PHYTOCHEMICAL INVESTIGATION OF EUPHORBIA, POUZOLZIA AND PAVETTA SPECIES FROM KUMAON HIMALAYAS

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By

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C E R T I F I C A T E

This is to certify that Mr. Kundan Prasad has carried out the research work under my supervision for the award of Ph. D. degree in Chemistry and has put in the required period of attendance in the Department of Chemistry, D.S.B. Campus, Kumaun University, Nainital.

The work included in the thesis entitled "Phytochemical Investigation of *Euphorbia*, *Pouzolzia* and *Pavetta* Species from Kumaon Himalayas" is the original work of Mr. Kundan Prasad and has not been submitted for any other degree elsewhere.

(G. Bisht)

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Nainital, 2008 (Kundan Prasad)

P R E F A C E

Medicinal plants are accessible, affordable and culturally appropriate sources of primary health care for more than 80% of Asia`s population (WHO). Plants have played dominant role in the introduction of new therapeutic agents. Drugs from higher plants continue to occupy an important niche in modern medicines.

The diversified topography of Kumaon and Garhwal region of Western Himalayas favoured the growth of a variety of medicinal and aromatic flora. Some of these are valuable for different industries and a number of these have been used indigenous in the folk medicines. The present study has undertaken the phytochemical analysis, biochemical composition, antioxidant composition and mineral analysis in selected medicinal plants.

The author has undertaken the study on three medicinally important plants:-

1. *Pouzolzia hirta* : Fam. *Urticaceae*
2. *Euphorbia thymifolia* : Fam. *Euphorbiaceae*
3. *Pavetta indica* : Fam. *Rubiaceae*

The thesis has been divided into four chapters, which are further subdivided into different parts.

Chapter I of the thesis is titled “General introduction” which deals with the relative vegetation found in Kumaon Himalayan region, pharmacological importance and work done on the sub species of the plants under investigation.

Chapter II of the thesis is titled "Phytochemical investigation" which deals with introduction of phytochemicals, review of literature, plant collection, their identification, general methods of extraction, chromatographic separation, identification, GC and GC-MS of plant oils and their results and discussion.

Chapter III of the thesis is titled "Biochemical analysis" which deals with biochemical, mineral, amino acid and antioxidant investigation including their general introduction, methods of extraction, HPLC and UV spectroscopic quantification and their results and discussion are described.

The references of literature concerned are given at the end of each chapter.

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C O N T E N T S

GENERAL INTRODUCTION

INTRODUCTION

Human society has been dependent for ages on different natural resources to meet its requirements of food, clothing, medicine, fodder, fuel wood, timber and other such needs. Several hundreds of plants and animals in our surroundings are effectively being used for these purposes for long. Among these, the plants used for food and medicines are usually considered important and attract attention because of their life supporting and life saving virtues. However, unlike the food plants, which are usually cultivated and lesser in number, the medicinal plants used by human societies are generally more in number and are quite often gathered from the wild habitats. Most of these plants, which possess valuable medicinal properties are used in different forms for curative, preventive and promotive purposes including hair care, skin care, dental care and body care.

The Himalayan vegetation which is regarded as the "Botanical Garden of the World" has been the source of every life saving plant derived drugs known till date. Most of the plants, which are being used in the Indian system of medicine since time immemorial, are found in Uttarakhand Himalayan region. Rigveda, one of the oldest repositories of human knowledge, written between 4500-1500 BC, mentions the use of 67 plants for therapeutic use and Yajurveda lists 81 plants whereas Atharveda written during 1200 BC describes 290 plants of medicinal value.

It has been estimated that 250,000 to 750,000 species of higher flowering plants exist on earth, some of these have not yet been botanically described and around

50-60% will be destroyed in ten years (**Ballick, 1990**). Although there is no way to determine accurately how many of these species have been used in traditional medicine, a reasonable estimate would be about 10% i.e. 25000 to 75000 species (**Farnsworth, 1985**).

Apart from fulfilling the primary need of food by their primary products such as carbohydrates, fats, proteins and vitamins, plants produce a number of secondary metabolites that serve as the novel molecules. These secondary metabolites are responsible for imparting medicinal properties to these plants. In this regard, nationwide surveys are being carried out to prepare a comprehensive database of medicinal plants and information on phytochemistry, pharmacology and pharmacognosy is being enriched. The present study is a step forward in this direction.

Plants have played an integral part in the sustenance of human life. Their physical and chemical properties provide not only an invaluable source of food but also a wealth of raw materials which fulfill daily requirements of fuel, fodder, medicine etc. Considering this, the global interest has increased to utilize the wild plant resources. As such, only 30 species out of 7500 edible species are providing 90% of the global nutritional requirement (**Walters & Hamilton, 1993**). This scenario has led us to search new alternatives.

The Indian Himalayan region supports 8000 species of flowering plants, of these 675 species are known for their edible value. These species form an important source as a supplement or substitute food at the time of scarcity and play a major role in meeting the nutritional requirement for the inhabitants of the hill region. A wide variety of fruits, roots and other parts of plants are collected from the nature and consumed by rural masses. The land holdings in the region are small and cannot afford optimum agriculture input therefore, they rely on a number of unconventional food plants.

The World Health Organization (WHO) has been promoting a movement for "Saving Plants for Saving Lives". This is because of the growing understanding of the pivotal role played by the medicinal plants in providing remedies to health maladies. India is the home of several important traditional systems of health care like Ayurveda, Siddha and Unani. All these systems depend on herbal products. Over 800 medicinal plant species are currently in use by the Indian herbal industry.

In recent years, there has been a growing interest to evaluate various wild edible plants for their nutritional value (**Arora & Pandey, 1996; Bhatt *et al.*, 2000 and Sundriyal & Sundriyal, 2001**) because nutrient sufficiency is the basis of good health and longevity for everyone and deficiency can create several problems to human beings. For example, the micronutrient deficiency affects over 3 billion people globally, mostly infants and children in developing countries. The consequences of micronutrient malnutrition are profound. Therefore alternative approaches for sustainable solution to this problem is urgently required (**Horst *et al.*, 2001**).

Uttarakhand lies between 28^0 45' 10" to 31^0 27' 42" N latitude and 77^0 34' 10" to 81^0 02' 11" E longitude, covering an area of 53484 km^2, with a highly complex and varied forest and vegetation types, stretching from subtropical to alpine through temperate region. Uttarakhand was carved out from the northern part of the state of Uttar Pradesh. It extends about 275 km east to west and about 200 km north to south. Nearly 87% of the total geographical area of Uttarakhand is mountainous in nature and comprises of high snow clad peaks of the greater Himalayas to mountains of lesser elevations which extend up to Bhabhar belt situated in the foot hills. The Uttarakhand region can be divided into three main climatic zones.

Trans-Himalayan Zone - Lying on the northern-most part of Uttarakhand, beyond the great Himalayan-peaks and bordering the Tibetan Plateau, this rain shadow region varies in elevation from 4600-6400 m above the sea level.

Greater Himalaya - It varies in elevation from 3000-7000 m above the sea level and stretches from east to west in the northern part.

Middle Himalaya - This zone lies between the higher Himalayas and the main boundary thrust, which extends along the entire length of the Himalayas and demarcates the northern limit of the Siwaliks. This belt, having a width ranging from 60 km to 90 km covers 56-21 percent of the total geographical area of Uttarakhand.

The topography of Kumaon hills possesses a great diversity in its natural vegetation. A large number of medicinal plants are used in folk possessing antiviral, antifungal, anticancer, antibacterial, antihelminthic, antimalarial, anti-inflammatory, febrifuge, spermicidal and various other properties. These plants grow wild from the tropical of high altitude region of Kumaon Himalayas. Kumaon hills have been considered a veritable emporium of useful medicinal plants since immemorial (**Kirtikar *et al*., 1923; Albert, 1980; Chopra, 1958 and Dollard, 1840**).

Pithoragarh town, being in the valley, is relatively warm during summer and cool during winters. During the coldest months of December - January, the tropical and temperate mountain ridges and high locations receive snowfall and has an average monthly temperature ranging between 5.5°C and 8°C. Pithoragarh district has extreme variation in temperature due to much altitude gradient. The temperature starts rising from mid March till mid June. The areas situated above 3,500 m remain permanently snow cover. The district receives

monsoon showers after June. Winter set for transhumance - seasonal migration among the Bhotiya tribe with their herds of livestocks to lower warmer areas.

The Pithoragarh town is located at a height of 1645 m above sea level. The district lies between 29.4° to 30.3° North latitude and 80° to 81° East longitude along the east. The Pithoragarh district is surrounded by the Almora, Champawat, Bageshwar and Chamoli districts.

Thus, this region offers a unique opportunity to undertake research on various groups of economically and medicinally important plants because of their abundant growth and development. Due to the availability and abundance of plant material in and around Pithoragarh, the present study was therefore, confined in and around Pithoragarh hills within the altitude of 1800-2611 m, which represents both the temperate and high altitude flora of Kumaun.

A rapid work is being done now a days to search new medicinal and economical plant resources in tribal areas for the socio-economic development of this region. Most of the plants of this region are either medicinal, toxic and used for edible purposes (**Mehrotra *et al.*, 1979; Bhattacharya, 1969 and Nadkarni, 1989**). A few plants like Nardostachys jatamansi (mansi), Skimmia laureola, Jurenia macrocephala, Pleurospermum densiflorum etc. are used as dhoop as well as in folk medicine (**Chopra *et al.*, 1956**).

In Kumaon region, a large number of plants are used by the native in their day to day life to cure several ailments or as edibles. The literature survey reveals that the plants of this region are still very inadequately explored and a number of plant species have not yet been investigated for their chemical and pharmacological aspect. Therefore, it was considered to be of great interest to take up the investigation on chemical constituents of certain plants of Kumaon region.

In the present investigation, three plants were selected on account of their medicinal importance, edible values and aromatic nature and are based primarily on the literature screening along with their several uses in day to day life of people. A brief description of the plants under study are described as follows:

1) *Pouzolzia hirta* Hassk
Family – *Urticaceae*
Local name – Chifaljari, Atenyaa

Pouzolzia hirta Linn. (*Urticaceae*) (Fig. 1.1 - 1.4) is found in weedy places, thickets by ditches and rice fields, mainly in India, S. China (including Taiwan) northwards to Yunnan westwards and also in tropics and sub tropics of Asia and Australia.

Pouzolzia hirta is erect monoecious herb with opposite, ovate, acute leaves; stipules free, persistent; inflorescence cymose or spike; male flowers subsessile; female flowers subsessile to sessile; tepals 5; perianth enclosing ovary; achene black, shiny, enclosed by broadly winged perianth.

The powder of the plant rhizomes has been used as binder to flour of maize and wheat by the local population of Uttarakhand. The tuberous roots of plant are eaten raw or roasted. The rhizomes of the plant are eaten as a vegetable to expel worms (**Ambasta, 1994**). The rhizomes of plant have been reported to possess good antihelminthic activity (**Ong & Nordiana, 1999**). The plant is used agriculturally as fodder for pigs and medicinally as treatment for boils and bone dislocations and fractures.

Fig.-1.1 *Pouzolzia hirta*

Fig.- 1.2 *Pouzolzia hirta* roots and shoots

Fig.-1.3 *Pouzolzia hirta* cultivated in the fields

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Fig. – 1.4 *Pouzolzia hirta* cultivated plants rhizomes for the study

2). ***Euphorbia thymifolia***

Family – *Euphorbiaceae*

Local Name – Dudhi grass

Euphorbia thymifolia (*Euphorbiaceae*), (fig. 1.5 -1.6) also known as *Chamaesyce thymifolia* L., occurs throughout India, as a weed in plains and low hills, ascending to 5500 ft. in Kashmir. It is found in all plains and on hills in Deccan and Carnatic at low elevations.

A small more or less pubescent, much branched prostrate annual herb, with opposite, oblong leaves; petioles very short; stipules fimbricate; involucres axillary, solitary or 2- 3 in an axil, campanulate; stalk very short; hairy capsules; quadrangular and wrinkled seeds with faint furrows (**Stone, 1970**).

The dried leaves and seeds of the plant are slightly aromatic. They are considered stimulant, astringent, antihelminthic and laxative, and given to children in bowel complaints. The plant juice is employed in S. India as a cure for ringworm. *E. thymifolia* has also shown beneficial effects when used in the treatment of diarrhoea and dysentery (**Singh R, 1983; Manickam *et al.*, 1998; Anonymous, 1952; Lin *et al.*, 2002 and Khan *et al.*, 1988**)

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Fig. -1.5 *Euphorbia thymifolia*

Fig. -1.6. *Euphorbia thymifolia* cultivated

3). *Pavetta indica*

Family – *Rubiaceae.*
Local Name : Pavetta , Pawatta
English name- Indian Pellet
Ayurvedic name- Pawatta Mul
Scientific name - *Pavetta indica*

Pavetta indica Linn. (*Rubiaceae*) (fig. 1.7 - 1.8) is distributed throughout the greater part of India, ascending to an altitude of 1500 m in the Himalayas. It occurs throughout Ceylon, S. China and N. Australia. It is seen as an undergrowth of deciduous forest in all forest districts in India. It comprises about 400 species of shrubs or small trees in tropical and subtropical regions of the world (**Mabberley, 1987**). In India the genus is represented by about 30 species (**Santapau & Henry, 1972**).

The plant is an erect, nearly smooth or somewhat hairy shrub 2 to 4 m or more in height. The leaves are elliptic-oblong to elliptic-lanceolate, 6 to 15 cm long, and pointed at both ends. The flowers are white, rather fragrant, and borne in considerable numbers in hairy terminal panicles which are 6 to 10 cm long. The calyx segments are very small and toothed. The corolla-tube is slender and about 1.5 cm long, with obtuse lobes about half the length of the tube. The fruit is black when dry, somewhat rounded, and about 6 mm in diameter.

The plant leaves are used in the treatment of liver dysfunction, pain of piles, urinary diseases and fever (**Kirtikar & Basu, 1933 and Thabrew *et al.*, 1987**). The roots of the plant together with dried ginger are given with water in case of dropsy. The leaves of plant are useful in relieving the pain of piles (**Nadkarni, 1998**). Methanolic extract of plant leaves have been reported antipyretic and anti-inflammatory activity (**Mandal *et al.*, 1998**). The plant is used for the preparation of important crude drug without isolation of target phytochemicals.

Fig.-1.7 *Pavetta indica*

Fig. -1.8 flower of *Pavetta indica*

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PHYTOCHEMICAL INVESTIGATION

INTRODUCTION AND CHEMICAL REVIEW OF PLANTS

Plants are probably the best cell factories on this planet from which more than 100 000 low molecular secondary metabolites have been discovered, with the estimated total number in plants exceeding 5,00,000 (**Hadacek, 2002**). Plant metabolites are not only used for food purposes but also serve as an important historical source of medicines.

Plant chemicals are classified as primary and secondary metabolites. The primary constituents include common sugar, proteins, amino acids and nucleic acids. They are obtained directly by the photosynthetic process in plants, whereas, secondary constituents of plants make up the entire set of remaining plant chemicals from alkaloids, terpenoids, flavonoids, coumarins, fumarocoumarins, aromatic and aliphatic compounds (**Monique *et al.*, 1999**). Secondary metabolites play an ecological role, they are pollination attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against micro organisms. Secondary metabolites are synthesized in specialized cell types at distinct developmental stages but in small quantities making extraction and purification difficult. Examples of commercially useful plant secondary metabolites are nicotine and rotenone which are used in limited quantities as natural pesticides. Certain steroids and alkaloids are used in drug manufacturing by pharmaceutical industry.

Biosynthesis of secondary plant metabolites

The main stream of secondary metabolism is outlined in chart-2.1. One remarkable feature is that most of the metabolites originate from a very limited number of precursors. They are the link to primary metabolism in which they

also play an important role. Acetic acid has a central position in the form of its thioester acetyl coenzyme A. It is produced in the cell from pyruvic acid or fatty acid or it may be directly formed from acetate and coenzyme A, with ATP as mediator. From acetic acid, mevalonic acid is derived from which via 3-Methyl-1-phosphoroso-but-2-ene, the terpenoids are formed. From carbohydrates, shikimic acid is derived which is the key to a wealth of aromatic amino acids as precursors of the great variety of nitrogen containing compounds. Flavonoids are derived from a polyketide (three acetate units) and a cinnamic acid (shikimic acid). The indole alkaloids come from shikimate and monoterpene (loganins).

Quality and quantity of secondary metabolites with respect of environmental factors

Quality and quantity of secondary metabolites vary greatly among plants and is reported to be influenced by environmental conditions **(Monique et al, 1999**). Effect of climatic factors like rainfall were observed in Ephedra species. Reports are also available about the influence of altitude and locations on alkaloid content (Ephedrine) in Ephedra intermedia, *E. major, E. gerardiana* (**Pandey, 2000**), and on intersite variation of phenolics in *Beutla nona* (**Eriico *et al.*, 2001**). Two types of plants growing in different habitats have been reported to exhibit difference in the quantum of physiologically active secondary metabolites, which should affect the quantities of crude drug (**Itho *et al.*, 1997**). Similarly, habitatwise phytochemical variation of *Echinacea angustifolia* is also reported (**Binns *et al.*, 2002**). Reports suggest that the relation between ecological conditions such as latitudes, annual precipitation, annual mean sunshine affect the flavonoid content of *Ginkgo biloba* leaves.

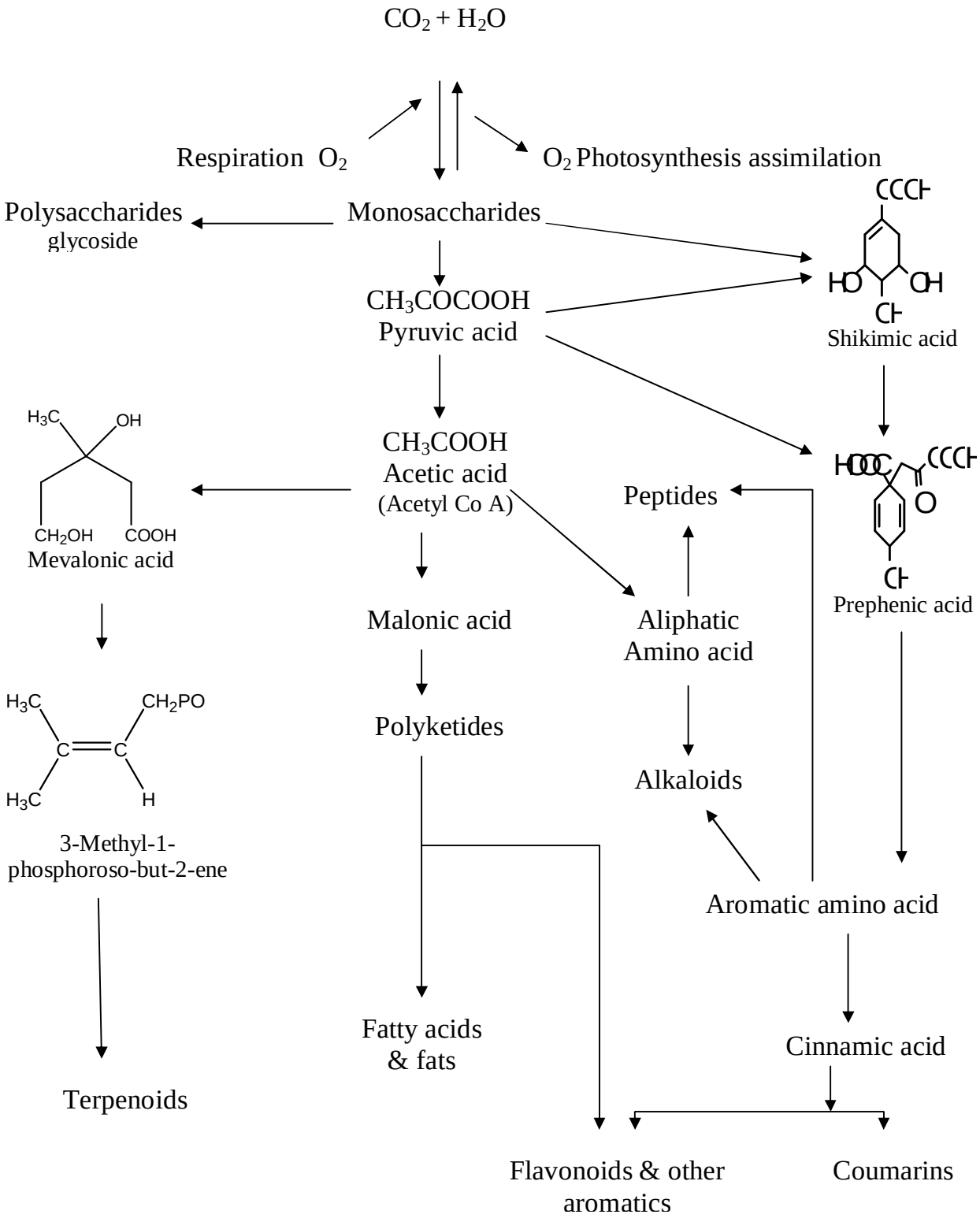


Chart -2.1 Main stream of Secondary metabolites (Torsell, 1983)

One of the largest groups of chemical arsenals produced by plants are alkaloids. Many of these metabolic by-products are derived from amino acids and include an enormous number of bitter, nitrogenous compounds. More than 10,000 different alkaloids have been discovered in species from over 300 plant families (**Raffauf, 1996**). Alkaloids often contain one or more rings of carbon atoms, usually with a nitrogen atom in the ring. The position of nitrogen atom in the carbon ring varies with different alkaloids and with different plant families. In some alkaloids, such as mescaline, the nitrogen atom is not within a carbon ring. In fact, it is the precise position of nitrogen atom that affects the properties of these alkaloids. Although they undoubtedly existed long before humans, some alkaloids have remarkable structural similarities with neurotransmitters in the central nervous system of humans, including dopamine, serotonin and acetylcholine. The amazing effect of these alkaloids on humans has led to the development of powerful pain-killer medications, spiritual drugs and serious addictions by people who are ignorant of the properties of these powerful chemicals.

