various bioactivities (201-203,211,245,246). It seems licorice could be utilized for an isolation of number of bioactive volatile compounds from its essential oil.

4.2 Effect of γ-irradiation on volatile organic compounds

GC-MS chromatographic profile of VOC's of non-irradiated and irradiated samples showed the similarity in their constituents (Fig 20). Equal numbers of VOC's detected in 1 kGy irradiated licorice as detected in non-irradiated sample. Above the dose of 1 kGy, one more compound related to aldehyde group was detected and a few kinds of compounds detected in non-irradiated and before 10 kGy irradiated samples were disappeared at 20 kGy irradiated sample. It may be due to the fact that the high dose irradiation splits the chemical bonds in molecules to form the free radicals and then promotes the combination of free radicals. Such chemical changes in different doses of irradiation therefore produce the variation in amounts and kinds of molecules (279). Accordingly in our result benzaldehyde was detected only after 3 kGy dose of irradiation. The data seemed to show that the volatile organic compounds were induced or produced from some precursors (large molecules) decomposed by low-dose irradiation. Recently we reported that one aldehyde compound was produced after 3 kGy dose of irradiation in dry Welsh onion (309). Jo and Ahn (2000) reported that several aldehydes produced in irradiated oil emulsion containing amino acids were increased in a dose dependent manner up to 10 kGy (333). On the other hand, higher the doses above 10 kGy, the decrement of few volatile organic compounds were also noted in previous study (334).

It was generally seen that irradiation doses did not affect significantly the yield of volatile organic compounds during the low doses of treatment (1 kGy) but some minor peaks (compounds) were increased as a result of irradiation. The number of compounds such as solavetivone, γ-nonalactone, [*E*]-2-tetradecenal, tetradecanol and ethanol were remarkably enhanced before the doses of 3 kGy but the moderate changes were detected by total yield of volatiles at such lower doses. These results are in agreement with general results reported in the literatures on sensory characteristics observed between non-irradiated and 0.05~1 kGy irradiated samples (335,336). Though the 10 kGy dose of irradiation induced the maximum yield of essential oil of licorice by 12.12%, the maximum dose given at 20 kGy inhibited the total yield by 6.11%. Highest numbers of the compounds were found to be highly enhanced at 10 kGy doses resulting that the total yield of volatile oil of licorice was maximum increased at this dose. Our result was found in agreement with the previous study of Al-Bachir *et al.* (2004) who reported that the

irradiation of licorice at 20 kGy dose significantly decreased the taste and flavor but doses of 5~15 kGy did not influence on these properties (337). It seems that irradiation will increase the yield of volatile organic compounds in increasing the doses but upto certain limit. Effect of gamma irradiation of several herbs has been previously reported that overall average dose of 10 kGy presents no toxicological hazards, no special nutritional changes and no microbial risk in food that would have an adverse effect on human health (305,381).

The level of major volatile constituents such as, 2-ethoxy-1-propanol, ethyl acetate, hexanal, hexanol, [E]-2-tetradecenal, γ-nonalactone, *p*-cymen-8-ol, acetic acid, 2-pentylfuran and α-terpineol in different dose irradiated samples were respectively 1.43, 1.12, 1.47, 1.19, 1.13, 1.35, 1.34, 1.12, 1.07 and 1.17 folds higher from the level of control. Though the content of several VOC's was increased after irradiation, the content of few major compounds such as 4-terpineol, myrtenal, tetramethylpyrazine, hexanoic acid, azulene and *p*-cymene were found decreased by the process. Some compounds related with same chemical group were found inhibited at different doses of irradiation by various proportions. In comparison with control, the high dose of irradiation (20 kGy) inhibited the content of majority of VOC's (53.47%) of licorice. Thus variation in content of the constituents upon irradiation was observed in the present study could presumably be due to the radiation sensitivity of these compounds at the dose employed. The above results were according to previous studies carried out on volatile organic compounds of some irradiated herbs (305,306,309,338,339) which determine the total yield of essential oil. It is therefore, a subject of interest in the future to determine the effect of γ-irradiation on pure compounds to explore the fact during irradiation.

The effect of high-energy radiation on nonhydrocarbon organic materials is determined by the functional groups present and will vary from compound to compound (340). The relative percentage of the functional groups related to identified volatile organic compounds of control and irradiated licorice clearly showed the effect of irradiation (Table 23). Alcohols were again detected as major volatile chemical classes (44.12~51.71%) of irradiated samples like in non-irradiated sample. Although remarkable differences in the contents of individual alcohol compounds between non-irradiated and irradiated samples were detected, the total proportion of these compounds was not influenced highly by irradiation. The relative content of total alcohol compounds from

volatile oil of irradiated licorice was increased by 5.47~11.44% from 1~10 kGy but decreased by 4.91% at 20 kGy dose of irradiation. In agreement with Kim *et al.*, (2004) (334), our result suggested that relative content of alcohols were increased upto 10 kGy but decreased above that dose. Linalool and α-terpineol also decreased above 10 kGy dose of irradiation which already was proved when Sjövall *et al.* (1990) irradiated some pure aroma compounds of spices (341). Similarly the relative content of aldehyde, ester and furan group were also increased but acid and N-containing (nitrogen-containing) compounds were found decreased after irradiation. Acids were also the group that showed the lowest variability on irradiation among the chemical classes. Our results were in agreement with general results reported in literature on the effect of irradiation on N-containing compounds (337,342).

Radiolysis of straight-chain hydrocarbon has little effect on the yield of product, which increased slowly with increasing chain length (343). Therefore, due to the high sensitivity of hydrocarbon compounds to irradiation treatment (344) their total contents were reduced even at lower dose (1 kGy) of irradiation. In present investigation, hydrocarbon compounds such as α-thujene, *p*-cymene, 2-methyl nonane and 3,5-dimethyl octane were highly sensitive and reduced after irradiation. A significant influence of irradiation causing a decrease in the quantity of essential oil and carbohydrate was previously noticed on black pepper, which was irradiated with 10, 20, 40 and 60 kGy doses (345). A noticeable reduction in the amount of terpinene such as α-terpinene and γ-terpinene present in licorice was observed after irradiation treatment. The similar result was reported in irradiated marjoram where terpenes were reported to converted into monoterpe-nesalcohols (346). Above results suggested that the contents of functional groups identified from volatile oil of licorice were changed after irradiations but their proportions were variable in dose dependent manner.

We conclude that γ-irradiation upto 20 kGy causes only slight (no significant) differences in the content and composition of volatile organic compounds of licorice. Therefore, the application of irradiation, if required for microbial decontamination of licorice is feasible as it did not undergo major qualitative and quantitative loses of volatile organic compounds when subjected to such irradiation doses.

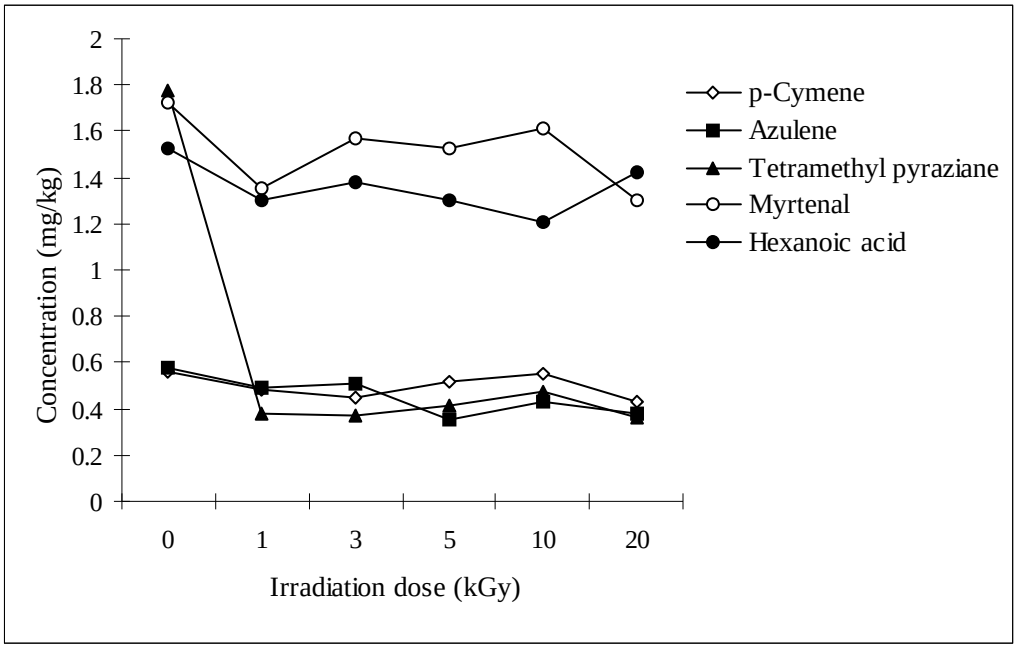

Fig. 18. Volatile organic compounds of licorice decreased after irradiation upto 20 kGy.

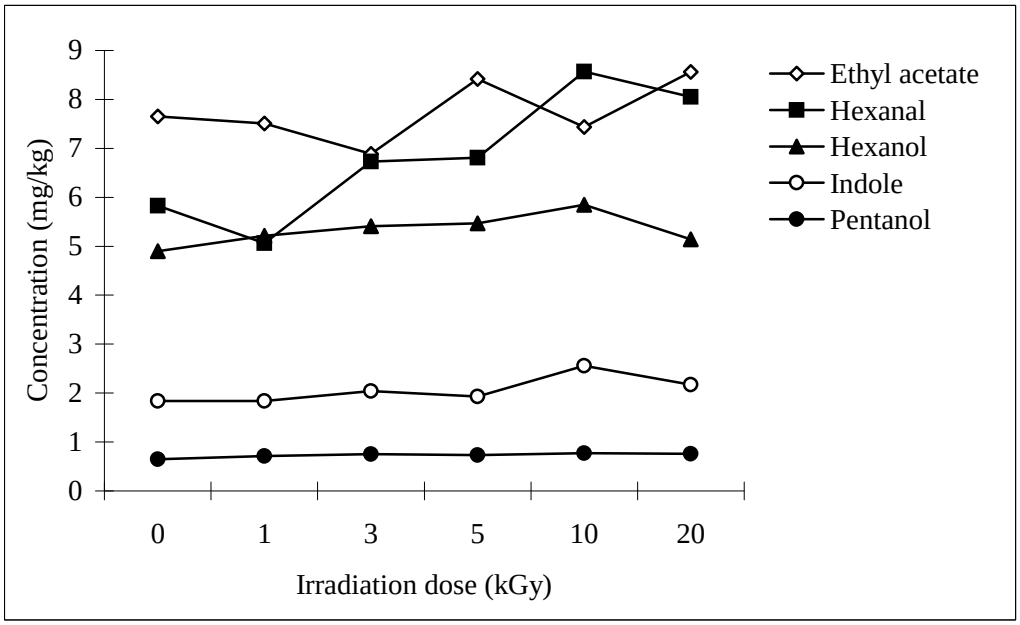

Fig. 19. Volatile organic compounds of licorice increased after irradiation upto 20 kGy.

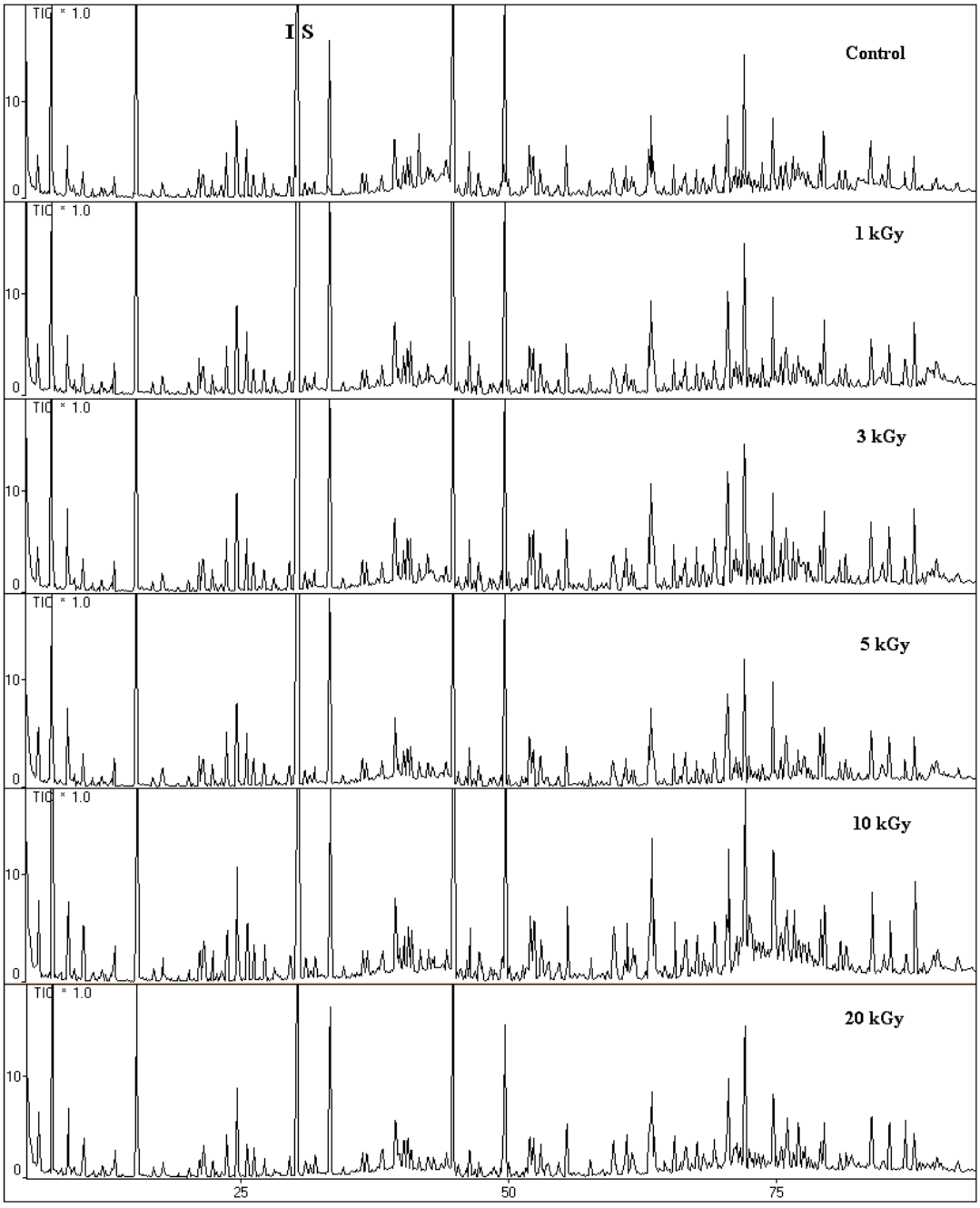

Fig. 20. GC/MS Chromatograms of volatile organic compounds obtained from non-irradiated and irradiated licorice.

Table 24. Volatile organic compounds identified in non-irradiated and irradiated licorice

Pk No.	RT[1)]	RI[2)]	Compound name	MF[3)]	mg/kg					
					0kGy	1kGy	3kGy	5kGy	10kGy	20kGy
1	6.20	804	Ethyl formate	$C_3H_6O_2$	1.38	1.27	1.27	1.50	1.28	1.42
2	7.18	853	Butanal	C_4H_8O	0.06	0.03	0.11	0.05	0.08	0.12
3	7.46	865	Ethyl acetate	$C_4H_8O_2$	7.65	7.51	6.89	8.42	7.44	8.56
4	8.96	925	Ethanol	C_2H_6O	1.53	1.34	2.02	2.05	1.44	1.76
5	9.41	941	2-Ethylfuran	C_6H_8O	0.14	0.13	0.13	0.08	0.12	0.08
6	9.57	946	3,5-Dimethyl octane	$C_{10}H_{22}$	0.33	0.22	0.21	0.22	0.18	nd
7	10.39	972	Pentanal	$C_5H_{10}O$	0.84	0.71	0.9	0.78	1.01	1.01
8	11.29	999	2-Methyl nonane	$C_{10}H_{22}$	0.20	0.18	0.11	0.18	0.11	0.05
9	12.45	1025	α-Thujene	$C_{10}H_{16}$	0.20	0.10	0.05	0.08	0.04	0.10
10	13.04	1038	2-Butenal	C_4H_6O	0.24	0.24	0.13	0.60	0.16	0.15
11	13.32	1043	2-Methyl-3-buten-2-ol	$C_5H_{10}O$	0.54	0.64	0.63	0.60	0.76	0.56
12	15.37	1081	Hexanal	$C_6H_{12}O$	5.83	5.06	6.73	6.81	8.57	8.05
13	17.83	1124	2-Pentanol	$C_5H_{12}O$	0.42	0.47	0.39	0.52	0.53	0.47
14	21.23	1177	α-Terpinene	$C_{10}H_{16}$	0.74	0.81	0.60	0.65	0.55	0.39
15	21.50	1181	2-Heptanone	$C_7H_{14}O$	0.49	0.48	0.47	0.43	0.37	0.50
16	23.80	1216	2-Hexenal	$C_6H_{10}O$	1.28	1.10	1.14	1.32	0.99	1.04
17	24.75	1231	2-Pentylfuran	$C_9H_{14}O$	2.14	2.02	2.13	2.22	2.26	2.29
18	25.68	1245	γ-Terpinene	$C_{10}H_{16}$	1.37	1.38	1.17	1.19	1.11	0.77
19	26.33	1255	Pentanol	$C_5H_{12}O$	0.65	0.71	0.75	0.73	0.77	0.76
20	27.31	1269	*p*-Cymene	$C_{10}H_{14}$	0.56	0.48	0.45	0.52	0.55	0.43
21	28.22	1281	Terpinolene	$C_{10}H_{16}$	0.31	0.34	0.26	0.28	0.21	0.15
22	29.67	1301	Tridecane	$C_{13}H_{28}$	0.40	0.39	0.56	0.35	0.43	0.38

[1)] retention time, [2)] retention index, [3)] Molecular formula, [nd)] not detected

Table 24. Continued

Pk No.	RT[1)]	RI[2)]	Compound name	MF[3)]	mg/kg					
					0kGy	1kGy	3kGy	5kGy	10kGy	20kGy
IS	30.48	1313	*Butylbenzene*	$C_{10}H_{14}$	-	-	-	-	-	-
23	32.03	1338	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	0.44	0.56	0.45	0.61	0.43	0.45
24	33.44	1358	Hexanol	$C_6H_{14}O$	4.90	5.21	5.41	5.47	5.85	5.14
25	36.49	1400	Tetradecane	$C_{14}H_{30}$	0.59	0.43	0.62	0.51	0.49	0.53
26	36.87	1407	3-Octen-2-one	$C_8H_{14}O$	0.63	0.58	0.65	0.44	0.44	0.17
27	39.50	1449	Acetic acid	$C_2H_4O_2$	2.15	2.13	2.21	2.16	2.41	1.82
28	40.34	1462	Furfural	C_6H_8O	0.75	0.74	0.63	0.73	0.76	0.79
29	41.78	1483	Tetramethyl pyraziane	$C_8H_{12}N_2$	1.78	0.38	0.37	0.41	0.47	0.36
30	44.33	1522	Benzaldehyde	C_7H_6O	nd	nd	0.39	0.44	0.53	0.51
31	44.99	1533	2-Ethoxy-1-propanol	$C_5H_{12}O_2$	23.38	29.38	29.61	33.32	31.51	21.67
32	46.17	1551	Linalool	$C_{10}H_{18}O$	0.29	0.35	0.31	0.13	0.34	0.28
33	46.962	1563	Octanol	$C_8H_{18}O$	0.13	0.09	0.24	0.17	0.06	0.20
34	47.55	1572	[*E,Z*]-3,5-Octadiene-2-one	$C_8H_{12}O$	0.26	0.27	0.32	0.25	0.27	0.12
35	49.46	1600	Hexadecane	$C_{16}H_{34}$	0.46	0.35	0.48	0.38	0.52	0.47
36	49.80	1606	4-Terpineol	$C_{10}H_{18}O$	7.77	6.88	6.70	6.29	6.71	4.76
37	50.14	1612	Hexyl hexanoate	$C_{12}H_{14}O_2$	0.47	0.45	0.46	0.28	0.29	0.35
38	52.09	1645	Myrtenal	$C_{10}H_{14}O$	1.72	1.35	1.57	1.53	1.61	1.30
39	52.45	1651	Pulegone	$C_{10}H_{16}O$	1.32	1.27	1.69	1.08	1.34	1.25
40	53.10	1661	2,3-Octanedione	$C_8H_{14}O_2$	0.82	0.83	1.05	0.92	0.94	1.00
41	55.54	1700	α-Terpineol	$C_{10}H_{18}O$	1.70	1.30	1.57	1.99	1.81	1.50
42	57.74	1741	Azulene	$C_{10}H_8$	0.58	0.49	0.51	0.35	0.43	0.38
43	60.89	1796	Butyrophenone	$C_{10}H_{12}O$	0.40	0.32	0.57	0.35	0.54	0.39