Phenolics are one of the most important phytochemical groups. The plant phenolics are antioxidants in many food systems (**Mau *et al.*, 2001 and Kahkpmem *et al.*, 1999**). Through the scavenging of free radicals or the quenching of radical reactions responsible for lipid rancidity, phenolics prevent food deterioration (**Shahidi & Naczk, 1995**). Many phenolics show both antioxidant activity and antimutagenicity and are functional food components possessing health benefits or being able to prevent diseases in human beings (**Yen & Chan, 1995**).

Plants phenolics are present in free ester and in insoluble bond form (**Shahidi & Naczk, 1995**, **Krygier *et al.*, 1982**). Free and esterified phenolics are soluble in some plants (**Krygier *et al.*, 1982**). Hydrolysis will convert the esterified

phenolics to simple phenolic acids. Plant phenolics include a great diversity of compounds such as simple phenols, phenolic acids, coumarins, flavonoids, tannins and lignins (**Robinson, 1963; Ribereau-Gayon, 1972 and Harborne, 1964**). Tannins comprise a large group of natural products widely distributed in plant kingdom. They have a great structural diversity but are usually divided into two basic groups the hydrolysable and the condensed type. Hydrolysable tannins include the commonly occurring gallic acid and ellagic acid. Now, the condensed tannins also have an important medicinal role, such as stable and potent antioxidants. In China, tannins containing substances, such as galls, pomegranate rinds and terminalia fruits are used in several medicinal preparations.

Tannins, commonly referred to as tannic acid, are water soluble polyphenols that are present in many plant foods. They have been reported to be responsible for the decrease in food intake, growth rate, feed efficiency, net metabolizable energy and protein digestibility in experimental animals. Therefore, food rich in tannins is considered to be of low nutritional value.

Saponins are thought to play an important role in various phenomena, which are of relevance for food technologists and nutritionalists. Saponins can bind to protein, which seems to increase their stability against heat denaturation; the sensitivity of protein to proteases is decreased when saponins are attached to proteins. The bitter taste of saponins is sometimes regarded as a problem, but there is also a range of health promoting effects that have been attributed to saponins (cholesterol-lowering, anti-viral, antimutagenic). It has also been suggested that saponins could interact with bile salts in small intestine, which may be an important factor in digestion of fat.

Terpenoids constitute one of the largest groups of natural products. More than 30,000 representatives from all biological kingdoms including a large number of

medically important compounds such as vitamins, hormones and cytostatic agents have been described in the literature (**Sacchettini & Poulter, 1997**). The plethora of terpenoid compounds is biosynthetically assembled from only two simple precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). The biosynthesis of these universal intermediates from acetyl-CoA via mevalonate has been elucidated by the classical studies of Bloch, Cornforth and Lynen using yeast and animal cells (**Qureshi & Porter, 1981; Bloch, 1992; Bach, 1995 and Bochar *et al.*, 1999**). For several decades, the mevalonate pathway was considered as a unique biosynthetic route conducing to IPP and DMAPP. The existence of a second isoprenoid pathway was discovered by the research groups of Rohmer and Arigoni in the course of stable isotope incorporation studies using various eubacteria and plants (**Rohmer *et al.*, 1993; Broers, 1994 and Schwarz, 1994**). These data suggest that pyruvate and triose phosphate serve as precursors for the formation of IPP and DMAPP via an alternative pathway (**Eisenreich *et al.*, 1998; Rohmer, 1999; Schwarz & Arigoni, 1999 and Lichtenthaler, 1999**).

Essential oils are valuable natural products, which are used as raw materials in many fields including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition (**Buchbauer, 2000**). Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure diseases, infections and indispositions by means of inhalation (**Buchbauer *et al.*, 1993**). This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects. Hopefully, this will lead to a new information on plant applications and new perspective on the potential use of these natural products.

Major classes of plant terpenoids

C_n	Class	Parent Unit	Sub. Group	Occurrence
5.	hemiterpenoids	IPP	tuliposides	essential oil
10.	monoterpenoids (hydrocarbon oxygenated)	GPP	iridoids	essential oil
15.	sesquiterpenoids (hydrocarbon and oxygenated	FPP	abscisic acid	essential oil, resins
20.	diterpenoids (non – oxygenated and oxygenated)	GGPP	gibberellins clerodanes	resins, bitteo - heart wood.
25.	Sesterterpenoids non- oxygenated and oxygenated.	GFPP	tiglianes	resins, bitteo- heart wood.
30.	triterpenoids.	squalene.	phytostereol, phytoecdysteroid, cardenolides, cucurbitacins saponins.	resin heart wood, latex
40.	tetraterpenoids.	phytone	carotenoids, xanthophylls.	pigments green tissue, latex fruit latex root
10^3-10^4	polyterpenoids	GGPP	rubber, balats, gutta.	latex root

GFPP – Geranyl farnesyl pyrophosphate, **IPP -** Isopentenyl pyrophosphate, **GPP -** Geranyl pyrophosphate, **FPP -** Farnesyl pyrophosphate, **GGPP -** Geranyl geranyl pyrophosphate.

The terpenoids form a group of compounds, majority of which occur in the plant kingdom. Structurally terpenoids are derived from isoprene units

(CH_2=C(CH_3)CH=CH_2) joined together generally in head to tail fashion. The simpler mono ($C_{10}H_{16}$) and sesqui ($C_{15}H_{24}$) terpenoids are the chief constituents of essential oils. These are the volatile oils obtained from the sap and tissues of certain plants and trees. The di and tri- terpenoids which are not steam volatile are obtained from plant tree gums and resins.

Individual compounds are more frequently used than oils, as such in medicinal preparations. Some widely used terpenoids in medicine preparation are:

Terpenoids	**:**	**Uses**
Camphor	:	Counter irritant.
Bornyl isovalerate	:	Sedative.
Menthol	:	In cough drops.
Terpinyl hydrate	:	Expectorant.
Chamazulene	:	Anti-inflammatory agent (**Brud *et al.*, 1989**).
β- Caryophyllene	:	Non-sterioda1 anti- inflammantory agent (**Hitronietal, 1996**)

Biosynthesis of Terpenoids

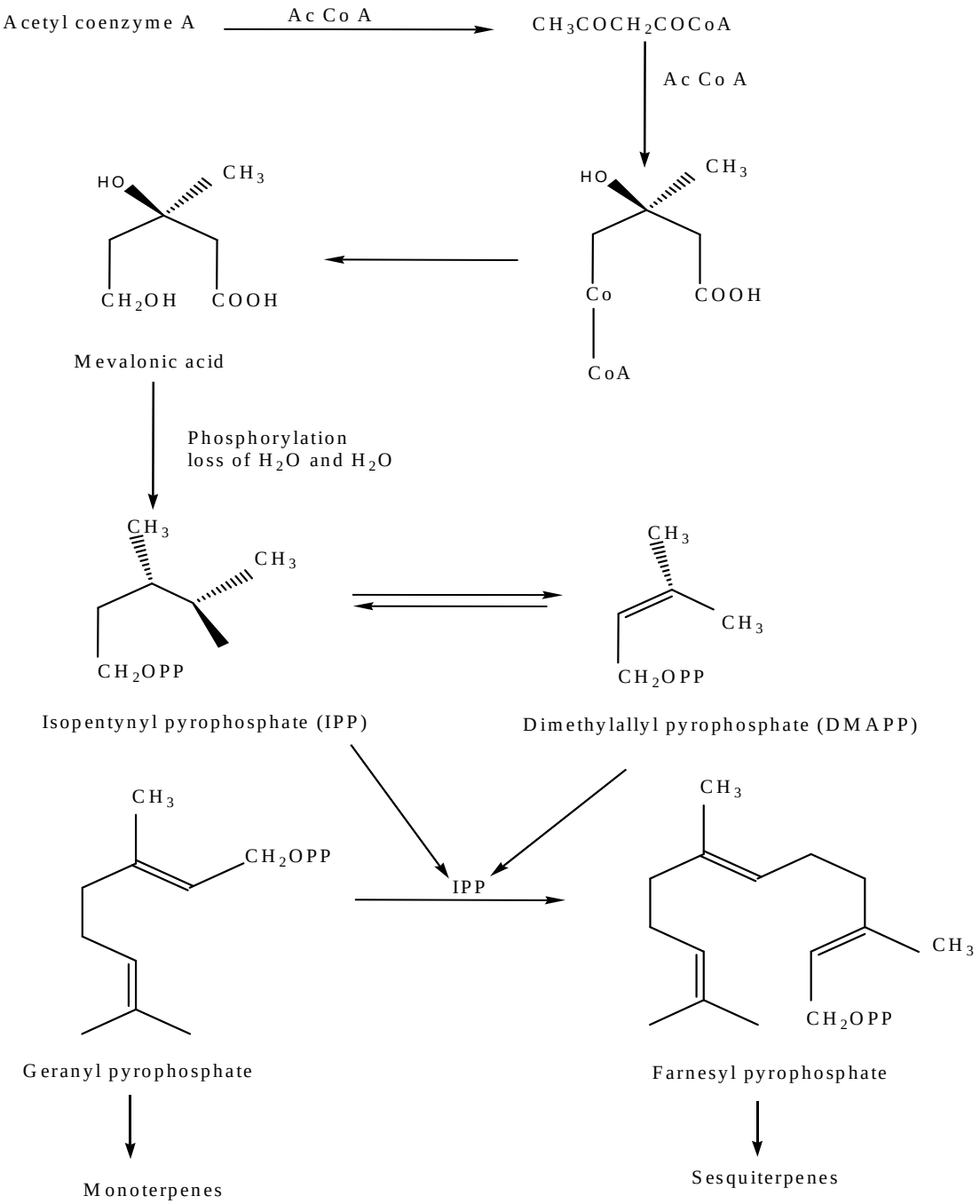


Chart 2.2- The mevalonate pathway of terpene biogenesis

Role of essential oils in plants

Sexual attractants: It has been reported that essential oils containing germacrene –D from the members of Compositeae and Rutaceae show activity like sex hormones. Eugenol and methyl eugenol have also been reported as attractants (**Ladd *et al.*, 1983; Allsopp *et al.*, 1991; Hee *el al.*, 1998 and Silva *et al.*, 2003**).

Defense and alarm compounds: Geraniol, nerol, geranyl acetate etc., act as defense secretions in *Centris adani* (bee) (**Vinson *et al.*, 1982**) and d- limonene α, β-pinene and β-myrcene have been reported as alarm and defense pheromone in *Myrmicaria natalensis.*

Antifeedant, repellant and antimicrobial: *Polygonam hydropiper* containing polygodial shows antifeedant property (**Beak *et al.*, 1986**). *Zanthoxyllum bungeanum* oil containing piperitone, terpinen-4- ol and linalool as active principles shows repellant activity against insects **(Bowers *et al.*, 1993**). Citronellol, β-pinene, isoborneol and p-cymene, have been reported to possess good insect repellent activity **(Tominaga *et al.*, 1984**). It has been reported that p-menthane, the active component in the essential oil of *Mentha puleleginum* and *Mentha spicata* shows antimicrobial activity.

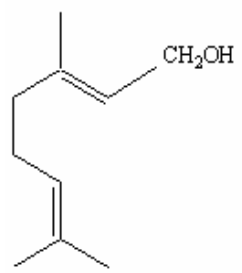
Growth and development : Sesamin, seamolin, juvocimene and juvadicene are reported in the essential oil of many plant families as juvenile hormones (**Bowers, 1991**) whereas sesquiterpenes, precocene I and II found in the oil of *Agevatum houtoniatum* are anti-juvenile hormones. Essential oil of *Citrus reticulata* shows toxic and growth inhibiting behaviour on *Culex quinquefasciatus.*

Social behaviour : *Teryphodendrane lineatum* containing a monoterpene (+)-lineatin has been reported as an aggregation pheromone (**Charlwood *et al.*, 1991**).

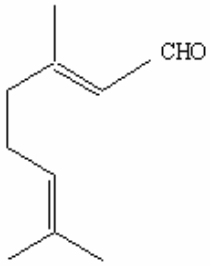
Allelopathy : Several chemicals are involved in plant–plant interaction and are called as allelopathic toxins or allelochemicals. The secondary plant metabolites are relatively low or medium molecular substances with usually simple structures. The volatile terpenoids or phenolic compounds which are extracted from leaf, stem, flower and roots are commonly associated with allelopathic activity. 1,8-cineole (eucalyptol) has been reported as a well known plant allelochemical (**Zhen *et al.*, 1996)**.

Guenther in his classical work "The Essential Oils, the Essential Constituents of Essential oils" presented a detailed account of the constituents in volatile oils. This appears to be early yet near complete description. He listed the following class of compounds in volatile oils:

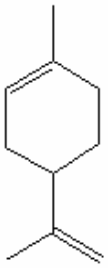
i) hydrocarbons, ii) alcohols, iii) aldehydes, iv) ketones, v) phenols and phenolic ethers, vi) quinones, vii) acids, viii) esters, ix) lactones, coumarins, x) furans, xi) oxides, xii) compounds containing nitrogen and sulphur.



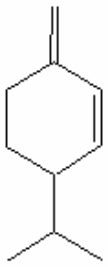
Geraniol



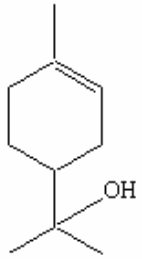
Citral



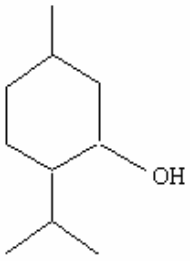
Linonene



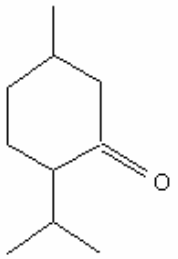
β-Phellandrene



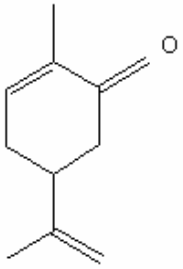
α-Terpineol



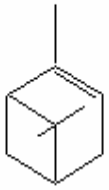
Menthol



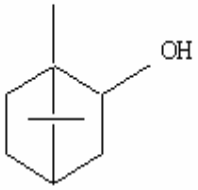
Menthone



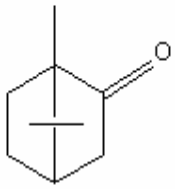
Carvone



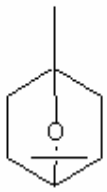
α-Pinene



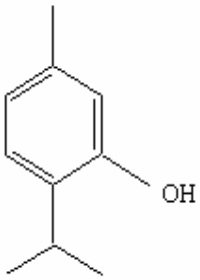
Borneol



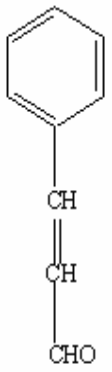
Camphor



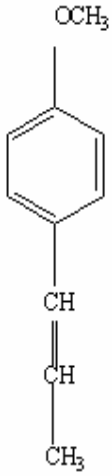
Cineole



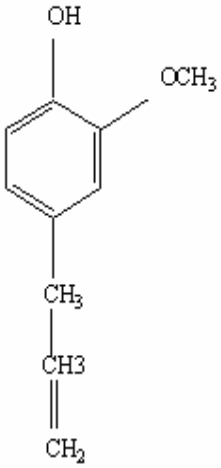
Thymol



Cinnamaldehyde



Anethole



Eugenol

Phytochemical review of plants

Soyeed *et al.*, (2002) isolated prenylated isoflavone, 5-methoxy-4-hydroxy-2, 2-dimethylpyrano (3",3",7,8) isoflavone from *Pouzolzia indica*.

Gupta and Garg, (1996) isolated myricyl alcohol, taraxerol and tirucallol from petroleum ether (B.P. 40-60 ^{0}C) extract and hentriacontane from ethanolic extract of *E. thymifolia*. **Baslas and Agrawal, (1980)** isolated 12-deoxyphorbol-13, 20- diacetate, 12-deoxy β-OH phorbol-13-dodecanoate 20-acetate, and 1-hexacosanol compoun from petroleum ether extract of *E. thymifolia*. **Agrawal and Baslas,** (**1981**) isolated epiteraxol, n-hexacosanol, euphorbol, 24-methylene cycloartenol, 12-deoxy 4β-OH phorbol-13-dodecanoate-20-acetate, 12-deoxy 4β-OH phorbol-13-phenylacetate- 20-acetate and quercetin -3 β-galactoside from petroleum ether and ethanolic extract of *E. thymifolia*. **Gupta and Garg, (1996)** isolated a new hydrolysable tannin named "Isomallotinic acid" in addition to 15 known tannins from the *E. thymifolia*. **Lee *et al.*, (1990)** reported 3-*O*-galloyl-4,6-*(S)*-HHDP-*D*-glucose, 1,3,4,6-tetra-*O*-galloyl-ß-*D*-glucose and rugosin B from *Euphorbia thymifolia*.

Balde *et al.*, (1991) isolated (+)-catechin (0.015%), (-)-epicatechin (0.010%), (+)-epicatechin, proanthocyanidin A-2 (epicatechin-(4β→8, 2β→O-7)-epicatechin), proanthocyanidin A-4 (epicatechine-(4β→8, 2 β →O)-ent-catechin) from EtOAc extract of Pavetta owariensis. **Balde *et al.*, (1995)** isolated pavetannins C-2 – C-6, five tetramaric proanthocyanidin, containing one or two double inrerflavanoid (A type) linkage, which have been isolated from stem bark of *Pavetta owariensis*.

MATERIALS AND METHODS

The selected plants for the present study were collected from different localities in different seasons. The name of locality, plant parts used and months when these materials were collected are as follows:

1. ***Pouzolzia hirta*:** Rhizomes of the plant were collected from Rai, Pithoragarh Distt. in the month of March.

2. ***Euphorbia thymifolia:*** Aerial parts of the plant were collected from the surroundings of Pithoragarh in the month of July.

3. ***Pavetta indica:*** The leaves of the plant were collected from Thal, Pithoragarh Distt. in the month of July-August.

The plants were first identified in the Department of Botany, Kumaun University, Nainital and then at B.S.I., Dehradun. The voucher specimen was deposited in the Herbarium section at B.S.I., Dehradun. The voucher no. 112173 for *Pouzolzia hirta* (Blume) Hassk and 112173 for *Pavetta indica*. *Euphorbia thymifolia* was matched with specimen 17195 at B.S.I. Dehradun. The collected plant materials were first washed with cold water to remove the soil particles and then shade dried. The dried material was finely powdered in the grinding machine and weighed in a rough balance.

2. Extraction

The finely powdered plant material was extracted, in a Soxhlet extractor with ethanol for about 16 h. The extract was cooled and concentrated under reduced pressure till a residual mass was obtained. Residue was chromatographed on silica gel column and eluted with variety of solvent systems with increasing polarity. Various fractions were analyzed further.

For the separation of essential oil, fresh plant material was cut into pieces and then subjected to hydro-distillation. The essential oil, from the distillate was obtained by extraction with n-hexane. The oil was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The oil thus obtained, was analyzed by GC-MS.

GENERAL EXPERIMENTAL PROCEDURE

Column chromatography: Column chromatography was used for the isolation of steroids, essential oil and triterpenoids.

A glass column was first washed with chromic acid and then dried in oven. It was adjusted vertically on the stand. A glass wool was fixed at the bottom. This column was packed with slurry of silica gel in n-hexane. The column was left overnight so that the adsorbent could settle.

The essential oil was adsorbed at the top of the column. It was then run with a variety of solvents starting from non-polar to polar one. The column was first run with n-hexane. Then the polarity was increased by adding to it small fractions of increasing volumes of solvent which lied next to it in the eluotropic series of solvents.

Eluotropic series of solvents

n-hexane	acetone
carbon tetrachloride	n-propanol
trichloroethylene	ethyl alcohol
benzene	methanol
dichloromethane	formamide
chloroform	water
diethyl ether	pyridine
ethyl acetate	acetic acid

Elution with different solvents was continued till the column was finally eluted with methanol. For the separation of essential oil, the column was first run with n-hexane and finally with ethyl acetate. Small fractions (each 25 ml) were collected in the beakers. The purity of the collected fractions was examined by TLC. Similar fractions were mixed. These isolated compounds were identified by different specific chemical and spectroscopic methods.

Thin layer chromatography: TLC plates were prepared by applying a slurry of silica gel G (Merck) in water on glass plates by using spreader to get homogeneous layers. The ratio of adsorbent to water was 1:2 w/v. These plates were dried at room temperature and activated by heating at 105^0C for 30 minutes in the oven.

Fractions were applied on the plates, in the form of fine drops by thin capillaries and dried in a current of air from blower. These spotted plates were developed in variety of solvent systems and dried at room temperature. Now these plates were examined in day light as well as under long range UV light and the positions of different spots were marked and noted down. The plates were then sprayed with different spraying reagents and treated according to the conditions

of reagents. The position of the spots which appeared after spraying was also noted down.

Different solvent systems were used for the best resolution of various classes of compounds.

Paper chromatography: Descending chromatography with 1-D method were applied on the Whatmann No. 1 and Whatmann No. 3 chromatographic filter paper sheets, for various classes of compounds. First of all paper strips were washed with 2% of (2N HCl) to remove impurity. The solution to be analyzed was spotted by means of capillary on a line, 8 cm distance and parallel from one edge of the filter paper strip at the intervals of 6 cm. After drying the spots, the paper sheet was folded sharply along a line 2.5 cm from the edge. Now paper sheets were fixed in a glass trough with the help of supporting glass tubes, along a 2.5 cm line. Now the solvent was added to the trough and the chamber was closed.

High performance liquid chromatography: High performance liquid chromatography was used for the separation of vitamins, carotenoids from the extracts of the plants. The method used is given below:

Detector : 1) SPD-10 AVP

Variable wavelength (190-750 nm)

UV- Vis detector.

Injector : 20 µl loop-manual Injector

Pump : LC- 10 AT vp.

Column: C_{18} Phenomenex® (150x4.60 nm,)and pore size 5 µm column.

Solvent Flow program: Isocratic.

Recorder and Printer : PC HCL- P-4 and laser 2100 series.

Gas chromatography: Gas chromatography is basically a separation technique in which components of vaporized sample are separated. Gas chromatography is mainly based on the distribution of components between the mobile phase and the stationary phase.

The partition ratio depends upon the nature of solute, stationary phase and temperature. Depending upon the partition ratio, the components distribute themselves between mobile and stationary phase. The retention time depends upon nature and amount of stationary phase, temperature of column and flow of gas. The area under peak is proportional to concentration of component and thus provides a basis for quantitative analysis. The gas chromatographs were recorded on Nucon 5765 model, Rtz-5 column (30m x 0.32mm i.d., 0.25µm film) under temperature programme 60^{o} C to 210^{o}C at 3^{o}C per minute rise. N_2 is used as carrier gas at 4.0. Kg/cm^2 outlet pressure. Injection volume 0.5µl was prepared in hexane.

SPECTROSCOPIC METHODS:

Ultra violet spectroscopy: All UV spectra were measured in a double beam UV visible spectrophotometer model UV5704-ss using methanol as solvent.

FT-IR Spectroscopy: Bruker model Vertex 70 was used for determining the FT-IR spectra. The sample crystals were mixed with solid KBr.

Mass spectroscopy: Mass spectroscopy is a powerful analytical technique for determining molecular weight, molecular formula, quantitative and qualitative determination of trace and ultra trace level of organic compounds in view of its high sensitivity, selectivity and specificity.

Many ionization methods are available and each has its own advantages and limitations.

APCI : Atmospheric pressure chemical ionization.

CI : Chemical ionization.

EI : Electron impact.

ESI : Electron spray ionization.

FAB : Fast atom bombardment.

FD/FI : Field desorption/Field ionization.

MALDI : Matrix assisted laser desorption ionization.

TSP : Thermospray ionization.

GC-MS: The gas chromatography coupled with mass spectrometry using 17A – Shimadzu interfaced with QP5050A ion trap mass spectrometer using Rtx-wax column (30m X 0.25 μm film coating). Helium was used as a carrier gas (1.5 ml/min flow). The ionization energy (70ev). The oven temperature was programmed 40 ^{0}C for 4 minutes and then rise 40 ^{0}C to 230 ^{0}C at the rate of 3^{o}C per minute, and final holding times was 10 minutes.

Nuclear Magnetic Resonance Spectroscopy

A wide variety of nuclei including ^{1}H, ^{13}C, ^{15}N, ^{19}F and ^{51}P have suitable magnetic properties and can be studied by ^{1}HNMR spectroscopy ^{1}H-NMR and ^{13}C-NMR are common for organic molecules.

COSY : A simple 2D experiment includes cosy (^{1}H- ^{1}H COSY). It is interpreted through off diagonal cross peaks for all protons.

DQF-COSY: It stands for "Double quantum filtered cosy".

TOCSY: TOCSY is the abbreviation for "Total correlation spectroscopy."

NOESY: "Nuclear overhauser enhancement spectroscopy".

ROESY: One in the rotating frame.

HMQ: " Hetero-nuclear multiple quantum correlation"

HMBC: "Hetero-nuclear multiple bond correlation."

HETCORE: It is termed as ^{1}H-^{13}C COSY. In a HETCORE spectrum, one can correlate ^{13}C nuclei with directly attached protons.

DEPT: "Distortionless enhancement polarization transfer" A DEPT spectrum is used to distinguish among CH_3, CH_2 and CH.

^{1}H Nuclear Magnetic Resonance Spectroscopy: The proton magnetic resonance spectra were measured in $CDCl_3$ using TMS as an internal standard in 400 MH_z JEOL NMR instrument.

^{13}C Nuclear Magnetic Resonance Spectroscopy: ^{13}C magnetic resonance spectra were measured in $CDCl_3$ using TMS as an internal standard in 400 MH_z JEOL NMR instrument.

Crystallization: The compounds isolated in the pure form were crystallized in different solvent systems. A few compounds were self crystallized in the solvent in which they were eluted. Mostly compounds were crystallized in chloroform, ethyl acetate and methanol.

Identification: The isolated pure compounds were identified by means of melting point. Mixed melting point, Co-chromatographying with authentic samples, specific chemicals and spectral methods.

Melting Points and Mixed Melting Points: Melting points were determined in H_2SO_4 bath, mixed melting points were determined by mixing the pure compounds with authentic samples.

RESULTS AND DISCUSSION

Phytochemical investigation of *Euphorbia thymifolia*

The finely powdered leaves of *E. thymifolia* were extracted in a Soxhlet apparatus with petroleum ether for about 16 h. The extract was cooled and concentrated under reduced pressure till a residual mass was obtained. The residual plant material was further extracted with 90% ethanol for 16 h. The extract was collected, dried under reduced pressure and partitioned with ethyl acetate: water. The ethyl acetate extract was collected and dried under reduced pressure and then it was subjected to column chromatography and eluted with variety of solvent systems with increasing polarity starting from petroleum ether, ethyl acetate, benzene and acetone. The detailed procedure is given in the chart 2.3.

COMPOUND – ETF-1

On eluting column with benzene: ethyl acetate (50: 50), yellow amorphous solid was obtained and it was purified by column chromatography and preparative TLC. It formed yellow needle shaped crystals (m. p. 315-316 ^{0}C) on recrystallization with ethyl acetate: petroleum ether (80: 20).

Colour reactions- The compound gave the following colour reactions:

The compound gave olive green colour with alcoholic ferric chloride, which showed the presence of phenolic group in the compound. The alcoholic solution of the compound gave red colour with magnesium and hydrochloric acid indicating the presence of flavonoid skeleton (**Shinoda, 1928**).

Extraction of *Euphorbia thymifolia* leaves

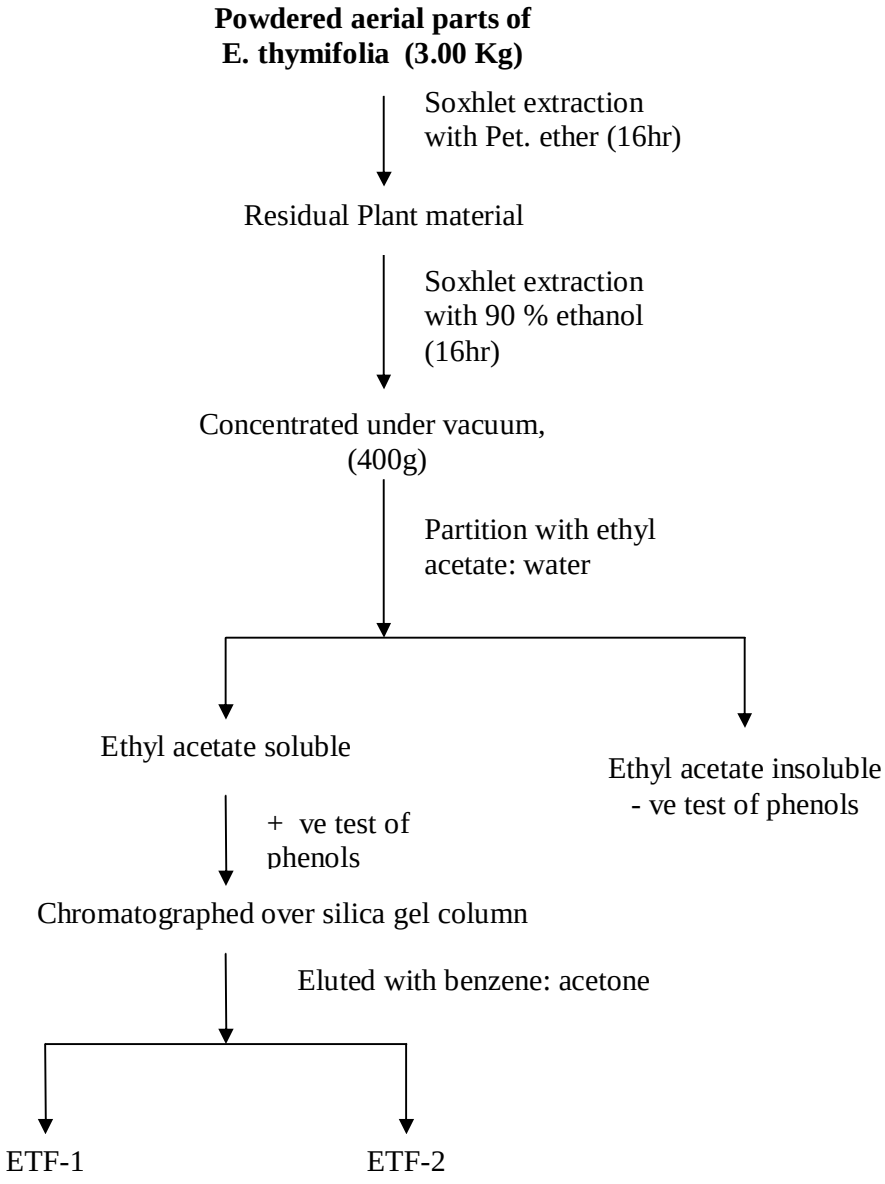


CHART- 2.3

Elemental analysis – Elemental analysis of the compound showed C - 59.65, H - 4.01 and O -36.34 %.

Spectral Studies of Compound ETF-1

UV spectra λ_{max} MeOH - 378, 329, 349, 358, 373, 275 nm.

Its UV spectrum showed λ_{max} at 378, 358, 329 and 353 nm, indicating its polyphenolic nature and addition to this in methanol it showed an absorption at 275 nm (band II) and 349 (band I) nm. The UV spectra of the compound ETF-1 is shown in fig-2.1.

FT-IR spectra - 3407,3325, 1666, 1611, 1562,1450, 941,721 cm^{-1}.

The IR spectrum of the compound exhibited absorption band at 3407 cm^{-1} for hydroxyl group, 1666 cm^{-1} for carbonyl group and 1562 cm^{-1} for unsaturation. The FT-IR spectra of the compound ETF-1 is shown in fig-2.2.

MS m/z: 304, 275, 165, **153**, 123, 77, 69, 51, 41.

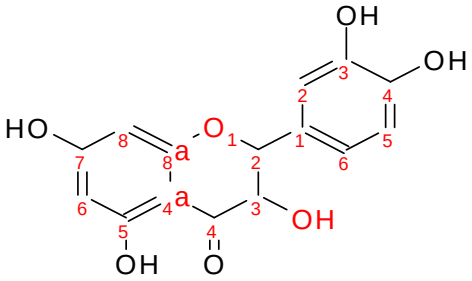
The MS of the compound showed M^+ ion peak at m/z 304 corresponding to the molecular formula $C_{15}H_{12}0_7$. The MS spectra of the compound ETF-1 is shown in fig-2.3.

^{1}H NMR ($CDCl_3$ 400 MHz) : The ^{1}HNMR spectrum (Table-2.1) showed the signals at δ 6.63 a doublet with integration of one proton, another doublet at δ 6.75 with integration of one proton assigned for proton at H-6 and H-8 respectively, suggesting the presence of hydroxyl group at C-5 and C-7 position. The doublet at δ 6.82 with integration of one proton, another doublet at δ 6.79 with integration of one proton and singlet at δ 7.29 with integration of one proton assigned for proton at H-5`, H-6` and H-2` respectively, suggesting the presence of hydroxyl group at C-3` and C-4` position. All these data suggesting

typical for 3`, 4`- disubstituted ring. Another pair of doublet at δ 3.45 and δ 3.16 (J= 8.44 Hz) was the characteristic of vicinal protons at C-2 and C-3 of 3-hydroxy flavanone having trans-diaxial stereochemistry. Singlet at δ 3.88 and δ 1.80 showed the presence of phenolic protons, alcoholic proton and phenolic protons respectively. The ^{1}HNMR spectra of the compound ETF-1 is shown in fig-2.4.

Table 2.1: ^{1}H NMR Spectral data of ETF-1.

Chemical Shift (δ)	Proton Count	Multiplicity of signals	Probable assignments
7.29	1	d (J=0.75 Hz)	H-2`
6.92	1	dd (J=8.20, 2.73 Hz)	H-6`
6.82	1	d (J=8.20 Hz)	H-5`
6.75	1	d (J=2.73 Hz)	H-8
6.63	1	d (J=2.73 Hz)	H-6
3.45	1	d (J=8.44 Hz)	H-2
3.16	1	d (J=5. 57 Hz)	H-3
3.88	4	s phenolic protons 4H	
1.80	1	s aliphatic alcoholic 1H	



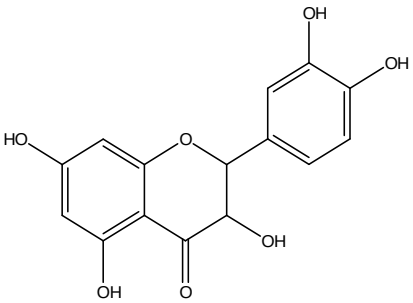
^{13}C NMR ($CDCl_3$ 400 MHz): The ^{13}C NMR spectra (Table-2.2) displayed signals for 15 carbons, corresponding to the molecular formula $C_{15}H_{12}O_7$.

Finally ETF-1 was characterized by comparison of its spectral data with those reported in literature (**Braune, 2001**).

Table 2.2 ^{13}C NMR spectral data of ETF-1

Chemical shift (δ)	Probable assignments	Nature of carbon
78.24	C-2	Aliphatic
92.59	C-3	Aliphatic
169.69	C-4	Carbonyl
102.62	C-4a	Aromatic
136.41	C-5	Aromatic
94.79	C-6	Aromatic
166.55	C-7	Aromatic
93.69	C-8	Aromatic
132.58	C-8a	Aromatic
127.59	C-1`	Aromatic
118.70	C-2`	Aromatic
108.98	C-3`	Aromatic
127.42	C-4`	Aromatic
115.67	C-5`	Aromatic
119.73	C-6`	Aromatic

Based on the above chemical and spectral results, the following structural formula could be assigned for the compound ETF-1.



3`,3,4`,5,7-pentahydroxy flavanone (Taxifolin).

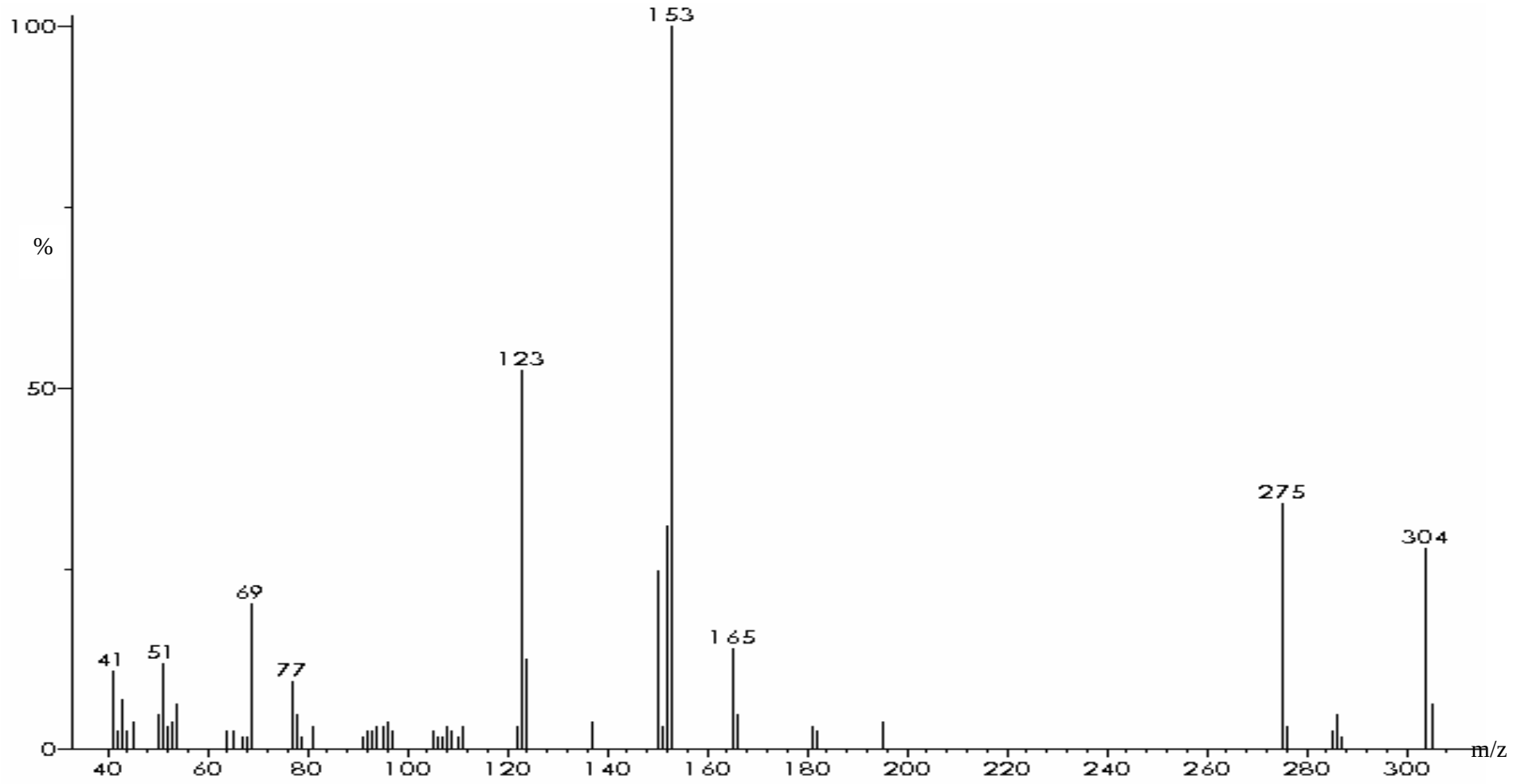


Fig. 2.3 MS spectra of ETF-1

COMPOUND – ETF-2

On eluting column with benzene: ethyl acetate after ETF-1, a yellow amorphous solid compound, m.p. 274 ^{0}C was obtained. It gave positive colour test for flavonoid as described above.