[1)] retention time, [2)] retention index, [3)] Molecular formula, [nd)] not detected

Table 24. Continued

Pk No.	RT[1)]	RI[2)]	Compound name	MF[3)]	mg/kg					
					0kGy	1kGy	3kGy	5kGy	10kGy	20kGy
44	61.12	1800	Octadecane	$C_{18}H_{38}$	0.73	0.64	0.79	0.61	0.99	0.57
45	63.20	1848	Hexanoic acid	$C_6H_{12}O_2$	1.53	1.30	1.38	1.30	1.21	1.42
46	63.46	1854	*p*-Cymen-8-ol	$C_{10}H_{14}O$	2.45	2.33	3.12	2.19	3.03	2.35
47	63.70	1859	Geranyl acetone	$C_{13}H_{22}O$	1.06	0.9	1.19	0.93	1.20	1.04
48	65.58	1900	Nonadecane	$C_{19}H_{40}$	0.74	0.62	0.83	0.67	0.92	0.52
49	66.16	1917	Phenethyl alcohol	$C_8H_{10}O$	0.25	0.28	0.30	0.27	0.40	nd
50	66.66	1930	Benzyl isocyanide	C_8H_7N	0.59	0.79	0.74	0.97	1.00	0.73
51	69.33	2002	Eicosane	$C_{20}H_{42}$	0.80	0.82	1.14	0.74	1.01	0.74
52	70.62	2039	γ-Nonalactone	$C_9H_{16}O_2$	2.56	2.62	3.45	2.34	2.92	2.57
53	71.72	2071	Tridecanol	$C_{13}H_{28}O$	0.63	0.36	0.74	0.42	0.97	0.73
54	72.17	2083	[E]-2-Tetradecenal	$C_{14}H_{26}O$	4.11	3.78	4.66	3.18	4.65	4.41
55	74.82	2137	Tetradecanol	$C_{14}H_{30}O$	2.11	1.68	1.79	2.20	1.99	1.56
56	75.07	2142	*p*-Cymen-3-ol	$C_{10}H_{14}O$	0.31	0.20	0.42	0.26	0.47	0.28
57	76.08	2160	Methyl hexadecanoate	$C_{17}H_{34}O_2$	0.70	0.74	1.11	0.86	0.52	1.08
58	77.16	2179	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	0.44	0.53	0.72	0.65	0.46	0.29
59	79.58	2234	Solavetivone	$C_{15}H_{22}O$	1.98	2.00	1.86	1.55	1.55	1.29
60	81.06	2275	Hexadecanol	$C_{16}H_{34}O$	0.48	0.42	0.62	0.51	0.79	0.42
61	83.99	2353	Indole	C_8H_7N	1.84	1.84	2.04	1.93	2.56	2.17
62	85.65	2395	Methyl linolelaidate	$C_{19}H_{34}O_2$	1.29	1.22	1.80	1.49	1.51	1.53
			Total		**102.44**	**102.04**	**111.61**	**110.46**	**114.86**	**96.18**

[1)] retention time, [2)] retention index, [3)] Molecular formula, [nd)] not detected

CHAPTER IV

Biological Activities of Few Medicinal Plants of Nepal

1. Introduction

Premature delivery is the most important problem in obstetrics both in developed and developing countries to vex clinicians and researchers. About 8% to 11 % of all pregnancies are born preterm or born before 37 weeks of gastation (347). This obstetric complication is responsible for 75% to 80 % of all neonatal deaths as well as a considerable infant and neonatal morbidity (348). Despite decades of investigation, the pathophysiology of premature labor is incompletely understood, and therapies or preventive strategies tailored to each of the many potential causes do not exist (349).

The etiology of preterm birth is related to the premature rupture of membranes in 30% of the cases, to maternal and fetal indications for early pregnancy termination in 20~25% and to spontaneous preterm births in about 4~45% of all cases (350). Spontaneous premature birth has been associated with multifactorial causes, including demographic factors, stress, infections and genital inflammations. Efforts are therefore being made to identify predictors of preterm birth, since some therapies, especially corticosteroids, are able to improve fetal prognosis (351). Uterine contractions are the most common symptom (352). Sub-clinical intra-amniotic infections possibly trigger preterm labor. Infection triggers cytokine production, as well as synthesis and release of prostaglandins, which are probably responsible for cervical ripening and uterine contraction. There is a sufficiently high level of evidence in the literature to suggest that an increased level of interleukin-6 in the amniotic fluid is related to preterm birth.

Most strategies for delaying preterm birth rely on the reduction of uterine contractions. It is well accepted that uterine contractile activity at birth involves uterine activation and increased levels of contractile stimulators. Uterine activation proteins include the oxytocin receptor, prostaglandin F2α (PGF2α) and PGE2 receptors (FP, EP1-4), and prostaglandin endoperoxide H synthase (cyclo-oxygenase; PGHS)-2 (353).

Medicinal herbs are also used to cure sexual diseases (leucorrhoea, gonorrhoea, menorrhagia, syphilis, alactorrhoea and to regularize menses). Traditional method of

protection of child during pregnancy and safe delivery of child by the use of herbal remedies exists among the tribal societies. The use of herbal medicines in pregnancy is extremely fashionable although there is very little real evidence of safety. Pregnant women usually use medicinal herbs as a panacea for all their symptoms, without understanding that there is a complex physiopathology and pharmacology involved. In the absence of adequate safety data concern against the use of herbal medicines during pregnancy there is a need for quality control legislation and further well-designed studies to establish safety and efficacy of the various remedies (354). We are interested to study how these plants of traditional botanical knowledge have been controlling their sexual disorders particularly preterm birth.

2. Justification of This Study

The aim of the present study was to investigate whether the plant extracts effects on rat spontaneously induced rat uterine smooth cell contractile activity in vitro to assist in determining activity of a plant-based preparation in the laboratory.

3. Material and Methods

3.1. Powerlab/4SP-polygraph

The PowerLab/4SP is a data acquisition and analysis system was used for research. The unit has 16 bit resolution (hardware and software supported). The unit incorporates four general purpose BNC analog inputs and four alternate pod (DIN) ports for measuring external signals. It also features a built-in analog output for stimulation or pulse generation (software controlled), and a trigger input. The activity was displayed on a channel recorder (Model 79 F Polygraph; Grass Inst., Quincy, MA, USA) with preamplifier (7P5B, Grass Instr.).

3.2 Plant material

As described in chapter II

Table 25. MAP's used for the study of biological activities

Name of Plants	Parts collected	Local name	Family
Dipsacus mitis D. Don	Roots	Banmula	Dipsaceae
Woodfordia fruticosa (L.) Kurz	Flowers	Dhairo	Lythraceae

3.3. Extraction of herbs

Extraction from medicinal plant was carried out using an Accelerated Solvent Extractor (ASE 200). 50 grams of samples were weighted and placed in the extraction cells in the oven of the instrument. Extraction was carried out at the temperature of 100 ^{o}C using methanol as extraction solvent. After the injection of the solvent into the cell, a pressurized static extraction phase lasting 5 min was carried out. After removal of the extracts (approx. 20 ml in each cell), they were filtered through a 0.45 μm filter (Waters

Millipore). The filtrate was evaporated to dryness using Rotavapour Apparatus (Buchi, Switzerland). Total 12.5 g and 10.25 g of MeOH extract was obtained form *Dipsacus mitis Woodfordia fruticosa* respectively.

3.4. Determination of uterine smooth muscle cells contraction

Uterine smooth muscle tissues were obtained from non-pregnant rats (n=21). The uterus of the rat was dissected and cut into 10 mm ring segments and placed immediately in Kreb's solution. The Krebs solution had been cooled previously at 4°C and aerated with carbogen (95% oxygen and 5% carbon dioxide) to maintain a pH of 7.4. The uterine ring segments were suspended in organ bath filled with Krebs solution. Each ring was suspended under an initial load of 2.0 g in 100 ml organ baths containing Krebs solution, temperature controlled at 37°C and continuously gassed with carbogen. The tissues were allowed to equilibrate for 1 h with Krebs solution washing every 10–15 min. After spontaneous uterine contractile activity had been accomplished, plant extracts were added cumulatively to the bath. Changes in isometric force were measured continuously with a channel recorder (Model 79 F Polygraph; Grass Inst., Quincy, MA, USA) with preamplifier (7P5B, Grass Instr.) and were displayed on recorders.

4. Results and Discussion

We studied the effect of the different plant extracts on the smooth muscle strips from rat uterus. Dramatic muscular relaxation on spontaneous contractility was obtained at a concentration of 6500 μg/ml of *Dipsacus mitis*, slight relaxation on spontaneous contractility was obtained by methanol extract of *Woodfordia fruticosa* upto concentration of 20000 μg/ml. Frequency of contraction in both cases was decreased as dose of drug was increased. The inhibition of Kreb's solution induced contraction could be due to an effect on one of the components of the vessel wall, namely the endothelium, the smooth muscle, or the extracellular matrix.