Elemental analysis - Elemental analysis of the compound showed C- 62.34, H- 3.42 and O- 34.24%.

Spectral Studies of Compound ETF-2

UV spectra λ_{max} MeOH - 275, 361, 341, 355, 332 nm.

Its UV spectrum showed λ_{max} at 361, 355, 341 and 332 nm, indicating its polyphenolic nature and addition to this in methanol it showed an absorption at 275 nm (band II) and 346 (band I) nm. The UV spectra of the compound ETF-2 is shown in fig- 2.5.

FT-IR spectra : 3316, 3188, 1662, 1612, 1568, 1440, 974, 722 cm^{-1}.

The IR spectrum of the compound exhibited absorption band at 3316 cm^{-1} for hydroxyl group, 1662 cm^{-1} for carbonyl group and 1612 cm^{-1} for unsaturation. The FT-IR spectra of the compound ETF-2 is shown in fig- 2.6.

MS m/z: <u>286</u>, 258, 229, 121, 93.

The MS of the compound displayed M^+ at m/z 286 corresponding to the molecular formula $C_{15}H_{10}O_6$. Other major mass fragments were observed at m/z 258, 229, 121, 93 indicating the presence of two hydroxyl groups on the ring A, one on the ring B and one at C-3 position of the flavonoid nucleus. The MS spectra of the compound ETF-2 is shown is shown in fig- 2.7.

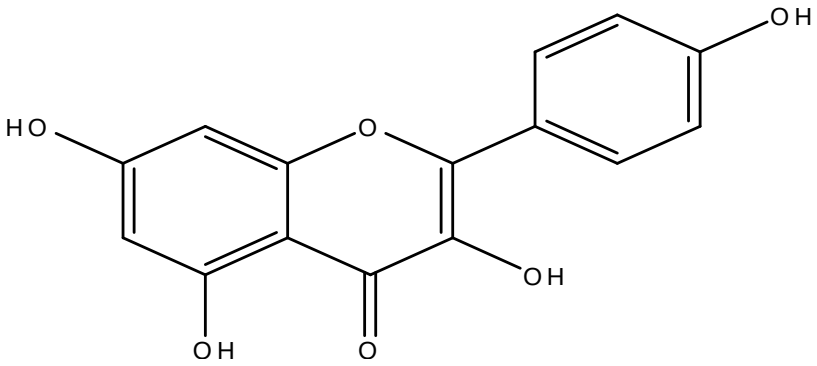
^{1}H NMR ($CDCl_3$ 400 MHz): The ^{1}H NMR spectrum (Table 2.3) of ETF-2 displayed two doublets at δ 6.25 (1H, J= 2.54) and δ 6.02 (1H, J= 2.0 Hz) for H-8 and H-6 protons respectively. Another two doublets at δ 7.38 (2H, J= 2.54 Hz) coupled with one more doublet at δ 6.56 (2H, J= 8.7 Hz) were typical of 4`-substitution on the ring with B indicated the presence of hydroxyl groups at C-4`. From the above mentioned data of compound, ETF-2 was suggested to be 3,4`,5,7-tetrahydroxy flavone (Kaempferol).

Table 2.3: ^{1}H NMR Spectral data of ETF-2.

Chemical Shift (δ)	Proton Count	Multiplicity of signals	Probable assignments
7.38	2	d (J=2.54)	H-2`, H-6`
6.56	2	d (J=8.7)	H-3`, H-5`
6.25	1	d (J=2.54)	H-8
6.02	1	d (J=2.00	H-6

^{13}C NMR ($CDCl_3$ 400 MHz): The ^{13}C NMR spectrum (Table-2.4) of ETF-2 and their multiplicity assignments also supported the structure. The compound was finally confirmed by comparison of its spectral data with those reported in literature (**Rao, 2007**).

Based on the above chemical and spectral results, the following structural formula could be assigned for the compound ETF-2.



3,4',5,7-tetrahydroxy-Flavone (Kaempferol).

Table 2.4: ^{13}C NMR spectral data of ETF-2

Chemical shift (δ)	Probable assignments
127.25	C-2
122.28	C-3
187.69	C-4
105.59	C-4a
160.61	C-5
97.25	C-6
165.79	C-7
96.61	C-8
156.29	C-8a
127.61	C-1`
130.29	C-2`, C-6`
115.72	C-3`, C-5`
156.69	C-4`

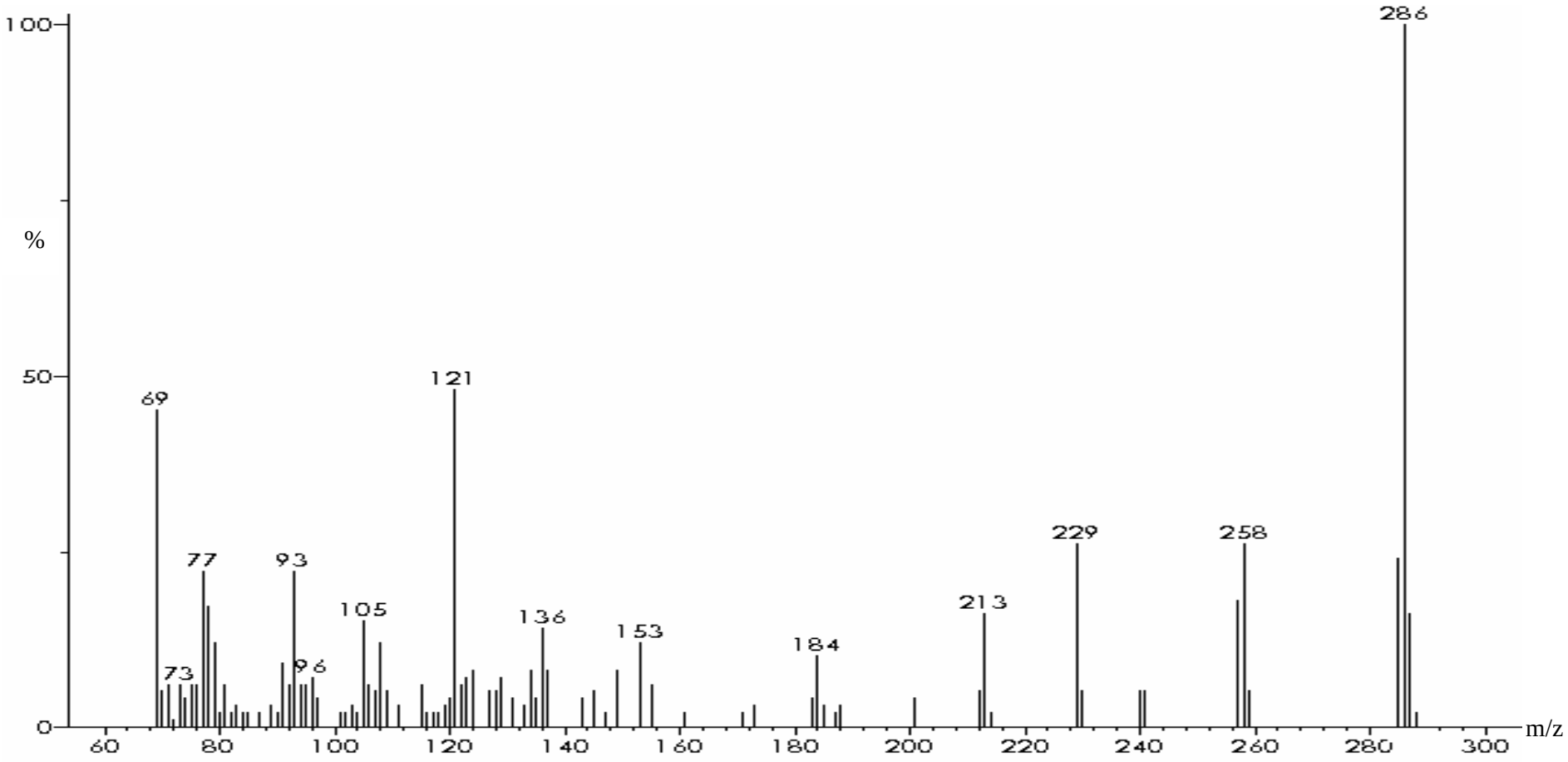


Fig – 2.7 MS spectra of ETF-2.

Phytochemical investigation of *Pavetta indica*

The leaves of *Pavetta indica* (3.0 kg) were extracted with MeOH (4L x 6) at room temperature for 24-48 h. The MeOH extract was evaporated under reduced pressure to yield dark-brown syrup. The MeOH extract (80.0 g) was chromatographed over silica gel (hexane/ethyl acetate /MeOH) to obtain 10 fractions. Every fraction was chromatographed over silica gel column and eluted with hexane, ethyl acetate and methanol. Fraction 10 was separated on silica gel column, eluted with ethyl acetate-MeOH (1:1) to obtain PI-1 (45 mg). The detailed procedure is given in the chart 2.4.

COMPOUND – PI-1

On eluting column with benzene: ethyl acetate, PI-1 formed a yellow amorphous solid compound, m.p. 308 ^{0}C.

Colour reactions - The alcoholic solution of the compound gave red colour with magnesium and hydrochloric acid indicating the presence of flavonoid skeleton (**Shinoda, 1928**).

Elemental analysis - Elemental analysis of the compound showed C- 62.63, H- 4.36 and O- 32.74 %.

Spectral Studies of Compound PI-1

UV spectra λ_{max} MeOH : 285, 272, 244, 229, 258,328, 321 nm.

The UV spectrum showed maxima at 272 nm band I, 328 nm band II and 321 nm band III for typical flavonoid. The UV spectra of the compound PI-1 is shown in fig- 2.8.

Extraction of *Pavetta indica*

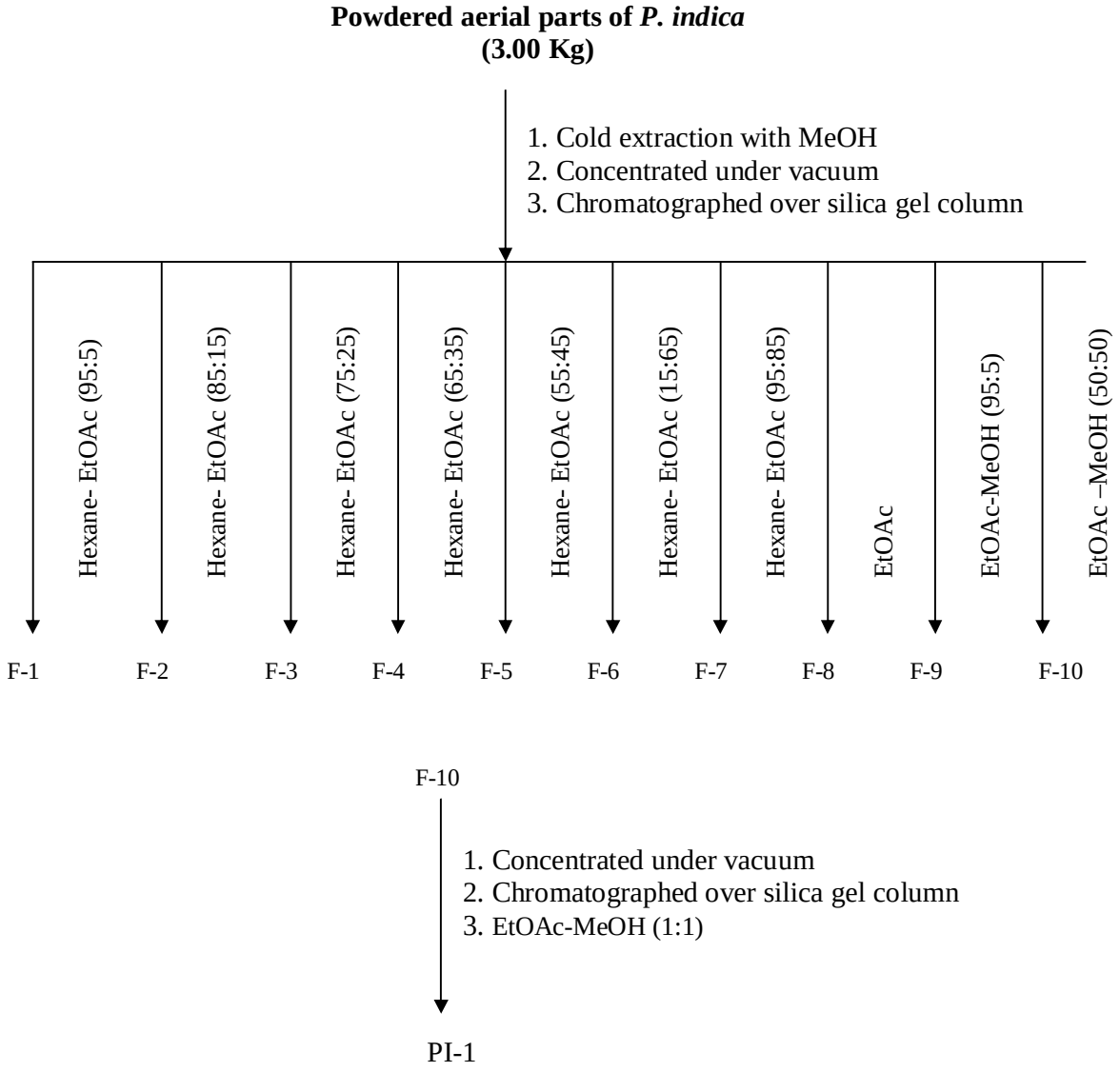


CHART 2.4

FT-IR spectra: 3377, 2919, 2850, 1630, 1605, 1520, 1455, 1029, 766 cm^{-1}.

The IR spectrum of the compound exhibited absorption band at 3377 cm^{-1} for phenolic group, 2919, 2850 cm^{-1} for - CH_3 str., 1630, 1605, 1520, 1455 cm^{-1} for unsaturation and aromatic ring. The FT-IR spectra of the compound PI-1 is shown in fig- 2.9.

MS m/z: 290, 152, **139**, 123, 77, 69, 55, 39.

The MS of the compound displayed M^+ at m/z 290 corresponding to the molecular formula $C_{15}H_{14}O_6$. The MS spectra of the compound PI-1 is shown in fig- 2.10.

^{1}H NMR ($CDCl_3$, 400 MHz): The ^{1}H NMR spectrum (Table 2.5) of PI-1 displayed overall 14 protons and their multiplicity assignments also support the structure. The compound was finally confirmed by the comparison of its spectral data with those reported in literature (**Yuko *et al.*, 1990**). The ^{1}H NMR spectra of the compound PI-1 is shown in fig- 2.11.

Table 2.5: ^{1}H NMR Spectral data of PI-1.

Chemical Shift (δ)	Proton Count	Multiplicity of signals	Probable assignments
7.27	1	d (J=1.62 Hz)	H-6`
7.26	2	d (J=8.20 Hz)	H-5`, H-2`
7.25	2	d (J=8.20 Hz)	H-6, H-8
1.64	1	d (J=2.73 Hz)	H-2
1.25	1	d (J=2.73 Hz)	H-3
0.85	1	d (J=8.44 Hz)	H-4
3.88	4	s phenolic protons 4H	
1.64	1	s aliphatic alcoholic proton 1H	

^{13}C NMR ($CDCl_3$, 400 MHz): The ^{13}C NMR spectrum (Table-2.6) of PI-1 and their multiplicity assignments also support the structure. The compound was finally confirmed by the comparison of its spectral data with those reported in literature (**Yuko *et al.*, 1990**). The ^{13}C NMR spectra of the compound PI-1 is shown in fig- 2.12.

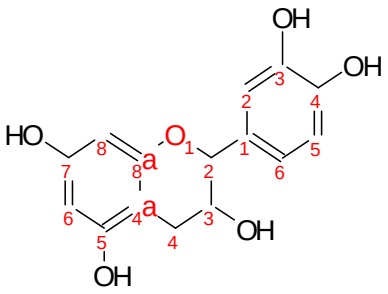
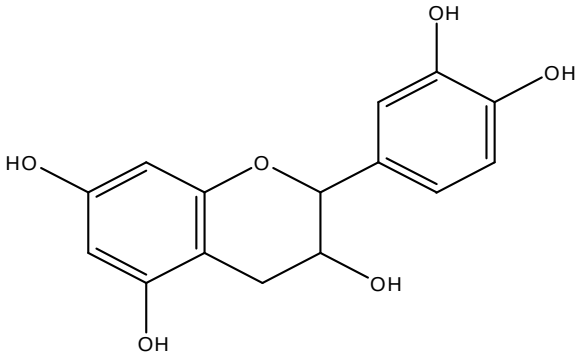


Table 2.6: ^{13}C NMR spectral data of PI-1

Chemical shift (δ)	Probable assignments
86.35	C-2
8240	C-3
21.42	C-4
102.95	C-4a
171.03	C-5
95.37	C-6
139.60	C-7
92.26	C-8
176.27	C-8a
119.74	C-1`
109.14	C-2`,
133.93	C-3`
127.96	C-4
102.99	C-5`
118.53	C-6`

Based on the above chemical and spectral results, the following structural formula could be assigned for the compound PI-1.



2-(3, 4-dihydroxy-phenyl)-chroman-3,5,7-triol (Catechin)

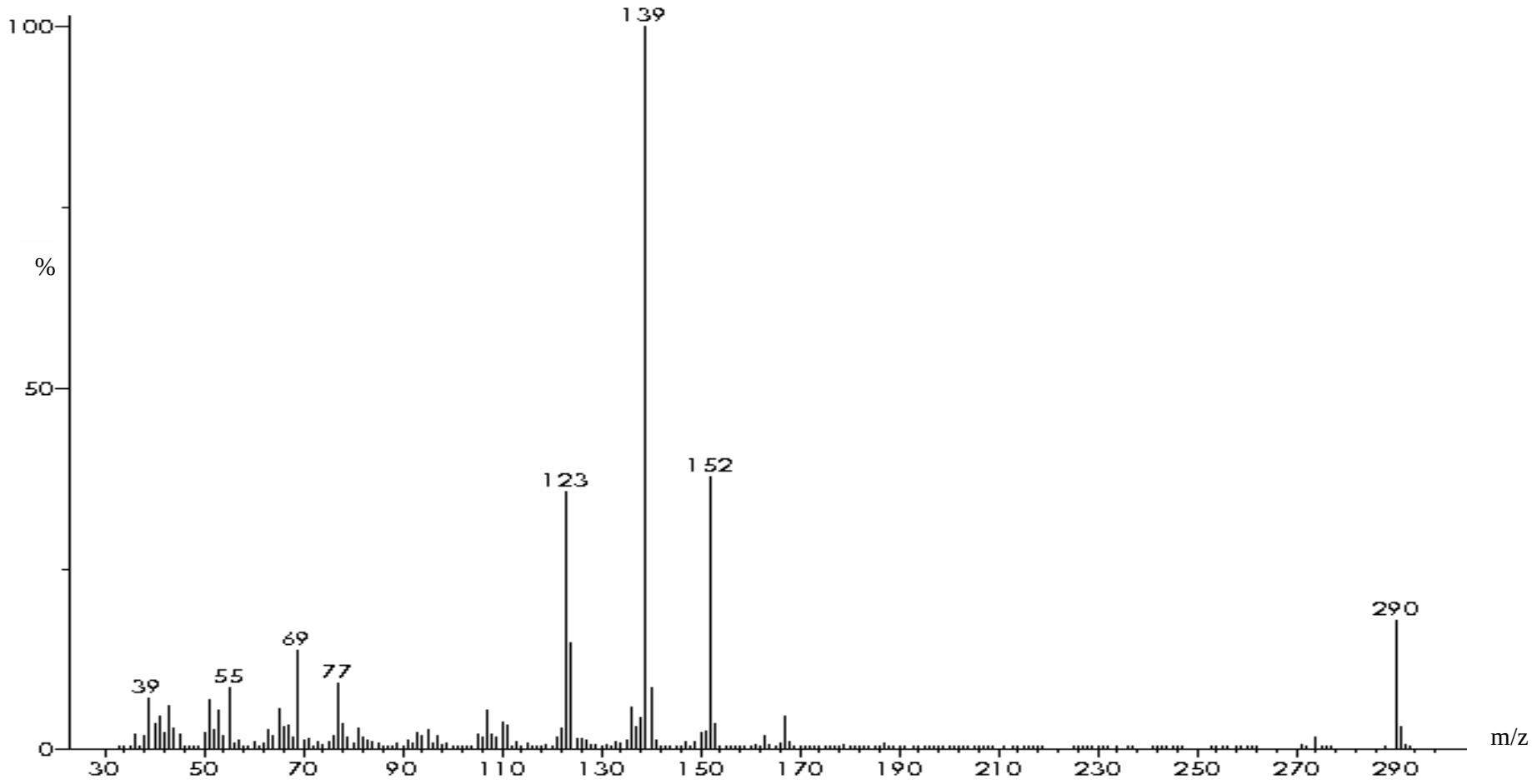


Fig 2.11: MS spectra of PI-1

Phytochemical investigation of *Pouzolzia hirta*

The leaves of *P. hirta* (3.0 kg) were extracted with petroleum ether (2 L x 4) at room temperature for 24-48 h. The petroleum ether extract was evaporated under reduced pressure to yield dark-brown syrup. The petroleum ether extract (90 g) was chromatographed over silica gel column (hexane/EtOAc/MeOH) to obtain 10 fractions. Fraction 3 was chromatographed on silica gel and active carbon, using hexane-EtOAc (30:1)) to obtain the compounds PE-1 (46 mg) and PE-2 (30 mg). The detailed procedure is given in the chart 2.5.

COMPOUND – PE-1

On eluting column with hexane-EtOAc (30:1), PE-1 formed white solid compound, m.p. 64 ^{0}C.

Colour reactions - The alcoholic solution of the compound gave red colour with by heating with 0.1 % ethanolic solution of 2, 6-dichorophenol indophenol indicting organic acid or keto acid (**Passera *et al.*, 1928**).

Elemental analysis - Elemental analysis of the compound showed C-74.94, H-12.58 and O- 12.48 %.

Spectral Studies of Compound PE-1

UV spectra λ_{max} MeOH: 201, 352, 577, 582 nm. The UV spectra of the compound PE-1 in fig- 2.14. The UV spectra of the compound PE-1 is shown in fig- 2.13.

FT-IR spectra : 2954, 2917, 2849, 2676, 1703, 1471, 1431, 1410, 1250, 940 cm^{-1}. The IR spectrum of the compound exhibited absorption band at 2849-2954 cm^{-1} for C-H str. (superimposed upon O-H str.), 1703 cm^{-1} for C=O str. for normal dimeric carboxylic acid. The IR spectra at 1408 cm-1 for C-O-H in

plane band, 1250 for C-O str. and 940 for O-H def. The FT-IR spectra of the compound PE-1 is shown in fig- 2.14.

Extraction of *Pouzolzia hirta* rhizomes

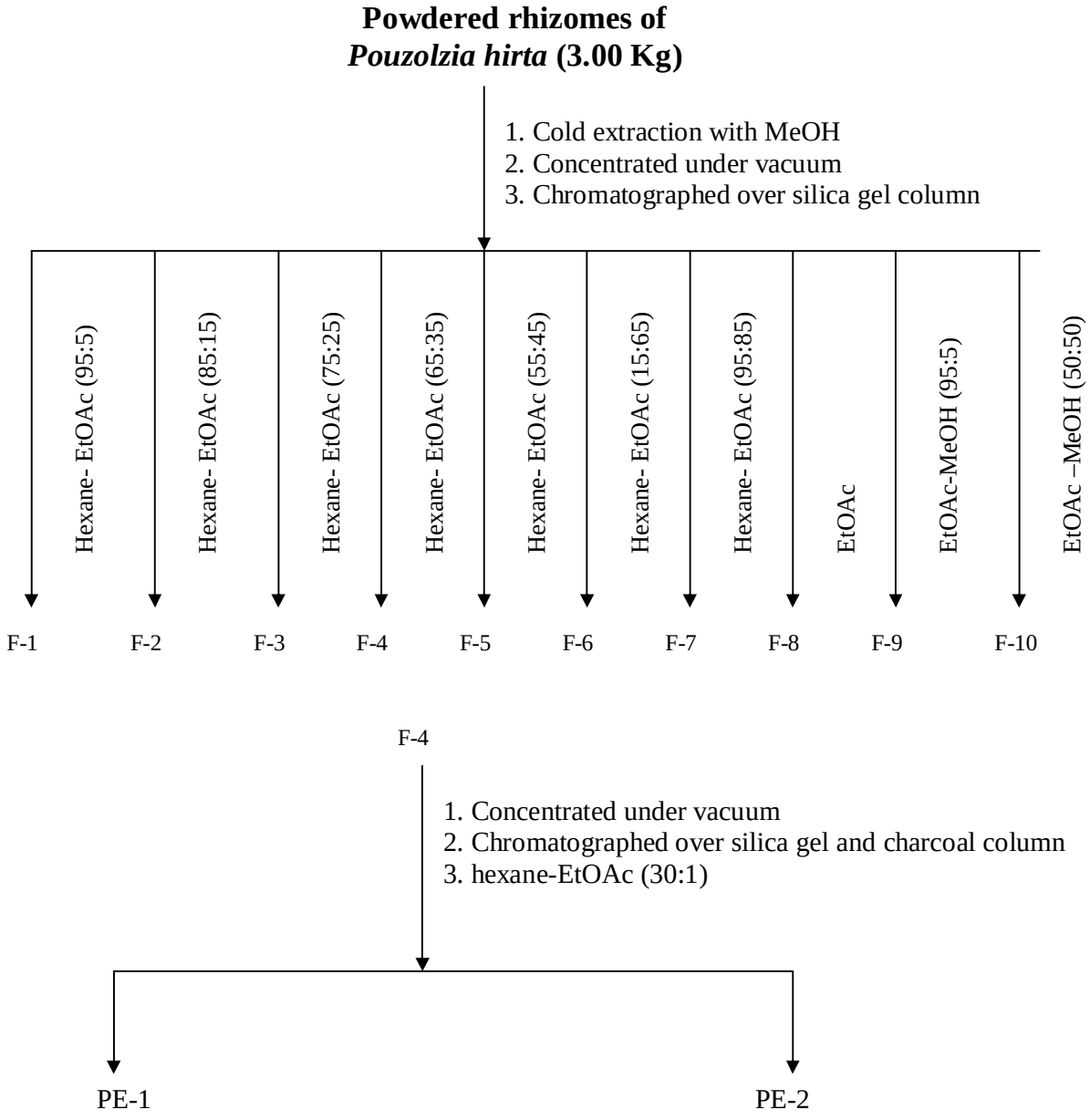


CHART 2.5

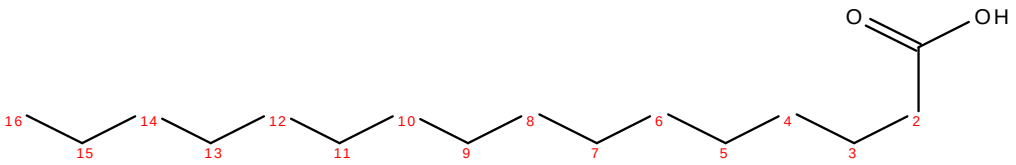
MS m/z: 256, 213, 129, 73, 60 57, **43.**

The MS of the compound displayed M^+ at m/z 256 corresponding to the molecular formula $C_{16}H_{32}O_2$. The MS spectra of the compound PE-1 is shown in fig- 2.15.

^{1}H NMR ($CDCl_3$ 400 MHz): The ^{1}H NMR spectrum (Table 2.7) of PE-1 displayed overall 32 protons and their multiplicity assignments also support the structure. The ^{1}H NMR spectra of the compound PE-1 is shown in fig- 2.16.

Table 2.7: ^{1}H NMR Spectral data of PE-1.

Chemical Shift (δ)	Proton Count	Multiplicity of signals	Probable assignments
0.87	3H	t (J = 2.38 Hz)	H-16
1.25	22H	m (J = 17.67 Hz)	H - 4-14
1.31	2H	-	H -15
1.69	2H	t (J = 1.00 Hz)	H- 3
2.34	2H	t (J = 1.00 Hz)	H-2
7.26	1	s, acidic OH proton	

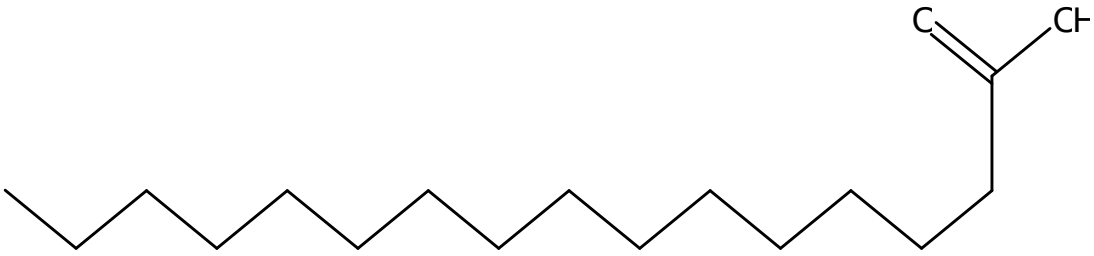


^{13}C NMR ($CDCl_3$ 400 MHz): The ^{13}C NMR spectrum (Table-2.8) of PE-1 and their multiplicity assignments also support the structure. The ^{13}CNMR spectra of the compound PE-1 is shown in fig- 2.17.

Table 2.8: ^{13}C NMR spectral data of PE-1

Chemical shift (δ)	Probable assignments
20.96	C-16
27.40	C-15
29.67	C-3
50.23	C-4
53.38	C-5
69.63	C-13
70.32	C-14
77.60	C-6 - C-12
130.17	C-2
170.30	C-1 Carbonyl group

Based on the above chemical and spectral results, the following structural formula could be assigned for the compound PE-1.



n-hexadecanoic acid (Palmitic acid).

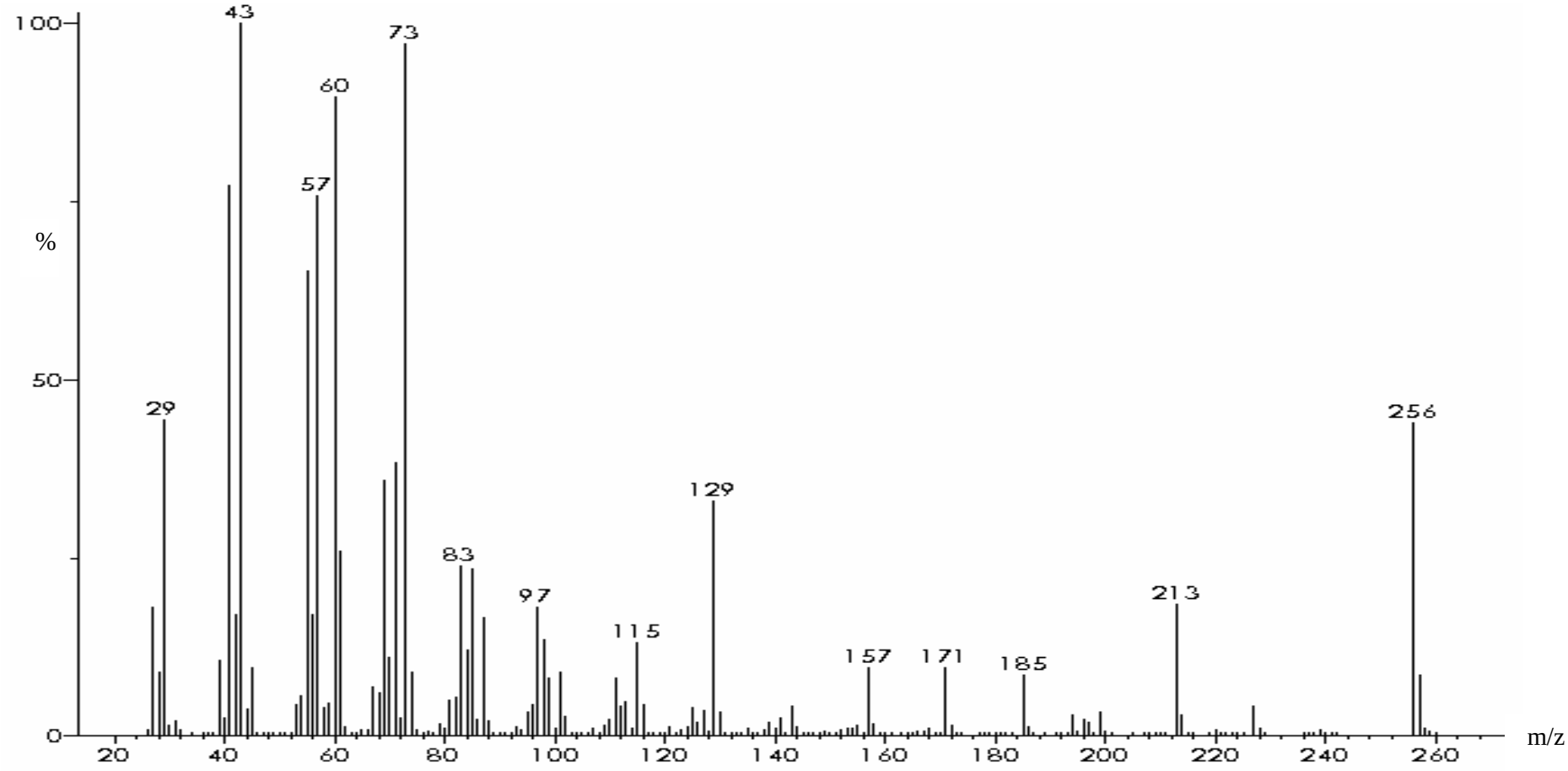


Fig- 2. 15: MS spectra of PE-1

COMPOUND - PE-2

On eluting column with hexane-EtOAc (30:1), after PE-1, white solid compound, m.p. 58 ^{0}C was obtained.

Colour reactions - The alcoholic solution of the compound gave red colour with by heating with 0.1 % ethanolic solution of 2, 6-dichorophenol indophenol indicting organic acid or keto acid (**Passera *et al.*, 1928**).

Elemental analysis - Elemental analysis of the compound showed C- 73.63, H- 12.36 and O- 14.01 %.

Spectral Studies of Compound 'PE-2'

UV spectra λ_{max} MeOH : 242, 221, 202, 268. The UV spectra of the compound PE-2 is shown in fig- 2.18.

FT-IR spectra: 2917, 2849, 2679, 1702, 1470, 1430, 1409, 1261, 940 cm^{-1}. The IR spectrum of the compound exhibited absorption band at 2849-2917 cm^{-1} for C-H str. (superimposed upon O-H str), 1702 cm^{-1} for C=O str. for normal dimeric carboxylic acid. The IR 1470-1409 cm-1 for C-O-H in plane band, 1261 for C-O str, and 940 for O-H def. The FT-IR spectra of the compound PE-2 is shown in fig- 2.19.

MS m/z: 228, 185, 171, 143,129, **73**, 60, 55, 43.

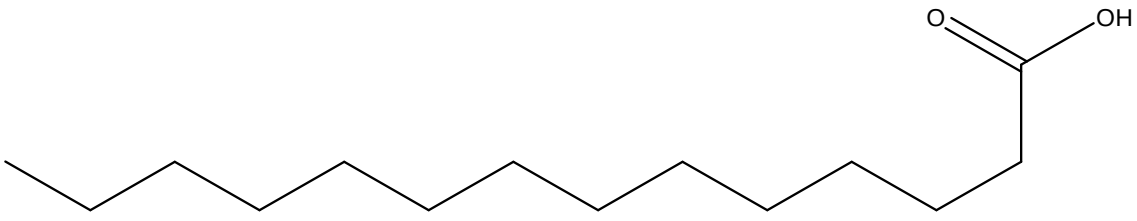
The MS of the compound displayed M^+ at m/z 228 corresponding to the molecular formula $C_{14}H_{28}O_2$. The MS spectra of the compound PE-2 is shown in fig- 2.20.

^{1}H NMR ($CDCl_3$ 400 MHz): The ^{1}H NMR spectrum (Table 2.9) of PE-2 displayed overall 32 protons and their multiplicity assignments also support the structure. The ^{1}HNMR spectra of the compound PE-2 is shown in fig- 2.21.

Table 2.9: ^{1}H NMR Spectral data of PE-2.

Chemical Shift (δ)	Proton Count	Multiplicity of signals	Probable assignments
0.86	3H	t (J = 1.60 Hz)	H-16
1.24	18 H	s (J = 10.72 Hz)	H - 4-12
1.31	2H	-	H -13
1.61	2H	m (J = 0.96Hz)	H- 3
2.33	2H	t (J = 1.00 Hz)	H-2
7.26	1	s acidic OH proton	

Based on the above chemical and spectral results, the following structural formula could be assigned for the compound PE-2.



Tetradecanoic acid (Myristic acid).

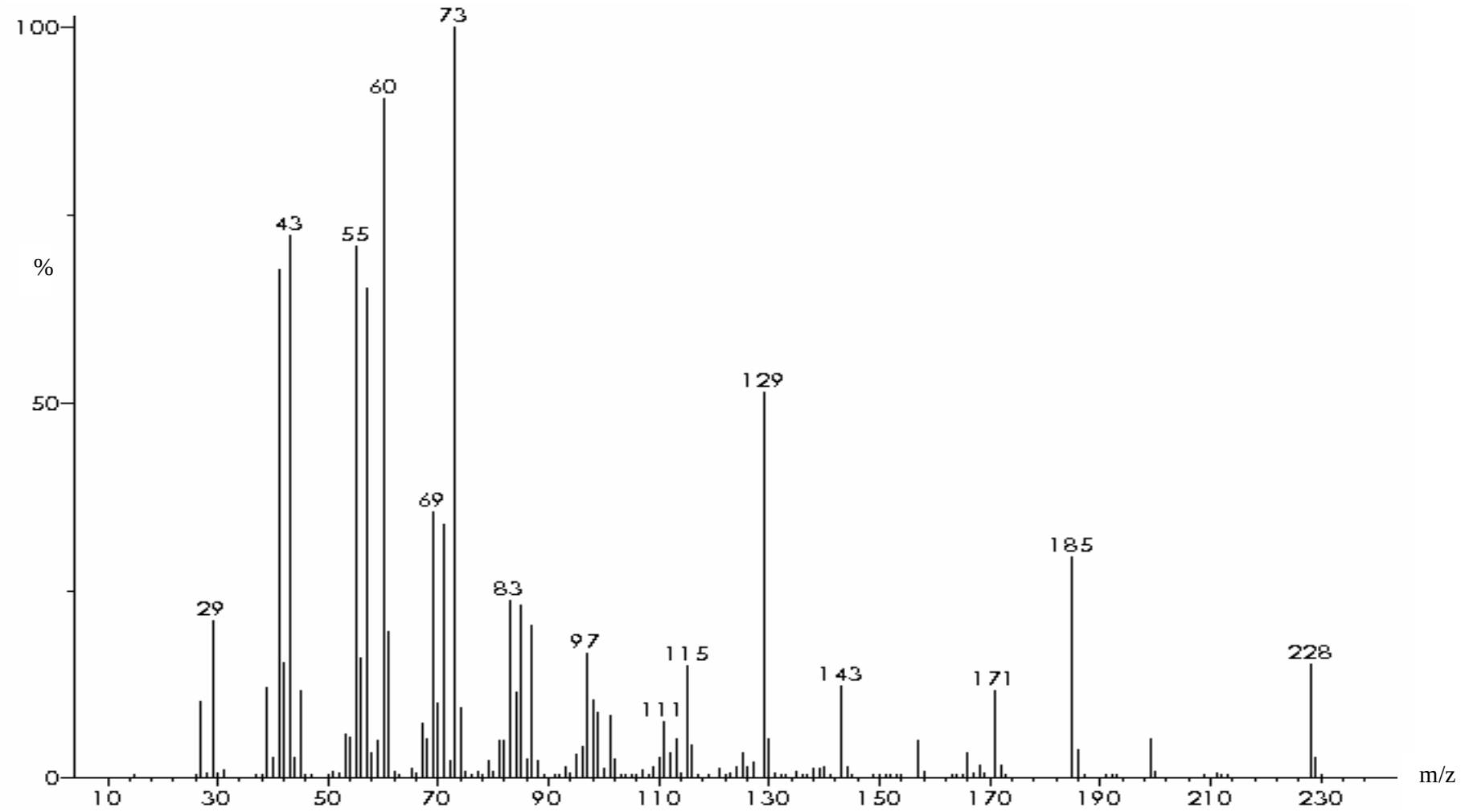


Fig. 2.20 MS spectra of PE-2

SECTION B

1. GC - MS study of *Pavetta indica* oil

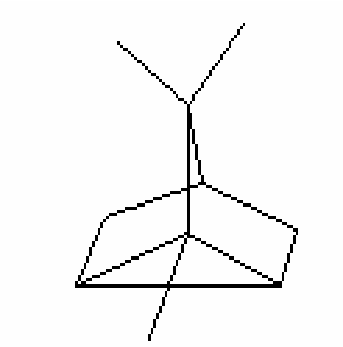
The composition of active principles in herbs has been the subject of many research studies. They are responsible for the wide aroma and therapeutic effects (**Gherman *et al.*, 2000**). The curative efficiency of herbs depends on their quality, time of harvesting, drying and storage procedure and on the climatic condition (**Robards &Antolovich, 1997**).