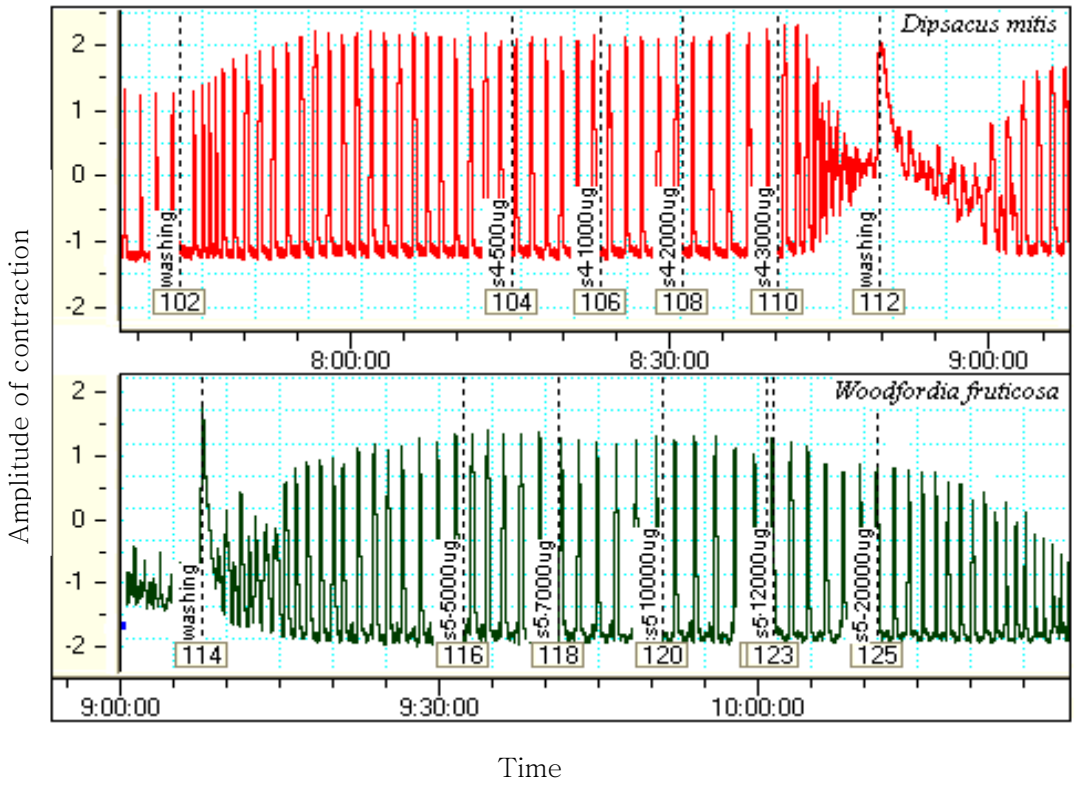

Fig. 21. Effect of methanol extracts of *Dipsacus mitis* and *Woodfordia fruticosa* on uterine smooth muscle tissues of non-pregnant rat.

After loading the tissue, it was runned for 30 minutes in Krab's solution to check and maintain contractibility. Normal Kreb's solution caused a significant contraction that reached a maximum within few minutes. Kreb's solution contains numerous nutrituents such as NaCl, KCl, $MgCl_2$, KH_2PO_4, $NaHCO_3$, $CaCl_2$ and Glucose. These nutrituents made cell active and could be seen on polygraphs. The observed inhibition of vascular contraction after adding MeOH extract was result of an effect of plant secondary metabolites. Efective weight 6,500 μg of plant is equivalent to 317 mg of its raw weight and 20,000 μg equivalent to 975 mg of its raw weight. This result appears to justify their traditional uses and give additional interest that these plants could be useful for controlling the preterm birth problem. For further confirmation and accurate report, dose dependent steps, in-vivo and in-vitro tests, ion-channel studies and more pre-clinical studies should be done.

We concluded that inhibited spontaneous induced uterine smooth muscle contraction by MeOH extract of *Dipsacus mitis* and *Dipsacus mitis* justify their traditional uses. *Dipsacus mitis* is the greatest and *Woodfordia fruticosa* is the least relaxation in rat uterine smooth muscle between them.

CHAPTER V

Conclusion

Phytochemical screening of 47 medicinal and aromatic plants (MAP's) of Nepal revealed the presence of plant secondary metabolites in all the species with different concentrations. Glycosides, tannins, terpenoids, flavonoids, alkaloids and saponins were the major secondary metabolites present in most of the species while cardiac glycosides and carotenoids were rarely detected among them. Total 8 species *Asparagus racemosus*, *Bergenia ciliata*, *Daphne bholua*, *Rhododendron arboretum*, *Schima wallichii*, *Terminalia chebula*, *Tinospora cordifolia* and *Woodfordia fructiosa* containing high concentrations of diverse phytochemicals are confirmed to be the potential species of medical value.

Studies on essential oil content of 8 MAP's of Nepal revealed that species *Acorus calamus*, *Centella asiatica*, *Swertia chirata* and *Woodfordia fruticosa* yielded high amount of essential oils than species *Asparagus racemosus*, *Bergenia ciliata*, *Dipsacus mitis* and *Terminalia chebula*. VOC's *β*-asarone, borneol, 5,6-dihydro-2-pyranone, [*Z*]-*β*-farnesene, 2-butenal, undecanoic acid, were major VOC's of *Acorus calamus*, *Asparagus racemosus*, *Bergenia ciliata*, *Centella asiatica*, *Dipsacus mitis*, *Swertia chirata*, respectively while furfural was dominant among *Terminalia chebula* and *Woodfordia fruticosa*.

Studies on effect of γ-irradiation revealed that irradiation doses 1-20 kGy did not highly influence to concentration and number of Volatile Organi Compounds (VOC's) of Licorice. Compounds were found highly enhanced at 10 kGy doses resulting that the total yield was increased by 12.12% at 10 kGy but the maximum dose 20 kGy inhibited the total yield by 6.11%. Major VOC's such as, 2-ethoxy-1-propanol, ethyl acetate, hexanal, hexanol, [E]-2-tetradecenal, γ-nonalactone, *p*-cymen-8-ol, acetic acid, 2-pentylfuran and α-terpineol were induced after irradiation treatments. Hence, the application of irradiation, if required for microbial decontamination of licorice is feasible as it did not undergo major qualitative and quantitative loses of VOC's when subjected to such irradiation doses.

We studied the effect of the different plant extracts on the smooth muscle strips from rat uterus. Dramatic muscular relaxation on spontaneous contractility was obtained at a concentration of 6,500 μg/ml of *Dipsacus mitis*, slight relaxation on spontaneous contractility was obtained upto concentration of 20,000 μg/ml of *Woodfordia fruticosa*.

CHAPTER VI

Summary

This study was performed to enumerate the plant secondary metabolites such as alkaloids anthocyanosides cardiac glycosides carotenoids coumarin glycosides flavonoids, saponins tannins, triterpenoids and essential oils of 47 medicinal and aromatic plants (MAP's) of Nepal. Volatile organic compounds (VOC's) of 8 MAP's were also investigated. This study also examined the effect of γ-irradiation on VOC of licorice. The screening testes were carried out on the aqueous and alcoholic extracts using standard procedures. Essential oils were extracted using SDE and VOC's were analyzed by GC/MS.

Phytochemical observations of medicinally important secondary metabolites showed that glycosides, tannins, terpenoids, flavonoids, alkaloids and saponins were the major secondary metabolites present in most of the plants while cardiac glycosides and carotenoids were rarely detected among these samples. Of the investigated plants, 81% plant speciescontained glycosides, 70% showed the presence of tannins, 66% terpenoids, 62% alkaloids, 60% flavonoids, 57% saponins, 45% volatile oils, 43% coumarins, 30% anthocyanosides, 17% cardiac glycosides and 15% carotenoids. Flowers and roots were rich in alkaloids, flavonoids, tannins and saponins. Total of 8 species *Asparagus racemosus*, *Bergenia ciliata*, *Daphne bholua*, *Rhododendron arboretum*, *Schima wallichii*, *Terminalia chebula*, *Tinospora cordifolia*, *Woodfordia fructiosa*, containing high concentrations of diverse phytochemicals are confirmed the potential species of medical value. There was definite co-relation between the traditional application of plants and possession of secondary metabolites, which supports the scientific basis for the traditional medicinal system.

The study on the essential oil content of 8 species revealed that species *Acorus calamus*, *Centella asiatica*, *Swertia chirata*, *Woodfordia fruticosa* yielded high amount of essential oils in comparision with species *Asparagus racemosus*, *Bergenia ciliata*, *Dipsacus mitis*, *Terminalia chebula*. GC/MS analysis of VOC's showed that *β*-asarone, borneol, 5,6-dihydro-2-pyranone, [*Z*]-*β*-farnesene, 2-butenal, undecanoic acid, were major compounds of *Acorus calamus*, *Asparagus racemosus*, *Bergenia ciliata*, *Centella asiatica*, *Dipsacus mitis*, *Swertia chirata*, respectively while furfural was dominant among *Terminalia chebula*, *Woodfordia fruticosa*. Aldehyde group was detected as a dominant

group in *Terminalia chebula* and *Woodfordia fruticosa*. Similarly ketone and alcohols were dominant in *Acorus calamus, Swertia chirata* and *Asparagus racemosus, Dipsacus mitis* respectively and hydrocarbon group was dominant in *Centella asiatica*. Some of these species could be important source of perfumery chemicals as well as medicinally active constituents.

Studies on effect of γ-irradiation on VOC's of licorice revealed that irradiation doses did not highly effect to yield and number of VOC's of licorice during the low doses of treatment. Sixty-one volatile organic compounds of the essential oil were detected in 1 kGy irradiated licorice as detected in non-irradiated sample. Above the dose of 1 kGy, one more compound related to aldehyde group was detected and a few kinds of compounds detected in non-irradiated and before 10 kGy irradiated samples were either disappeared or/and not detected at 20 kGy irradiated sample. Highest numbers of the compounds were found highly enhanced at 10 kGy doses resulting that the total yield was maximum increased by 12.12% at 10 kGy but the maximum dose 20 kGy inhibited the total yield by 6.11%. Major VOC's such as, 2-ethoxy-1-propanol, ethyl acetate, hexanal, hexanol, [E]-2-tetradecenal, γ-nonalactone, *p*-cymen-8-ol, acetic acid, 2-pentylfuran and α-terpineol were induced after irradiation treatments. Hence, the application of irradiation, if required for microbial decontamination of licorice is feasible as it did not underwent major qualitative quantitative and lose of VOC's when subjected to such irradiation doses.

We studied the effect of the different plant extracts on the smooth muscle cells from rat uterus. Dramatic muscular relaxation on spontaneous contractility was obtained by methanol extract of *Dipsacus mitis* at concentration of 6,500 μg/ml and slight relaxation on spontaneous contractility was obtained upto concentration of 20,000 μg/ml of *Woodfordia fruticosa*. These results appear to justify their traditional uses.

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APPENDICES

Appendix I Medicinal and Aromatic Plants (MAP's) Collected from Nepal

Appendix II Mass spectra of some bioactive volatile compounds identified in Nepalese medicinal plants

Appendix III Food items permitted to irradiation in different countries

Appendix I: Medicinal and Aromatic Plants (MAP's) Collected from Nepal

Name of Plants	Local name	Plant parts	Family	Traditional uses
Abies spectabilis (D.Don) Spach	Talish patra	Leaf	Pinaceae	Carminative, tonic, expectorant, stomachic, astringent, in asthma
Acacia catechu Willd.	Khayer	Wood	Leguminosae	Cooling, digestive, cough, diarrhea
Acorus calamus L.	Bojho	Rhizome	Araceae	Emetic, in dyspepsia, bronchitis, nauseant, stomachic, dysentery,
Adhatoda vasica Nees	Asuro	Leaf	Acanthaceae	Cough, ulcer, asthma, antispasmodic
Aegle marmelos Corr.	Bael	Fruit	Rutaceae	Cooling, laxative, dysentery, digestive, stomachic, diarrhea
Aneilema scapiflorum Wight.	Musali	Root	Commelinaceae	Aphrodisiac and in snake-bite (antipoison), colic, piles.
Asparagus racemosus Willd	Kurilo	Root	Liliaceae	Refrigerant, demulcent, diuretic, antispasmodic, aphrodisiac, galactagogue
Azadirachta indica A. Juss.	Neem	Twig	Meliaceae	Bitter, cough, intestinal worms, malarial fever, diabetes, antiseptic
Berberis aristata DC.	Chutro	Wood	Berberidaceae	Heart tonic, jundice, diarrhea deobstruent, menorrhagia,
Bergenia ciliata- (Haw.) Sternb	Pashanved	Rhizomes	Saxifragaceae	Aphrodisiac, fever, tonic, diarrhea, pulmonary problem, antiscorbutic,
Betula utelis D.Don	Bhoj patra	Bark	Betulaceae	Antiseptic, carminative and in hysteria
Cassia fistula L.	Raj-briksha	Pods	Leguminosae	Cathartic, emetic, astringent, tonic, febrifuge, laxative and purgative

Appendix I: Continued

Name of Plants	Local name	Plant parts	Family	Traditional uses
Centella asiatica (L.) Urb.	Ghodetapre	Whole	Apiaceae	Tonic, leprosy, skin diseases, nerves and blood purifier, diuretic, nervousness, indigestion
Crataeva religiosa auct. Non Forst.	Sipligan	Wood	Capparidaceae	Stomachic, laxative, diuretic, antipyretic, demulcent
Daphne bholua Buch.-Ham. ex D. Don	Lokata	Aerial part	Thymelaeaceae	Purgative, febrifuge,
Dipsacus mitis D. Don	Banmula	Root	Dipsaceae	Used in pregnancy interceptions, abortificant
Emblica officinalis Linn.	Amala	Fruit	Euphorbiaceae	Acrid, cooling, refrigerant, diuretic and laxative
Entada phaseoloides (L.) Merr.	Pangra	Seed	Leguminosae	Tonic, emetic, antiperiodic, anthelmintic
Glycyrrhiza glabra Linn.	Jethi-madhy	Root	Leguminosae	Tonic, demulcent, emollient, laxative, urinary diseases, coughs
Juglans regia L.	Okhar	Bark	Juglandaceae	For aegilops, cancer, carbuncles, bleeding, mouth rinse, alternative in rheumatism
Juniperus recurva Buch.Ham. ex D.Don	Dhupi	Wood	Cupressaceae	Smoke from green wood-emetic, producing long-continued vomiting
Lindera nessiana Benth.	Sil-timur	Fruit	Lauraceae	Efficaciious in treating the poisoing
Myrica esculanta Buch.-Ham. ex D. Don	Kafal	Bark	Moraceae	Decoction wash for ulcers, in leucorrhoea, gargle in salivation
Nyctanthes arbor-tristis Linn.	Rudilo	Steam	Oleaceae	For worms, sciatica, arthritis

Appendix I: Continued

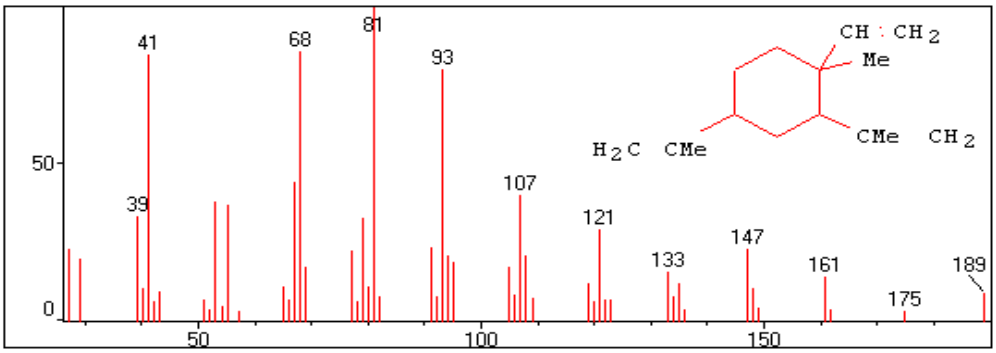
Name of Plants	Local name	Plant parts	Family	Traditional uses
Nardostachys jatamansi DC.	Jatamansi	Root	Valerianaceae	Aromatic, stimulant, satiseptic, mental disorders, carminative,
Ocimum sanctum Linn.	Tulsipatra	Herb	Labiatae	Expectorant, diaphoretic, antiperiodic, scorpion-sting
Operculina turpethum (Linn.)Silva Manso	Nisotha	Root	Convolvulaceae	Purgative, in snake-bite and scorpion-sting
Petrocarpus santalinus Linn.f.	Rakta-chandan	Wood	Leguminosae	Astringent, tonic, skin diseases, fever, boils, applied in headache
Picrorhiza scrophulariiflora Pennel	Kutki	Steam	Scrophulariaceae	Cathartic, dyspepsis, purgative, bitter, fever, in scorpion-sting
Piper longum L	Pipla	Fruit	Piperaceae	Sleeping problem, stomachic, carminative, antidote snake-bite ,
Podophyllum hexandrum Royle	Laghu patra	Root	Berberidaceae	Hepatic stimulant, cholagogue and purgative,
Rhododendron anthopogon D.Don	Dhupi	Aerial	Ericaceae	Aromatic, stimulant
Rhododendron arboreum Sm.	Laliguransh	Flower	Ericaceae	Tonic, juice is prinked in summer
Rheum emodi Wall	Padamchal	Rhizomr	Polygonaceae	Purgative, astringent, tonic, diarrhea
Sapindus mukorossi Gacrtn	Ritha	Fruit	Sapindaceae	Expectorant, used in salivation, chorosis and epilepsy, fish poison
Schima wallichii (DC.) Korth.,	Chilaune	Bark	Ternstroemiaceae	Anthelmintic, rubefacient
Semicarpus anacardium Linn.f.	Bhalayo	Fruit	Anacardiaceae	Nut applied to uteri to produce abortion, given as vermifuge

Appendix I: Continued

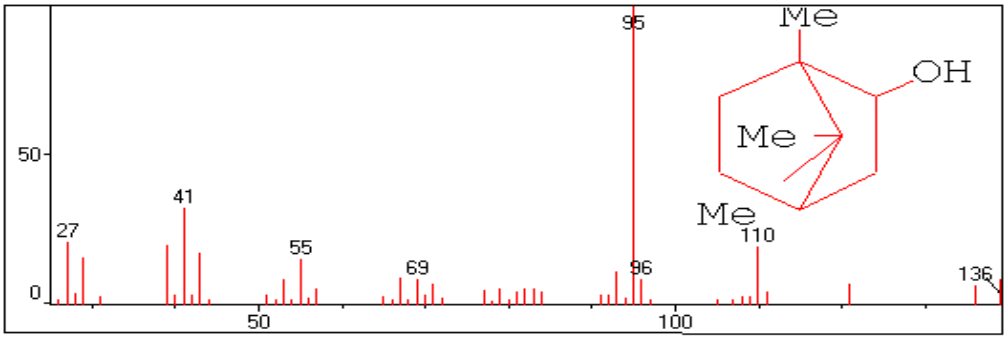
Name of Plants	Local name	Plant parts	Family	Traditional uses
Shorea robusta Gaertn. f.	Sal	Wood	Dipterocarpaceae	Astringent, tonic use in diarrhea, dysentry, splenomegaly
Swertia chirata Hamilt	Chirato	Whole	Gentianaceae	tonic, stomachic, febrifuge, anthelmintic, antidiarrhoetic, dyspepsia antimalaria
Terminalia belerica Roxb.	Barro	Fruit	Combretaceae	Astringent, laxative, antipyretic, used in piles, dropsy, diarrhea, leprosy, biliousness
Terminalia chebula Retz.	Harro	Fruit	Combretaceae	Astringent, alternative, dentifrice, bleeding
Tinospora cordifolia (Willd.) Miers	Gurjo	Stem	Menispermaceae	Bitter, stomachic, antiperiodic, antipyretic, aphrodisiac
Urtica dioica Linn.	Sisno	Leaf	Utricaceae	leaf: in nephritis, heamaturia, jaundice, menorrhagia
Viola serpens Wall.	Ghatte- ghans	Herb	Violaceae	Antipyretic, diaphoretic, febrifuge
Withania somnifera Dunal	Aswagandha	Root	Solanaceae	Aphrodisiac, deobstruent, diuretic, narcotic, abortifacient, rheumatism, consumption,
Woodfordia fructicisa (L.) Kurz	Dhairo	Flower	Lythraceae	Astringent, in dysentery, menorrhagia, liver disfunction, safe stimulant in pregnancy
Xanthoxylum armatum DC	Timur	Fruit	Rutaceae	Aromatic, cholera, tonic, dyspepsia, toothache, stomachic, carminative