Extraction of essential oil from the leaves of *Pavetta indica*

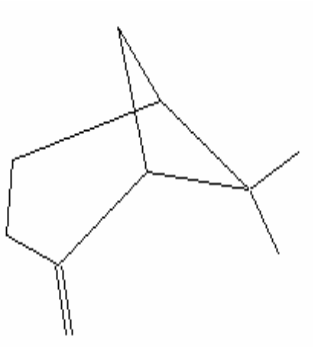
Extraction of oil: The leaves (5.0 Kg) of the plant were used for essential oil extraction by hydro distillation. The distillate was saturated with NaCl and the distillate was extracted with n-hexane. The n-hexane layer was separated and dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residual oil was used as such for analysis by GC-MS.

GC-MS study of essential oil from the leaves of *Pavetta indica*

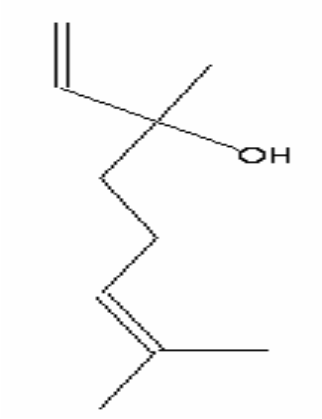
The gas chromatogram shows the presence of 24 compounds and 74.00 % of essential oil has been identified (Table 2.1). The major constituents of oil were β-pinene (25.45 %), β-eudesmol (7.06 %) and tricyclene (5.74 %). The oxygenated monoterpenes and sesquiterpene hydrocarbons found in the oil as minor components. The oxygenated monoterpenes were 4.99, monoterpene hydrocarbons were 33.58, oxygenated sesquiterpes were 8.93 and sesquiterpene hydrocarbons were 6.79 % of the total identified compounds. The objective of present study was to analyse the volatile component of the leaves of *Pavetta indica* by GC-MS. Following are the structures of the major compounds and their MS spectra of the compounds are shown in fig-2.22- 2.27. The GC chromatogram of *P. indica* oil is shown in fig. 2.28.



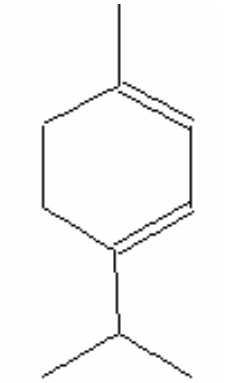
Tricyclene



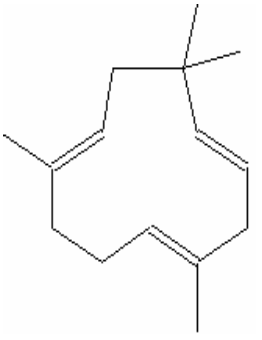
β- pinene



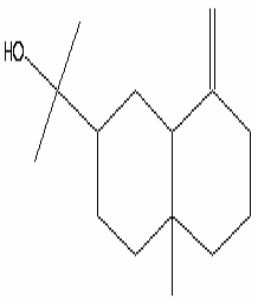
Linalool



α-terpinene



α-humulene



β-eudesmol

Table 2.10: Composition of essential oil from leaves of *Pavetta indica*

S. No.	Compound	Area (%)	Mol. formula	Mol. wt.	Mode of identification
1.	Tricyclene	5.74	$C_{10}H_{16}$	136	a, b
2.	α-thujene	1.23	$C_{10}H_{16}$	136	a, b
3.	Benzaldehyde	0.68	C_7H_6O	106	a, b
4.	Sabinine	2.23	$C_{10}H_{16}$	136	a, b
5.	β- pinene	25.45	$C_{10}H_{16}$	136	a, b
6.	Trans-meta-mentha-2,8-diene	0.88	$C_{10}H_{16}$	136	a, b
7.	α-terpinene	3.93	$C_{10}H_{16}$	136	a, b
8.	o-cymene	1.23	$C_{10}H_{14}$	134	a, b
9.	Limonene	2.14	$C_{10}H_{16}$	136	a, b
10.	γ-terpinene	2.36	$C_{10}H_{16}$	136	a, b
11.	Acetophenone	0.56	C_8H_8O	120	a, b
12.	Linalool	3.68	$C_{10}H_{18}O$	154	a, b
13.	Perillene	2.09	$C_{10}H_{14}O$	150	a, b
14.	Cis-pinan-2-ol	0.25	$C_{10}H_{18}O$	154	a, b
15.	3-hexenyl isobutyrate	0.23	$C_{10}H_{18}O_2$	170	a, b
16.	Piperitone	0.46	$C_{10}H_{16}O$	152	a, b
17.	β-caryophyllene	1.56	$C_{15}H_{24}$	204	a, b
18.	α-gvaiene	2.26	$C_{15}H_{24}$	204	a, b
19.	α-humulene	3.69	$C_{15}H_{24}$	204	a, b
20.	germacrene D	0.84	$C_{15}H_{24}$	204	a, b
21.	γ-cadinene	1.78	$C_{15}H_{24}$	204	a, b
22.	δ-cadinene	1.89	$C_{15}H_{24}$	204	a, b
23.	β-eudesmol	7.06	$C_{15}H_{26}O$	222	a, b
24.	Thujapsonone	2.08	$C_{15}H_{24}O$	220	a, b
	Total %	74.30			