Appendix II: Mass spectra of some bioactive volatile compounds identified in Nepalese medicinal plants

β-Elemene



Borneol



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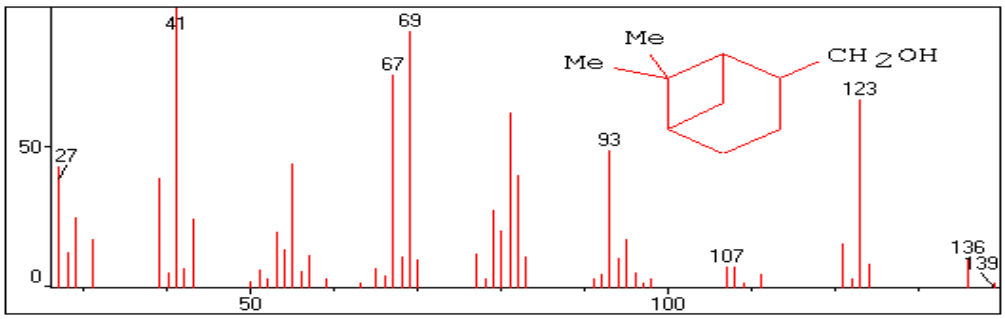
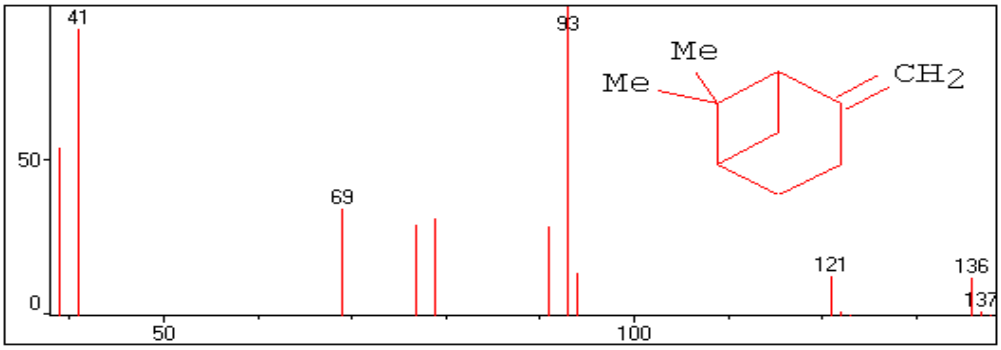


Appendix II: continued

***β*-Pinene**



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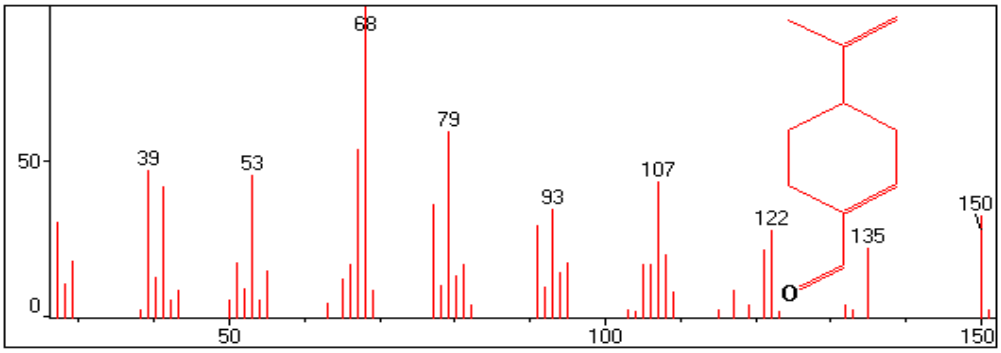
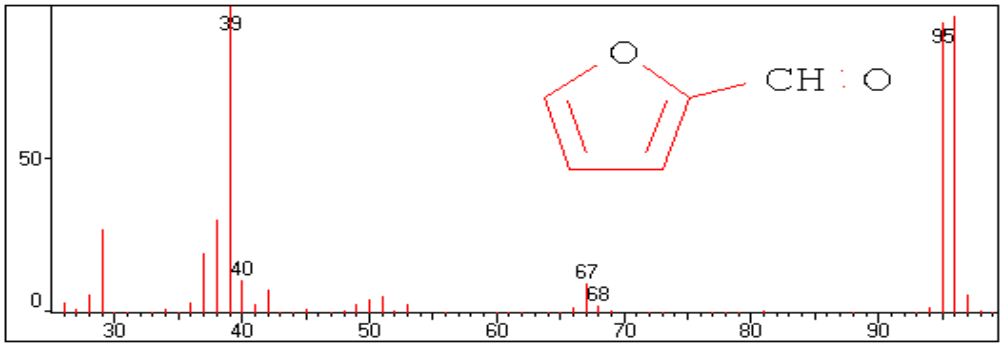
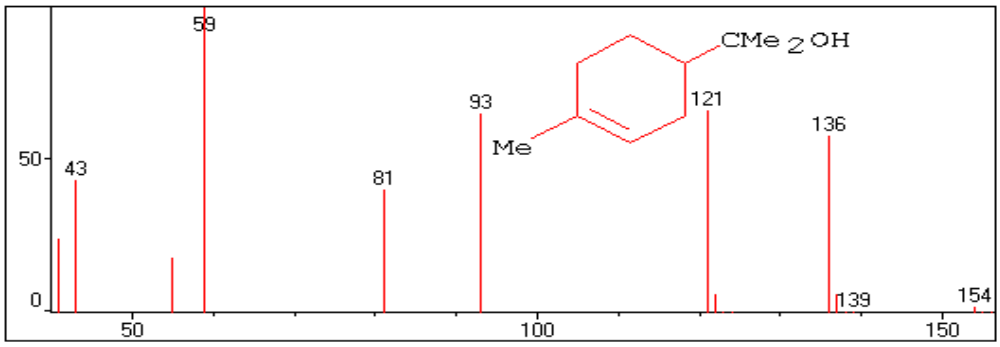


Furfural

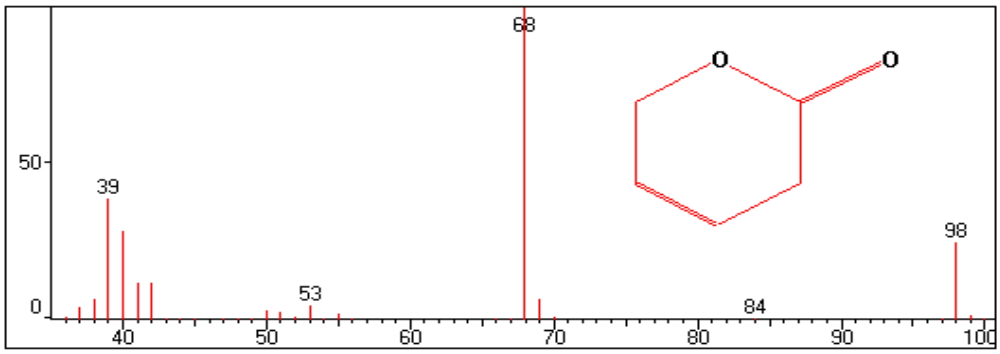


Appendix II: continued

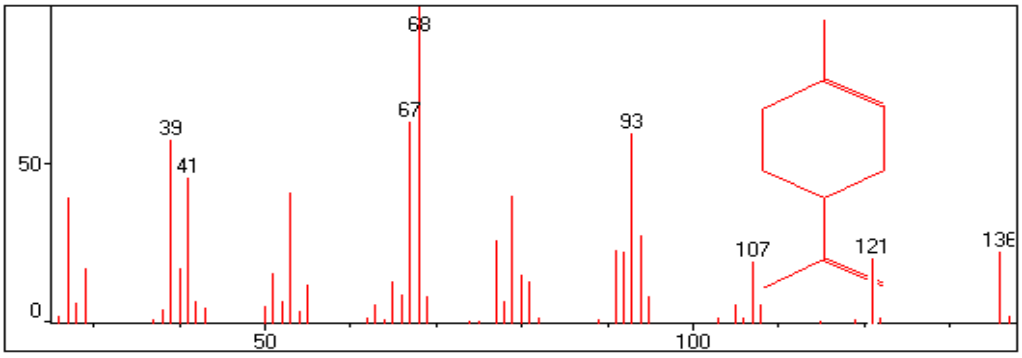
α-Terpineol



5,6-Dihydro-2-pyranone

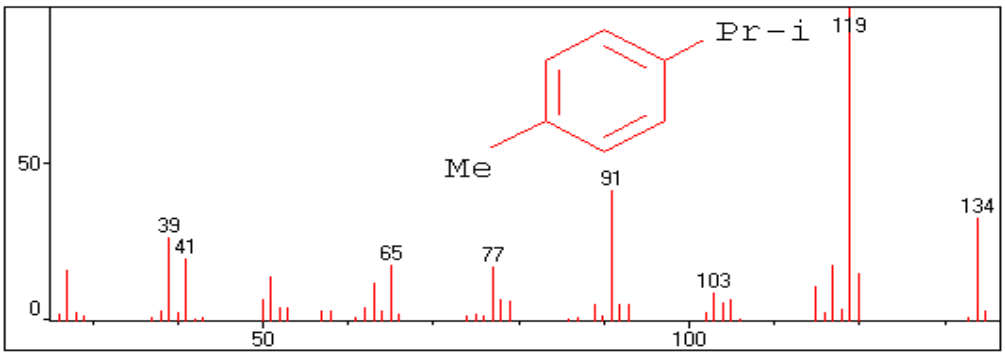


Limonene

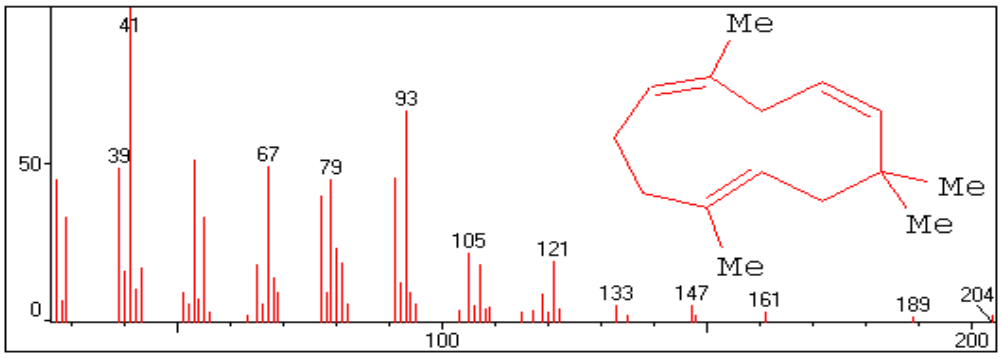


Appendix II: continued

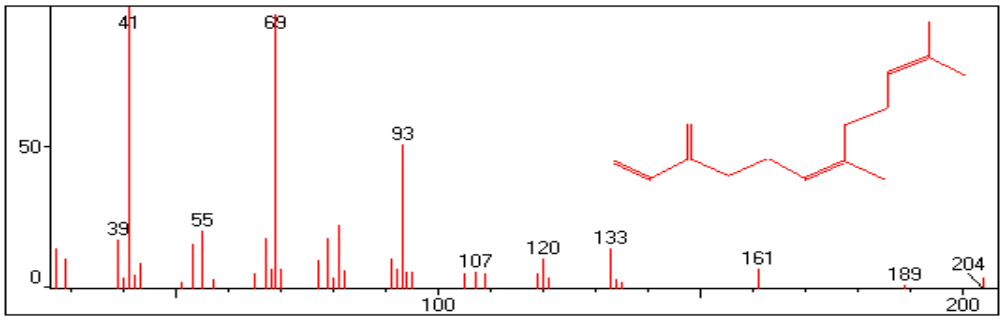
***P*-Cymene**



α-Humulene

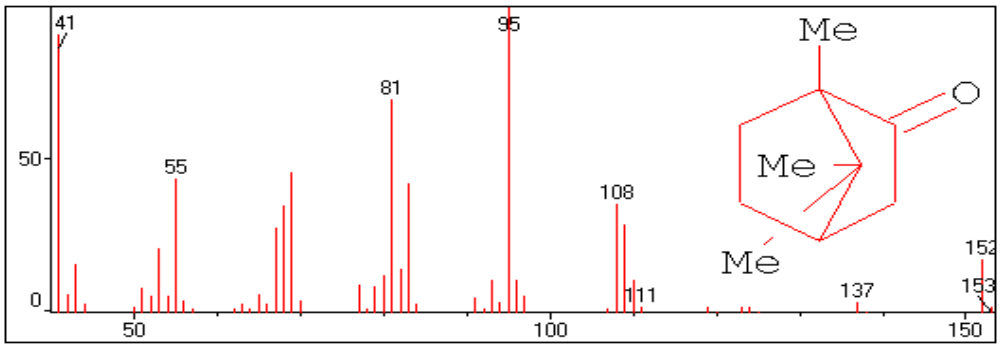


[*Z*]-*β*-Farnesene

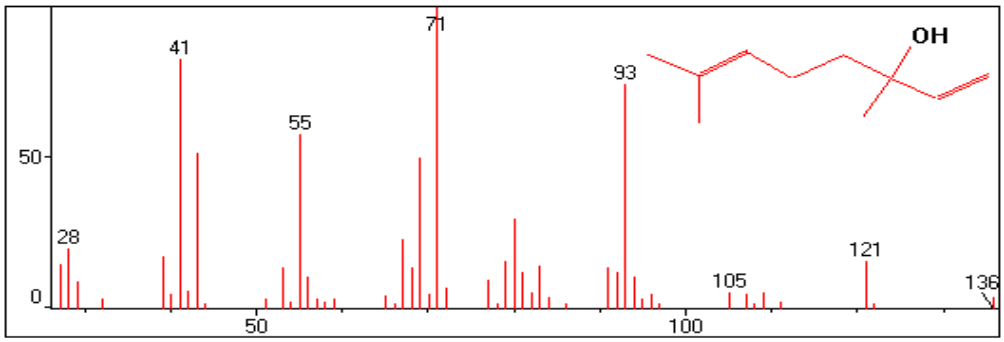


Appendix II: continued

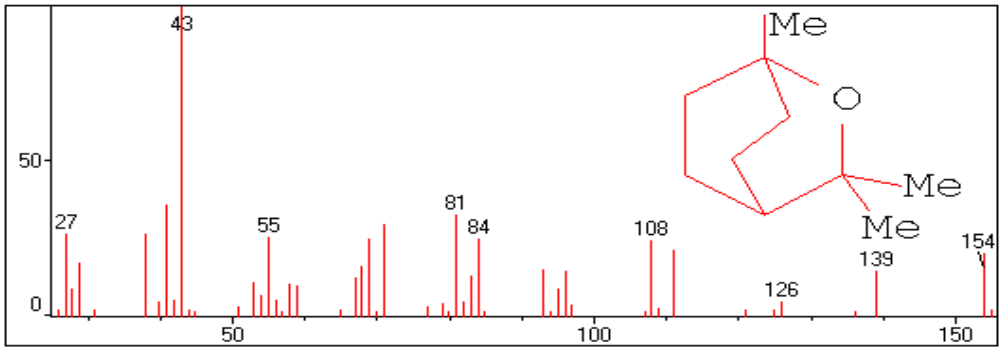
Camphor



Linalool

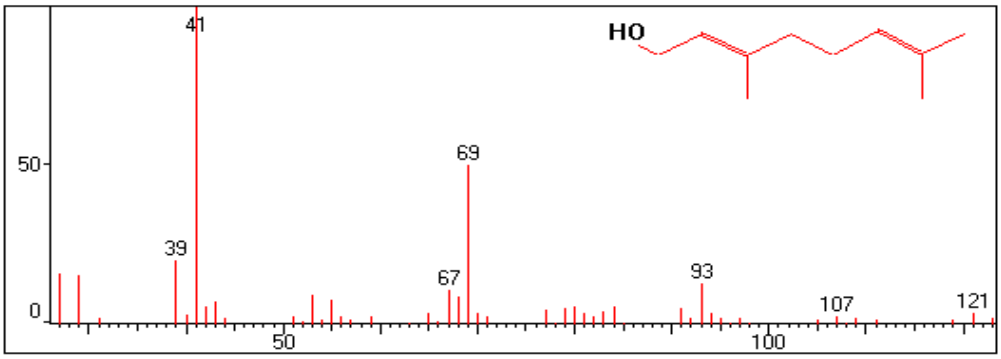


1,8-Cineole

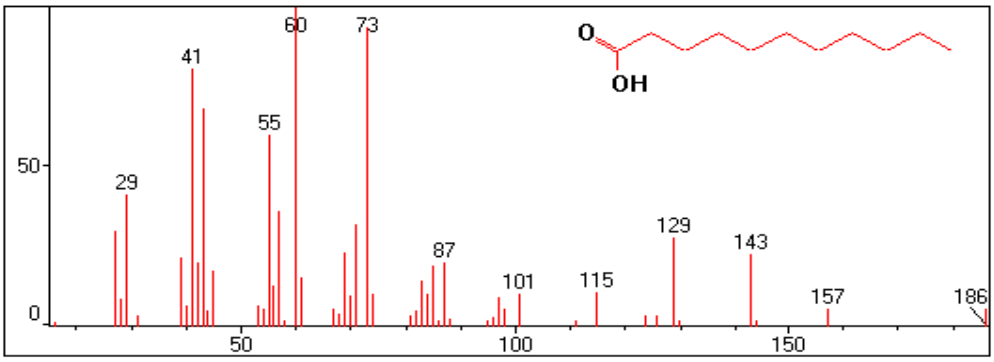


Appendix II: continued

[Z]-Geraniol



Undecanoic acid



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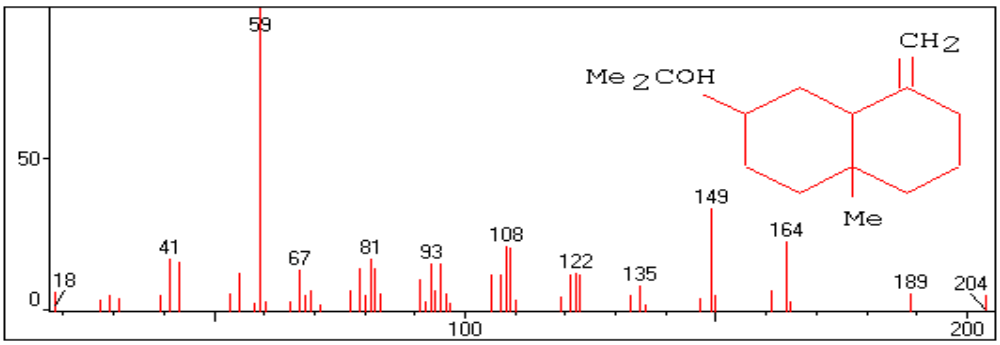
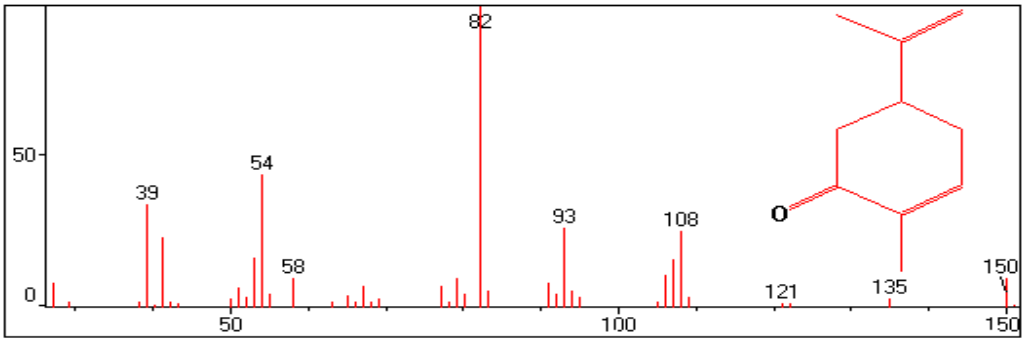
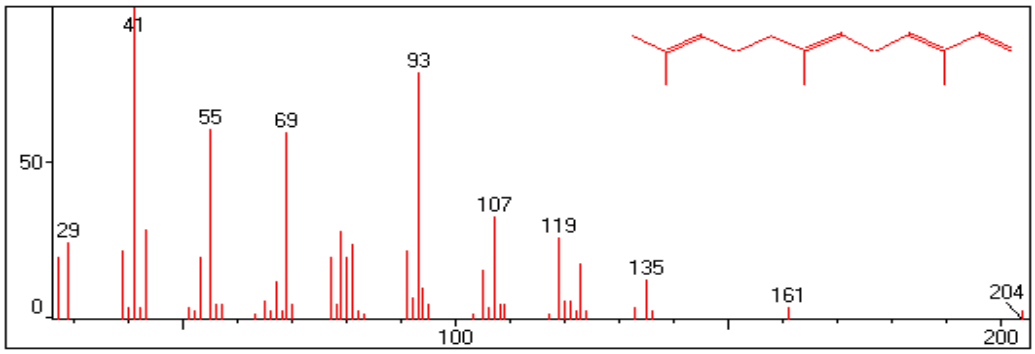