a = Retention index of gas chromatogram, b= GC-MS

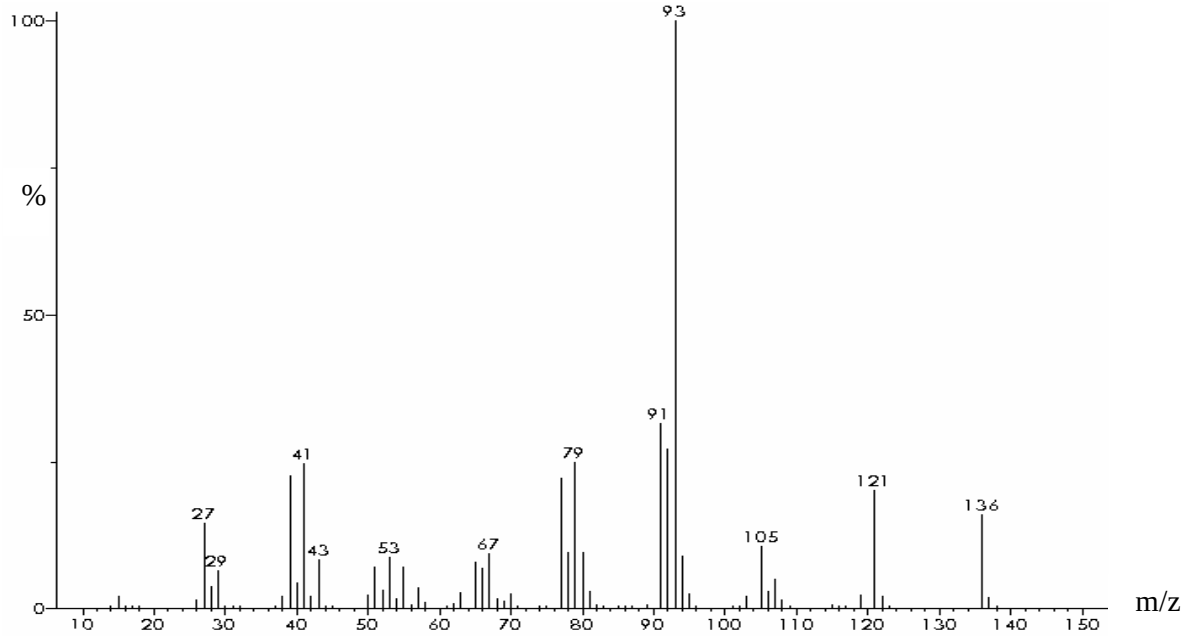


Fig-2.22 – Tricyclene

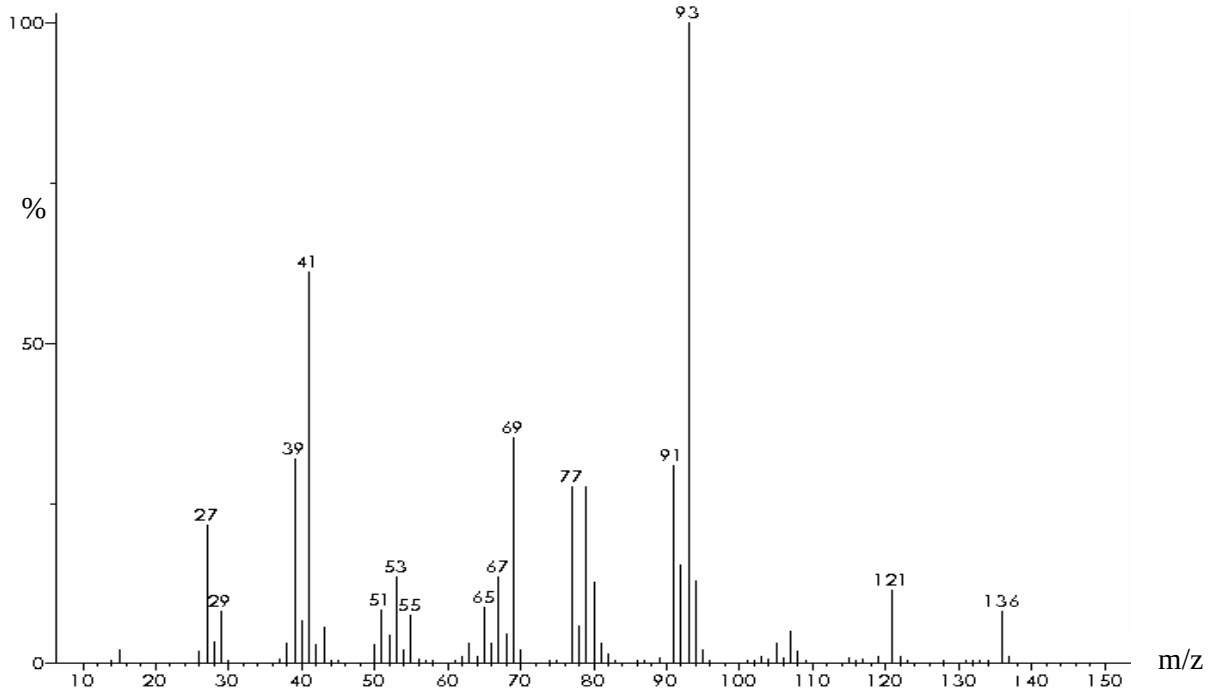


Fig-2.23 – β- pinene

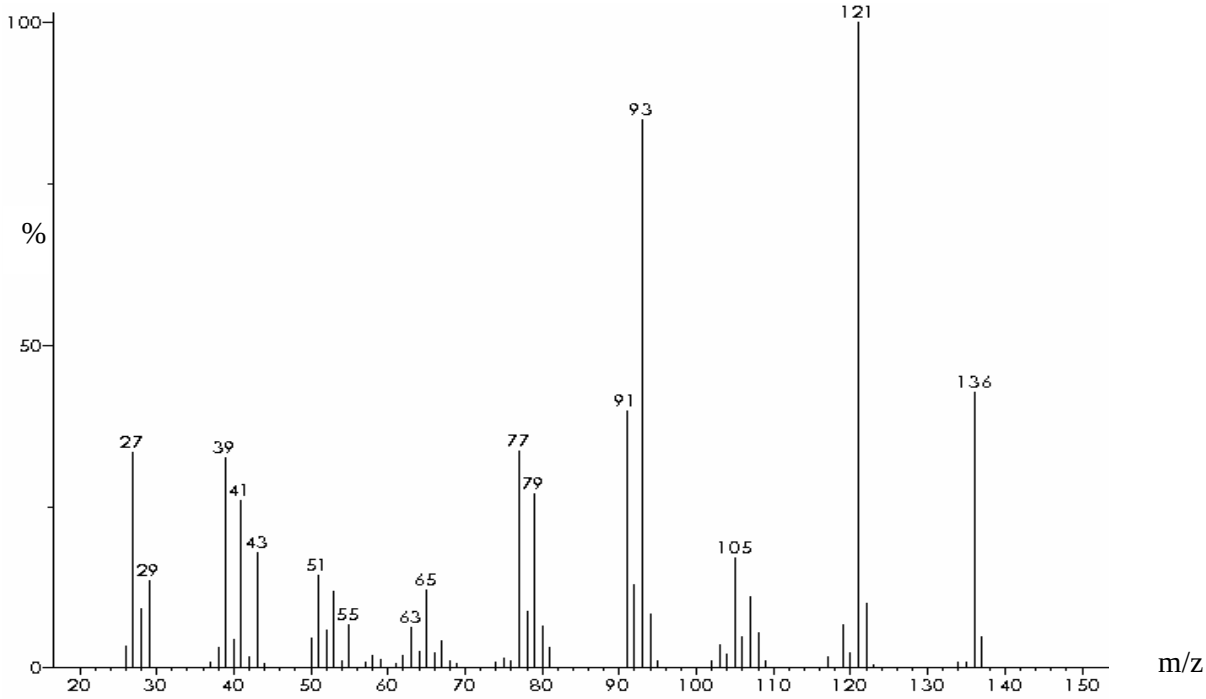


Fig-2.24 – α-terpinene

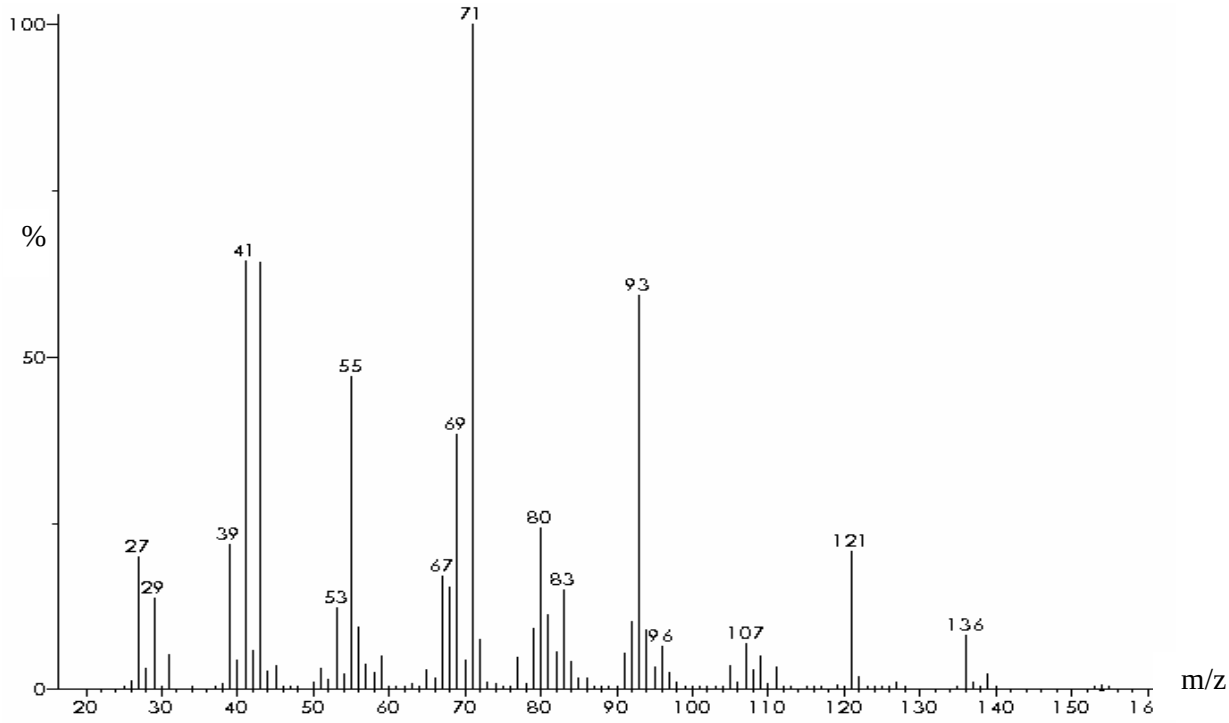


Fig-2.25– Linalool

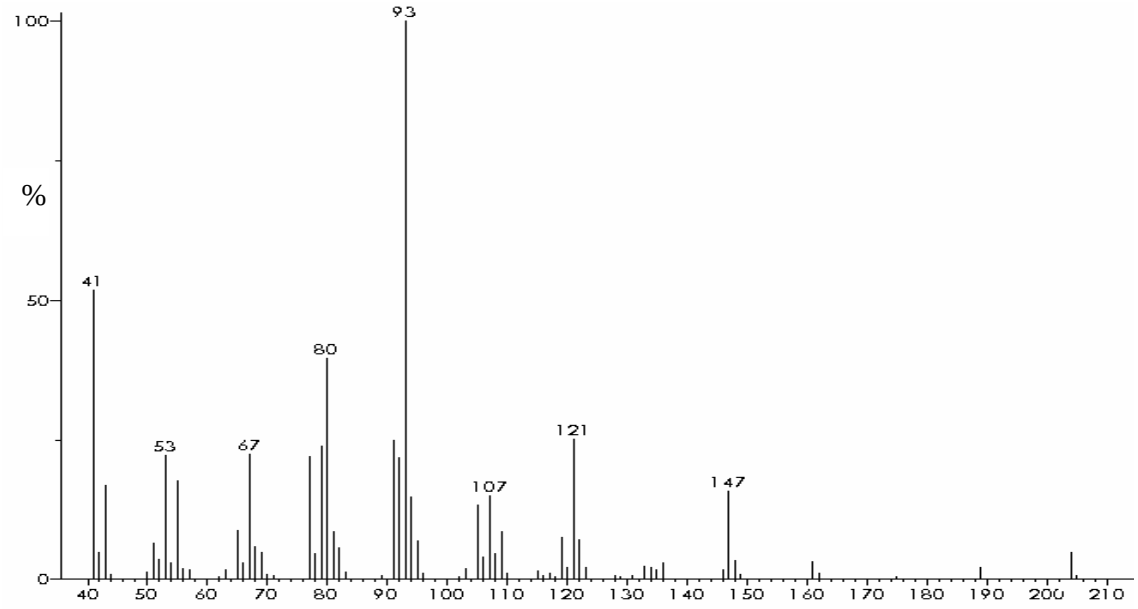


Fig-2.26 α-humulene

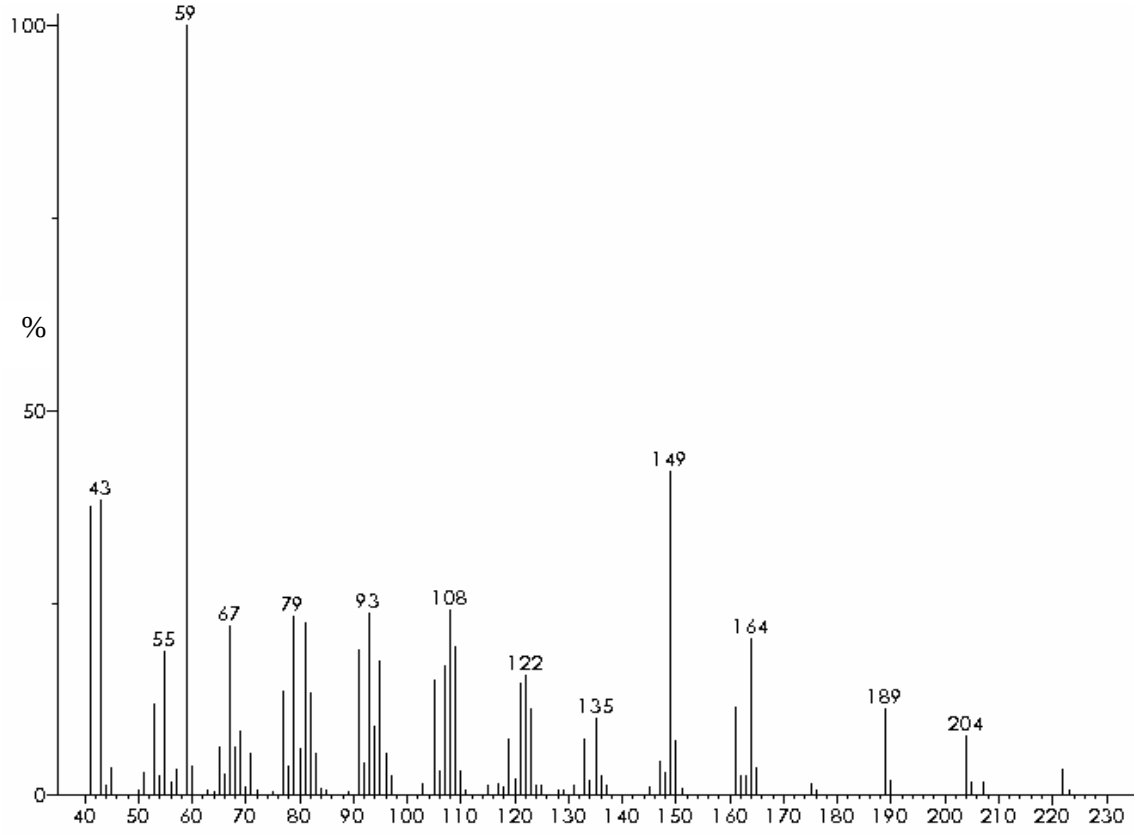


Fig.2.27 β-eudesmol

10 g of the oil was chromatographed over silica gel (150g). The column was eluted with n-hexane followed by a mixture of n-hexane: ether (5 to 50 % ether in n-hexane) and finally the column was washed with ethyl acetate. From this procedure, we obtained nine fractions. Fraction 4 was re-chromatographed on silica gel column (n-hexane-ether (90:10)) to get the compounds FPI-1, (45 mg) and FPI-2 (60 mg). The detailed procedure is given in the chart 2.6.

Compound FPI-1

On eluting column with hexane-ether (90:10), FPI-1 compound was isolated.

Spectral Studies of Compound 'FPI-1'

UV spectra λ_{max} MeOH : 244, 262, 252, 275. . The UV spectra of the compound FPI-1 is shown in fig- 2.29.

FT-IR spectra - 3024, 2919, 1658, 1468, 1445, 771 cm^{-1}.

The IR spectrum of the compound exhibited absorption band at 3024 -2919 cm^{-1} for C-H str., 1658 cm^{-1} for C=C str. The IR 1468- 1445cm^{-1} for C-H bending. The FT-IR spectra of the compound FPI-1 is shown in fig- 2.30.

MS m/z: 136, 121, **93**, 91, 77, 69, 53, 41.

The MS of the compound displayed a M^+ at m/z 136 corresponding to the molecular formula $C_{10}H_{16}$. The MS spectra of the compound FPI-1 is shown in fig- 2.31.

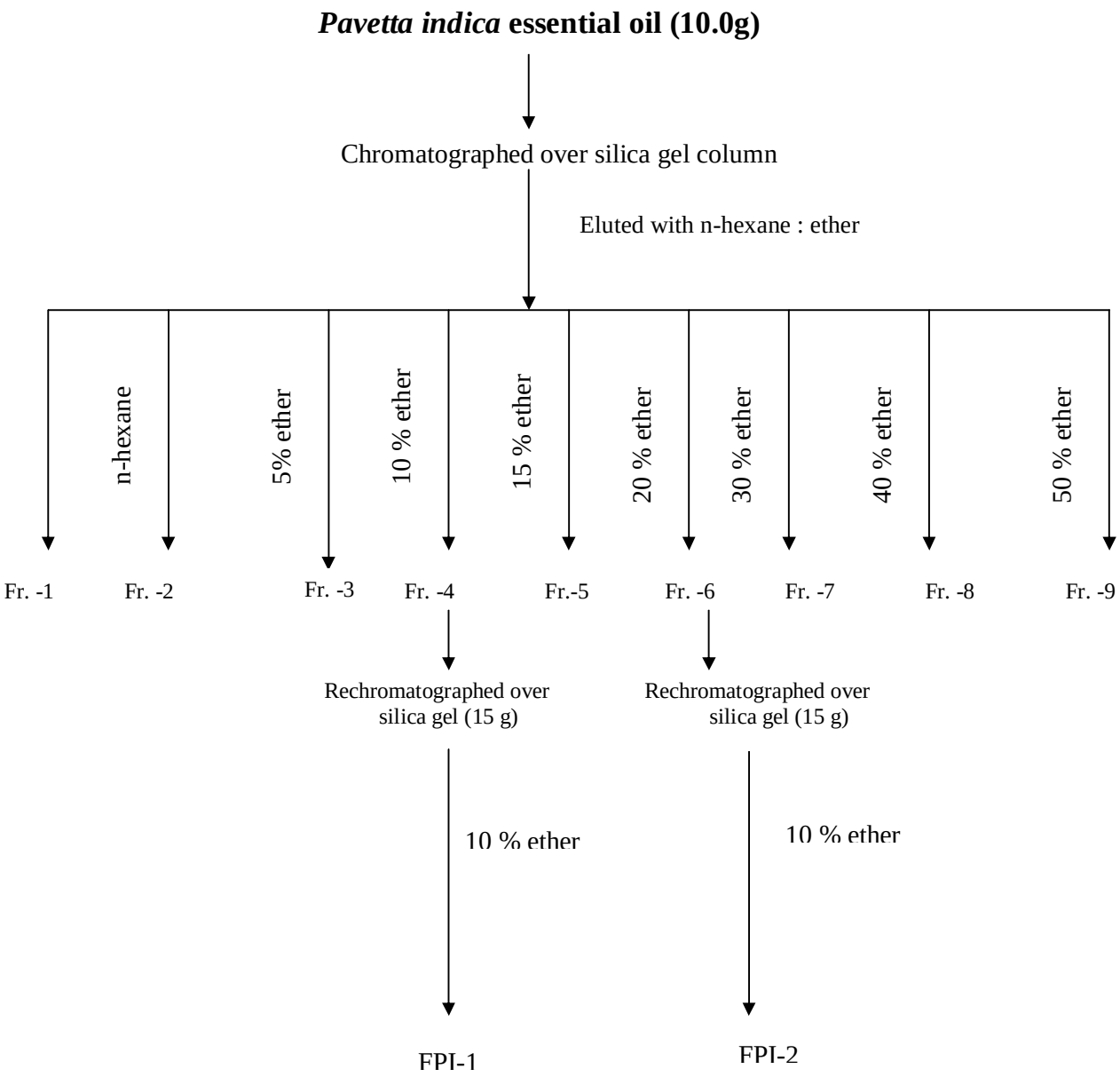
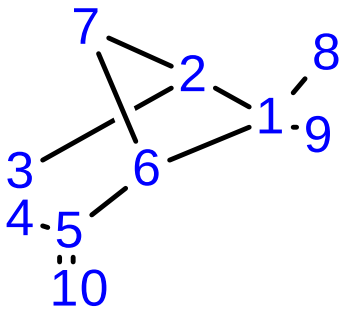
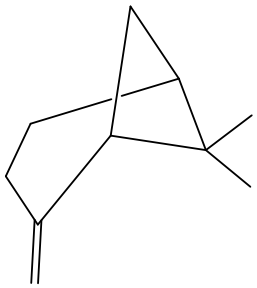

Pavetta indica essential oil (10.0g)
Chromatographed over silica gel column
Eluted with n-hexane : ether
n-hexane
5% ether
10 % ether
15 % ether
20 % ether
30 % ether
40 % ether
50 % ether
Fr. -1
Fr. -2
Fr. -3
Fr. -4
Fr.-5
Fr. -6
Fr. -7
Fr. -8
Fr. -9
Rechromatographed over silica gel (15 g)
Rechromatographed over silica gel (15 g)
10 % ether
10 % ether
FPI-1
FPI-2

CHART 2.6

^{1}H NMR ($CDCl_3$ 400 MHz): The ^{1}H NMR spectrum (table 2.9) of FPI-1 displayed overall 16 protons and their multiplicity assignments also support the structure. The ^{1}HNMR spectra of the compound FPI-1 is shown in fig- 2.32.

Based on the above chemical and spectral results, the following structural formula could be assigned for the compound FPI-1.





6, 6-dimethyl-2-methylene-bicyclo[3.1.1] heptane (β-pinene)

Table 2.9: ^{1}H NMR Spectral data of FPI-1.

Chemical Shift (δ)	Proton Count	Multiplicity of signals	Probable assignments
0.85	6	t (J = 19.77 Hz)	H-8, H-9
1.03	2	q (J = 1.0 Hz)	H – 3
1.18	2	d (J = 1.99 Hz)	H -7
1.30	1	t (J = 7.0 Hz)	H- 2
1.67	2	t (J = 7.21 Hz)	H-4
2.19	1	s (J= 34.67 Hz)	H-6
5.20	2	t (J=6.49 Hz)	H-10

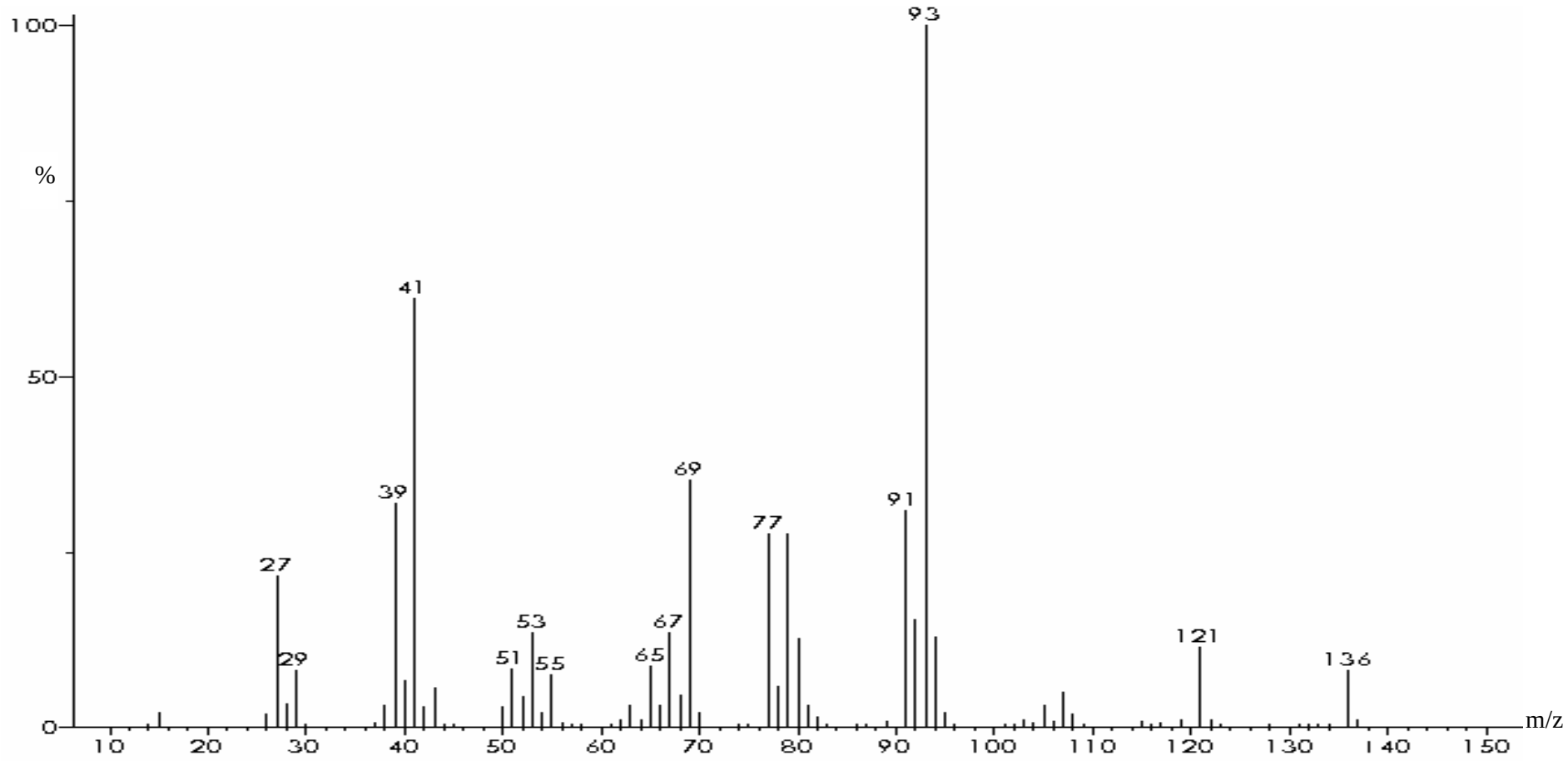


Fig-2.31 – MS spectra of FPI-1

Compound FPI-2

On eluting column with hexane-ether (90:10), after FPI-1, the compound FPI-2 was obtained in liquid form.

UV spectra λ_{max} MeOH : 257, 250, 266, 273.

The λ_{max} at 257 and 250 shows the compound homoannular conjugated diene. The UV spectra of the compound FPI-2 is shown in fig- 2.33.

FT-IR V_{max} cm^{-1} : 781, 947, 1446, 1465, 1662, 2873, 2960, 3019 cm^{-1}.

The IR spectrum of the compound exhibited absorption band at 3019 -1873 cm^{-1} for C-H str., 1662 cm^{-1} for C=C str. The IR 1446- 1465cm-1 for C-H bending. The FT-IR spectra of the compound FPI-2 is shown in fig- 2.34.

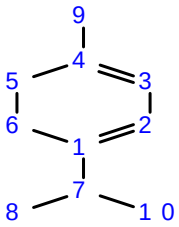
MS spectra: 136, **121**, 105, 93, 91, 77, 41.

The MS of the compound displayed a molecular ion peak at m/z 136 corresponding to the molecular formula $C_{10}H_{16}$. The MS spectra of the compound FPI-2 is shown in fig- 2.35.

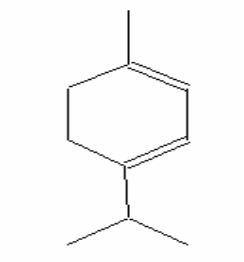
^{1}H NMR ($CDCl_3$ 400 MHz): The ^{1}H NMR spectrum (table 2.10) of FPI-2 displayed overall 16 protons and their multiplicity assignments also support the structure. The ^{1}HNMR spectra of the compound FPI-2 is shown in fig- 2.36.

Table 2.10: ^{1}H NMR Spectral data of FPI-2.

Chemical Shift (δ)	Proton Count	Multiplicity of signals	Probable assignments
1.04	6	d (J = 5.04 Hz)	H-8, H-10
1.68	3	s (J = 2.96 Hz)	H -9
2.21	1	s (J = 1.00 Hz)	H-7
2.61	4	s (J = 3.39 Hz)	H- 5, H-6
5.45	2	s (J= 1.90 Hz)	H-2, H-3



Based on the above chemical and spectral results, the following structural formula could be assigned for compound FPI-2.



1-isopropyl-4-methyl-cyclohexa-1,3-diene (α-terpinene)

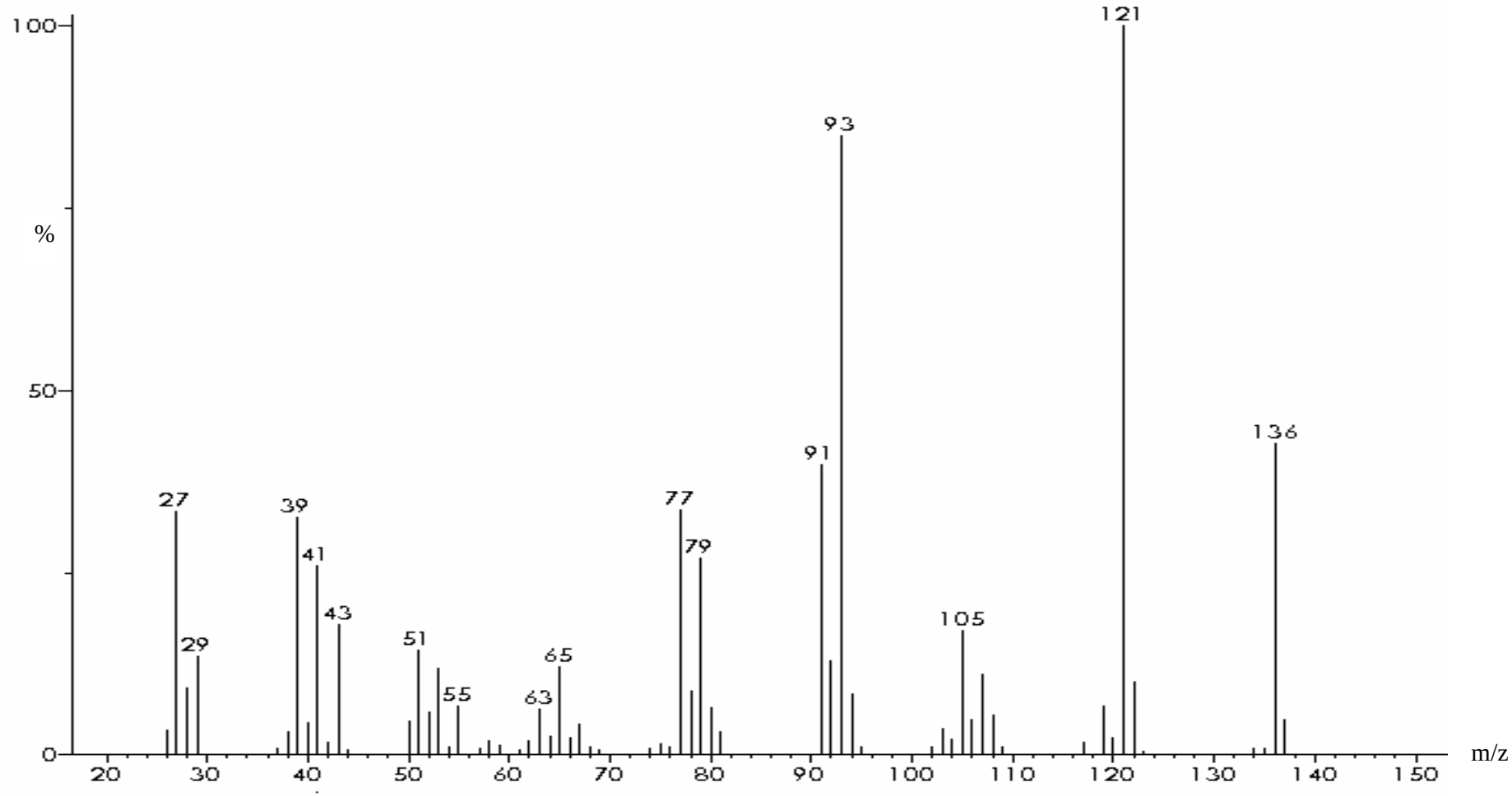


Fig. 2.35 – MS spectra of FPI-2

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BIOCHEMICAL ANALYSIS

INTRODUCTION

Kumaon region is gifted with rich natural vegetation, viz., grasslands and forests. The livelihood of most of population of this region depends upon livestock and animal based products, due to poor farming conditions and low output from crops. The output from crops is low as compared to the cost involved in growing them.