Appendix II: continued

Carvone



Farnesene



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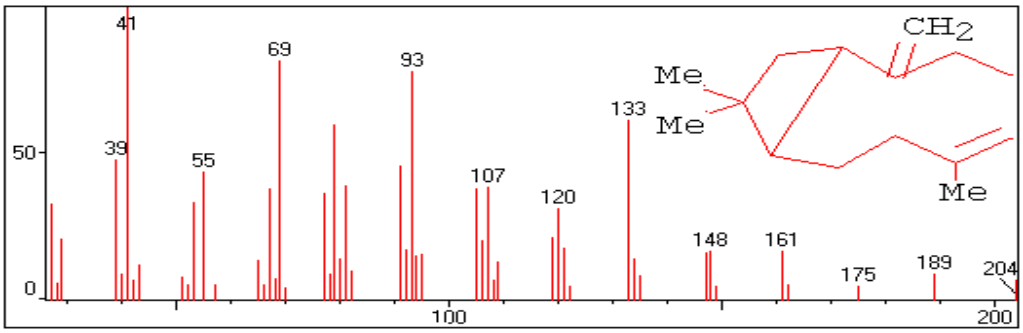
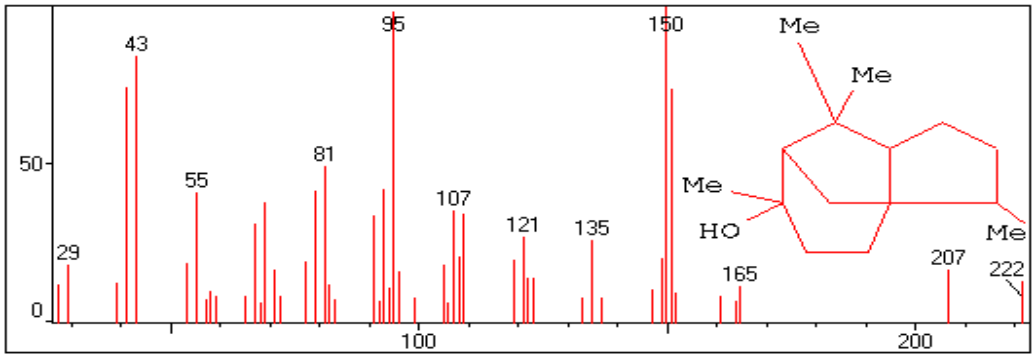


Appendix II: continued

Cedrol



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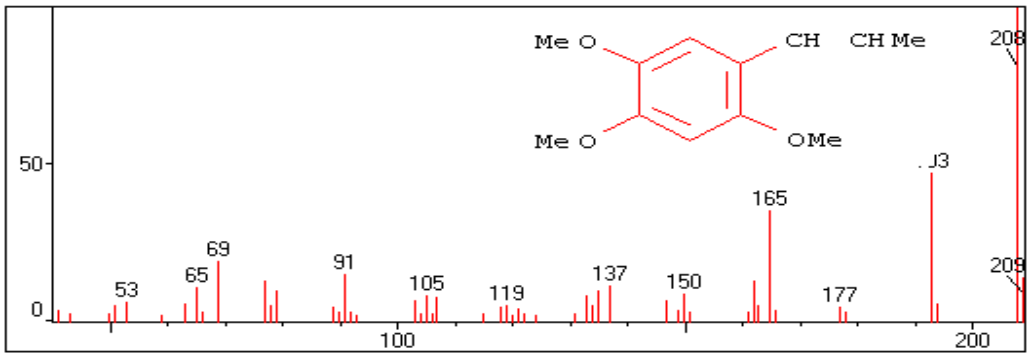
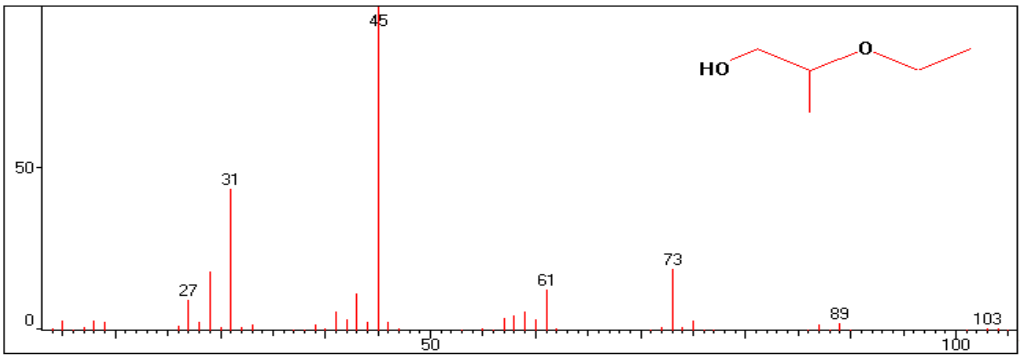


2-Ethoxy-1-propanol



Appendix III: Food items permitted to irradiation in different countries

Country	Number of foods	Country	Number of foods
Argentina	14	Italy	6
Australia	15	Japan	1
Austria	3	Korea	19
Bangladesh	21	Libya	6
Belgium	12	Luxembourg	3
Brazil	117	Mexico	101
Canada	7	Netherlands	19
Chile	20	New Zealand	15
China	24	Norway	3
Costa Rica	21	Pakistan	86
Croatia	72	Philippines	3
Cuba	18	Poland	5
Czech Republic	2	Portugal	3
Denmark	3	Russian Federation	48
Egypt	13	South Africa	94
Finland	4	Spain	5
France	30	Sweden	3
Germany	3	Syria	20
Ghana	171	Thailand	25
Greece	3	Turkey	97
Hungary	12	Ukraine	47
India	30	United Kingdom	55
Indonesia	22	Uruguay	1
Iran	1	USA	47
Ireland	3	Viet Nam	8
Israel	46	Yugoslavia	23

Source: http://www.iaea.org/icgfi/data.htm

CURRICULUM VITAE

of

RAJENDRA GYAWALI

1973	Born on November 23 in Daugha-9, Gulmi District, Middle Mountain of Nepal as a son of Prashu Ram and Radha Gyawali
1980-1990	School level education at Hem Raj MV, Kapilvastu
1991-1993	Intermediate degree (Biology) at Tribhuvan University, Nepal
1993-1996	Bachleor's degree (Biology) at Tribhuvan University, Nepal
1997-1998	Master's degree at Tribhuvan University, Nepal
2001-2003	Biology lecturer at Amrit Science Campus, Tribhuvan University, Nepal
2001-2004	Biology lecturer at Kathmandu Community College, Nepal
2002-2003	Biology lecturer at Prasady Academy, Nepal
Since, 2004	Study of Ph.D. under the supervision of Prof. Dr. Kyong-Su Kim at the Department of Applied Science, major in Food science and Biotechnology, Chosun University, Gwangju, Republic of Korea **(Student ID: 20047558)**
Feb. 2007	Final examination to obtain for the Ph.D. degree

List of Publications during Ph.D.

1) Rajendra Gyawali and Kim Kyong-Su, Effect of γ-irradiation on the volatile compounds of Licorice (*Glycyrrhiza urelansis* F.) *European Food Research and Technology*, Article submitted, 2006.

2) Rajendra Gyawali, Keun-Young Ryu, Sung-Lye Shim, Jun-Hyong Kim, Hey-Young Seo, Kyu-Jae Han and Kyong-Su Kim. Essential oil constituents of *Swertia chirata* Buch.-Ham., *J. Food Sci. Nutr.* **11(3)**: 232-236, 2006.

3) Hey-Young Seo, Ki-Mi No, Seong-Lye Shim, Keun-Young Ryu, Kyu Jae Han, Rajendra Gyawali and Kim Kyong-Su. Analysis of enantiomeric composition of chiral flavor components from dried ginger (*Zingiber officinale* Roscoe), *J. Korean Soc. Food Sci. Nutr.* **35(7):** 874-880, 2006.

4) Su-Hyeong Yang, Sung-Lye Shim, Ki-Mi No**,** Rajendra Gyawali, Hye-Young Seo, Hyun-Pa Song, and Kyong-su Kim. A Comparative study of the changes in volatile flavor compounds from dried leeks (*Allium tuberosum* R.) following γ-irradiation, *Food*

Sci. Biotechnol. **15(3):** 341-346. 2006.

5) Rajendra Gyawali, Hye-Young Seo, Hyun-Ju Lee, Hyun-Pa Song, Dong-Ho Kim, Myung-Woo Byun and Kyong-Su Kim. Effect of γ-irradiation on volatile compounds of dried welsh onion (*Allium fistulosum* L). *Radiat. Phys. Chem.,* **75(2):** 322-328, 2006.

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