The study of wild edible plants is important for the identification of the potential source which could be utilized as alternative food source. So, the need of the hour is to study the biosphere for conservation and making available the indigenous source for domestic and commercial consumption.

Concerned professionals in the field of animal nutrition particularly in the developing countries had for long identified the cost of finished livestock feed as the most economically limiting factor in the industry (**Ademosun, 1973; Obioha, 1976; Balogun, 1988 and Fasuyi & Aletor 2005a**). The greens (green plants of various sources) have long been recognized as the cheapest and most abundant potential source of proteins because of their ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials such as water, CO_2, atmospheric N_2 (as in legumes) (**Byers, 1961; Oke, 1973 and Fasuyi & Aletor 2005b**).

A few world compilations on useful wild and cultivated plants and other regional work of this kind have listed such edible plants (**Tanaka, 1976; Uphof, 1986; Clute, 1943 and Burkill, 1935**). Of about 15,000 species of Angiosperms available in India, only about 1,000 fall in the above categories to be used directly or indirectly as food stuff. Many articles on the use of wild

edible plants have appeared since the first comprehensive publication dealing with this aspect as also on other economic plants but the main source of such information remain scattered in the various regional/ floristic works dealing with the flora of India (**Watt, 1971; Duthie, 1960; Gamble, 1957; Kanjilal *et al.*, 1934; Prain, 1963; Santapau, 1958 and Collett, 1971**). Attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy (**Glombitza *et al.*, 1993**). Botanical richness in wild edible plants of India are listed in table 3.1.

Table 3.1. Wild edible plants of India.

Useful plant parts	Floristic richness			Species domesticated/ semi-domesticated
	Families	Genera	Species	
Root and tubers, underground parts	42	98	145	33
Leafy vegetable/ greens/ pot-herbs	156	377	521	72
Bud and flower	47	88	101	15
Fruits	112	357	647	107
Seeds and nuts	53	91	118	25

Source - **Singh and Arora, 1978**.

In developing countries, one of the greatest problems affecting millions of people, particularly children is the lack of adequate protein intake in terms of quality and quantity. Evidences have shown that protein deficiency is a major nutritional problem among the children which has hindered their health, mental capability, school performance and productivity, thus affecting the economic growth (**Spur *et al.*, 1977; Martorell, 1992; NHCD, 1998; Chapin, 1999;**

Berkman & Kawachi, 2000; Ivanovic *et al.*, 2002; Braveman & Gruskin, 2003 and Ishara, 2005).

Carbohydrates

Carbohydrates (starch and sugar) are the rich source of energy, which provide the bulk of the calories in most diets. Age, sex, size, health, and the intensity of physical activity strongly affects the daily need for calories. Surplus carbohydrates are also converted to glycogen and fat by the body. Most health professionals recommend that carbohydrates should comprise 50% to 60% of the dietary calories, of which most of them (80% of all carbohydrates eaten) should be complex carbohydrates, such as cereals and vegetables. Complex carbohydrates are preferred because the fast-acting simple carbohydrates, such as honey and sugar, are difficult for the body (especially the pancreas) to handle in large doses. Simple carbohydrates also lack vitamins, minerals, proteins, and fibers that are generally present in complex carbohydrates.

In plants, sugars are mainly found as mono and disaccharides form of carbohydrates. These include (i) monosaccharides - aldohexoses, ketoses (glucose and fructose) and (ii) disaccharides - sucrose and maltose etc. Monosaccharides, mainly D-glucose is the first product of photosynthesis in plants and is responsible for the production of various biochemical compounds of organic origin.

In plants, the free form of sugar is found as reducing and non-reducing, which in combination make the total sugar contents. Formation of sugar depends on photosynthetic reactions which depend on CO_2 intake, photolysis of water, chlorophyll content of leaves, intensity and amount of enzymes.

Proteins and Amino Acids

Proteins in the diet provide amino acids for the synthesis of body proteins, including structural proteins for building and repairing tissues, and the enzymes

for carrying out various metabolic processes. In addition, proteins may be used as source of energy when the preferred fat and carbohydrate supply runs low. A body that is in the process of building itself (such as that of a growing child or an adult recovering from illness) requires greater proportion of protein than one that is fully grown and utilizes protein merely for repair of worn-out tissues. An average adult requires 1 g of protein per kg of body weight per day, whereas a child may require two to three times of this amount of protein.

Alpha-amino acids are the building blocks of proteins. Proteins are formed by the condensation of amino acids. Each type of protein has a unique sequence of amino acid residues; this sequence is the primary structure of the protein. Just as the letters of the alphabet can be combined to form an almost endless variety of words, amino acids can be linked in varying sequences to form a huge variety of proteins.

The term kwashiorkor refers to a disease, which occurs when there is inadequate protein intake with reasonable calorie intake (**Ibukun Olu-Alade, 2001**). Studies suggest that marasmus represents an adaptive response to starvation whereas kwashiorkor represents a maladaptive response to starvation (**Berkow & Robert, 1999**). Children may present with a mixed picture of marasmus and kwashiorkor or with milder form of malnutrition, for this reason, the term protein energy malnutrition was suggested to include both entities (**Berkow & Robert, 1999**).

Fats

Fats in the diet provide a concentrated source of energy. Their energy content (9 kcal/g) is about twice as great as carbohydrates and proteins (4 kcal/g). In addition to acting as a source of stored energy, they provide physical protection and insulation for tissues and form important portions of cell membrane structure. Fats also aid in the absorption of the fat-soluble vitamins (vitamins A, D, E and K) from the intestine. Ingested fats provide the precursors from which

we can synthesize our own fat as well as cholesterol and various phospholipids. Humans can synthesize fats from carbohydrates, however, three essential fatty acids cannot be synthesized and must be incorporated in the diet.

Vitamin C

Vitamin C or ascorbic acid is one of the important water-soluble vitamins essential for health. Most plants and animals can synthesize ascorbic acid for their own requirement. However, humans cannot synthesize ascorbic acid. Hence, ascorbic acid has to be supplemented mainly through fruits and vegetables. The biological function of vitamin C is based on its ability to donate electrons. It is essential for collagen, carnitine and neurotransmitters biosynthesis. Many health benefits have been attributed to ascorbic acid such as antioxidant, anti-atherogenic, anti-carcinogenic and immunomodulator etc. Another important indirect function of vitamin C is its ability to regenerate other biologically important antioxidants such as glutathione and vitamin E.

Vitamin C is an important antioxidant in human body fluid (**Halliwell & Gutteridge, 1989**). This vitamin is also required for the proper formation and maintenance of intracellular material, especially collagen (**Latham, 1997**). It has been found that vitamin C plays a preventive role in the development of cardiovascular diseases (**Mehra *et al.*, 1995 and Benzie & Stain, 1997**).

Fibers

Fiber is a group of substances chemically similar to carbohydrates. It is only found in the foods derived from plants, and never occurs in animal products. A diet rich in fibers may help to prevent colon cancer and other types of cancer. Isolated dietary fibers from vegetables and fruit sources, particularly pectins, have been shown to have hypocholesterolemic action in humans. Most health agencies recommend that adults should consume 20-35 g of fiber daily. Some

excellent sources of fiber are kidney beans, cereals, baked potatoes with skin, apples, oranges, pears, spinach, oatmeal and popcorn.

Minerals

The mineral elements constitute an important group of nutrients required by the human body for optimal functions (**WHO, 1996**). They can be divided into macro minerals (major elements) and micro minerals (trace elements). The macro minerals such as sodium, potassium, magnesium, calcium and phosphorus are required by the body in amounts greater than 100mg per day whereas the micro minerals such as iron, copper, zinc and manganese are required in amounts less than 100mg per day (**Murray *et al.*, 2000**).

Mineral elements have a great diversity of uses within the animal body. The following mineral elements are recognized as essential for human body functions - calcium, phosphorus, sodium, molybdenum, chlorine, magnesium, iron, selenium, iodine, manganese, copper, cobalt and zinc. To these may be added fluorine and chromium which have also been shown to be essential for land animals.

The prominence of each mineral element in body tissues is closely related to its functional role. As constituents of bones and teeth, minerals provide strength and rigidity to skeletal structures. In their ionic states in body fluids they are indispensable for the maintenance of acid-base equilibrium and osmotic relationship with the aquatic environment, and for integration activities involving the nervous and endocrine systems. As components of blood pigments, enzymes and organic compounds in tissues and organs, they are indispensable for essential metabolic processes involving gas exchange and energy transactions.

Medicinal herbs used in indigenous medicines in crude forms for the management of diabetes mellitus, contain both the organic and inorganic

constituents. It is known that certain inorganic mineral elements (potassium, zinc, calcium, traces of chromium, etc.) play an important role in the maintenance of normal glucose-tolerance and in the release of insulin from beta cells of islets of Langerhans. Although silicon is not an essential nutrient, its application is beneficial for plant growth and development (**Hamilton *et al.*, 2004**). Animals also require iron to maintain the activity of many important enzymes and for vital haem proteins such as haemoglobin, myoglobin and cytochromes which are involved in oxygen transport and energy metabolism, respectively.

Calcium

Calcium is the most promoted nutrient by proponents of conventional, nutritional and alternative medicine. Calcium is also the important constituent of middle lamella in cell wall and plays an important role in the formation of cell membrane. Its deficiency in plant causes chlorosis, malformation of younger leaves, disintegration of growing meristematic regions of root etc. In human body the desirability of about 0.4 to 0.6 g calcium has been suggested.

Sodium

Sodium is concentrated mainly in the extracellular fluid. The human diet must contain a sensible amount of sodium. The sodium cation is the main extracellular cation and is important for nerve function in animals. In animals the ratio sodium/ potassium concentration in intercellular and extracellular fluids is responsible for the transport of ions through the cellular membranes and the regulation of the osmotic pressure inside the cell.

Potassium

Potassium cation has an extremely important role in several biological reactions and vital manifestations, such as transmission of nervous pulses to produce muscular contraction in animals. Plants can absorb potassium ions by the soil through the humus compound, or through clay or natural Zeolites. Potassium is the major mineral in most root crops while sodium tends to be low. This makes some root crops particularly valuable in the diet of patients with high blood pressure, who have to restrict their sodium intake. In such cases the high potassium to sodium ratio may be an additional benefit (**Meneely & Battarblee, 1976**). However, high potassium foods are usually omitted in the diet of people with renal failure (**McCay *et al.*, 1975**).

Iron

Iron is main constituent of haemoglobin and myoglobin. It is also needed in nonhaem iron proteins and intercellular haem protein cells i. e. cytochromes. A deficiency of dietary iron causes anemia. Iron deficiency in plants also causes decrement in protein content, increment in soluble nitrogen compound and disturbs Chlorophyll production (**Montgomery *et al.*, 1990**). The nutrition accept group of the Indian Council of Medical Research recommended on allowance of 20-30 mg of iron in the balance diet for an adult. The RDAs for iron are 10 mg/day for men and 15 mg/day for women (**NRC, 1989**). The deficiency of iron is a problem of global concern. Iron deficiency reduces learning and working capacity as well as appetite (**Pollit, 1993**). Hence the inclusion of iron as one of the food fortifants which is aimed at reducing micronutrient deficiencies (**Blum, 1997**).

Copper

Copper acts as a catalyst in the storage and release of iron to form haemoglobin (**American Medical Association, 1995**). Its ability to catalyse the oxidation of ferrous ion into ferric state (ferroxidase activity) makes it an important

antioxidant in vivo (**Scholes, 1983**). Copper is an essential component of many enzymes including the antioxidant enzyme, superoxide dismutase (**Valentine & De Freitas, 1985**). The antioxidant defense protects the body against the deleterious effects of free radicals (**Halliwell & Gutteridge, 1989**), hence, a healthy body requires stores of copper. Unfortunately, suboptimal intake of copper is common in developing countries (**Olivares & Uauy, 1996**).

Zinc

Zinc shows an antioxidant role in defined chemical system. Administration of pharmacological doses of zinc in vivo, has protective effects against general and liver specific peroxidants. Dietary Zinc deficiency causes increased susceptibility to oxidative damage (**Niri, 1991**). The RDA for zinc in adults is 15 mg (**NRC, 1989 and Sandstead, 1995**). The effects of Zn deficiency include delayed wound healing, suboptimal immune functions, increased plasma lipid peroxides and reduced taste/smell acuity (**Fortes *et al.*, 1997**).

Manganese

Manganese shows a free radical scavenging activity. The chain breaking antioxidant capacity of manganese seems to be related to the rapid quenching of peroxyl radicals (**Caussin, 1992**). In the biological functions, manganese act as cofactor of enzymatic reaction-peptidase, polymerases, carboxylase, pyruvate. Manganese also function as phosphorylation chlolesterol and in fatty acid synthesis.

Cobalt

Cobalt is the important constituent of Vitamin B_{12} (cyanocobalamin) which is needed to maintain normal bone marrow function for producing erythrocytes. Therefore, deficiency of cobalt gives rise to limitation of Vitamin B_{12}.

Antioxidants

Antioxidants are the chemicals that oppose or neutralize oxidation in cells. Some of the most important ones are essential vitamins but not all; secondary plant metabolites are also antioxidants. Normal physiological processes synthesize most of the antioxidants in the human body (at any one time), while the remaining comes from food. All dietary sources of antioxidants come from secondary plant metabolites. Some are polyphenols, α-carotene, β-carotene and lycopene. These carotenoid antioxidants lack the phenolic ring structures that characterize polyphenols.

Vitamin A is essential for normal growth, vision, immune response and cell differentiation (**Sommer &West, 1996**). The intake of vitamin A recommended by FAO is 750µg retinol per day for adults, with lactating mothers require 50% more whereas children and infants require less **(FAO, 1988 and Latham, 1997**). The deficiency of this vitamin is of public health concern in many developing countries. Available data (**UNICEF, 1994**) indicates that in Nigeria, vitamin A deficiency affects 9.2% of children and 7.2% of mothers. Vitamin A deficiency has been associated with increased respiratory infections, risk of diarrhoea and decreased immune response (**Sommer & West, 1996**).

Free Radicals

Free radicals are oxygen-based or nitrogen-based molecules with unpaired electrons that are generated by a number of metabolic processes within the body. For example, when the body turns foods into energy, free radicals are formed by normal oxidation reactions. Vigorous exercise increases free radical production, as does inflammation, exposure to certain chemicals, cigarette smoke, alcohol, air pollutants and high-fat diets. Normally a body can handle free radicals, but if antioxidants are unavailable, or if the free-radical production becomes excessive, damage can occur.

- They can damage cell membranes and interact with genetic material, possibly contributing to the development of a number of disorders including cancer, heart disease, cataract, and even the ageing process itself.
- Oxygen-free radicals can also enhance the dangerous properties of low-density lipoprotein (LDL) cholesterol, a major player in the development of atherosclerosis.

Major Free radicals in biological systems

- Peroxyl radical ($ROO^{\cdot}$), which is the most common radical in biological systems.
- Hydroxyl radical ($^{\cdot}OH$), which is always harmful.
- Superoxide radical ($O_2^{\cdot -}$), which is produced by phagocytic cells and can be beneficial in inactivating viruses and bacteria.
- Nitric oxide is a vasodilator agent and can function as a neurotransmitter and can be produced by macrophages and act to kill parasites. Nitric oxide may also be harmful when it reacts with superoxide to form the peroxynitrite anion.
- Hydrogen peroxide (H_2O_2), which is not a free radical, but can cause damaging oxidative events in cells.

Reactive oxygen species (ROS) or free radicals are generated as byproducts or intermediates of aerobic metabolism and through reactions with drugs and environmental toxins. The elevated cellular levels of free radicals cause damage to nucleic acids, proteins, and membrane lipids and are associated with many ageing related problems including carcinogenesis and heart diseases (**Halliwell *et al*., 1992; Halliwell, 1996 and Wang & Jiao, 2000**). The balance between the production and scavenging of ROS can therefore determine the susceptibility of the body to oxidative damage. Although almost all organisms

possess antioxidant defense and repair systems, which quench or minimize the production of oxygen-derived species, thus protecting them against oxidative damage, these protective systems are insufficient to entirely prevent the damage (**Simic, 1988**). The phenolic compounds, which are widely distributed in plants, were considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living systems (**Block, 1992 and Hertog & Feskens, 1993**). The study of numerous compounds that could be useful antioxidants has generated increasing interest in the field of food or medicine (**Usoh *et al.*, 200**5).

Vitamin E is a lipid soluble free radical scavenger which protects the membrane from lipid peroxyl radical (**Buttner & Burns, 1996**). Similarly, vitamin C is the water soluble antioxidant which reacts with peroxyl radicals formed in the cytoplasm before they reach the membrane (**Khoja & Marzouki, 1994**) and serves to regenerate the reduced Vitamin E (**Tanaka *et al.*, 1997**).

A good number of studies have established the effectiveness of antioxidant Vitamins against oxidative stress (**Farris, 1991; Verma & Nair, 2001; Ognjanovic *et al.*, 2003**). However, in diabetics, the production of free radicals is accelerated due to an abnormal metabolic regulation function, thereby easily causing oxidative damage (**McCord *et al.*, 1969**).

Vitamin E is a naturally occurring antioxidant available in the biological system. It was postulated that tocotrienol are more mobile and less restricted in their interactions with lipid radicals in the membrane than tocopherol (**Serbinova *et al.*, 1991**). The biological activity of vitamin E is believed to be due to its antioxidant action to inhibit lipid peroxidation in biological membranes by scavenging the peroxyl chain reaction. Studies have shown that tocotrienol to be a more potent antioxidant as compared to tocopherol (**Serbinova & Packer, 1994**).

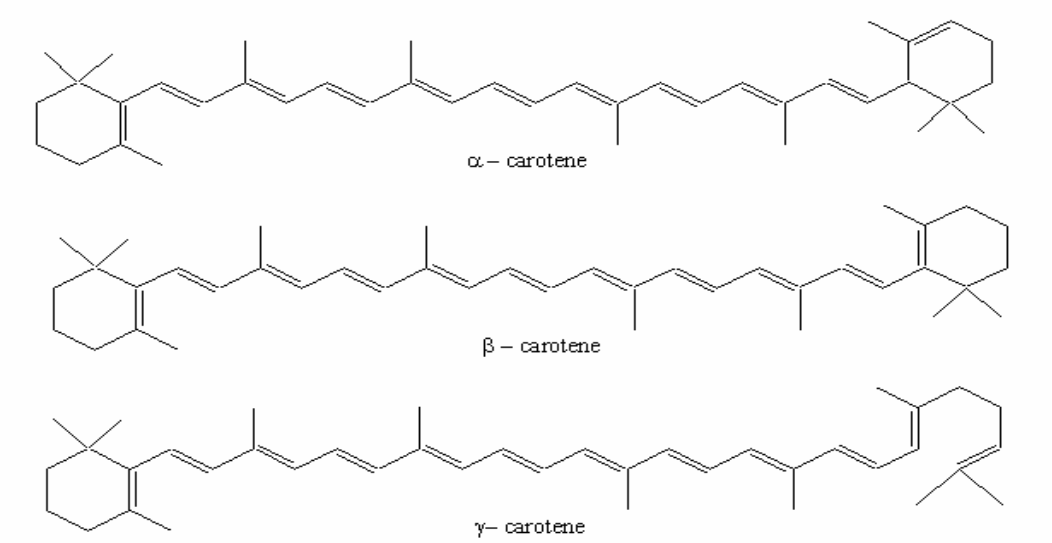
Nearly 50% of all deaths in Germany and other industrial countries are related to cardiovascular diseases (CVD) (**Statistisches Bundesamt, 2003**). Most of the cardiovascular diseases are caused by atherosclerosis, which itself depends upon a multifactorial process, with hypertension, smoking, obesity, and elevated serum low-density lipoprotein concentrations as important associated risk factors (**Hennekens, 1998**). Although it is not yet known whether an elevated homocysteine concentration is a causative factor in the development of atherosclerosis, some authors consider hyperhomocysteinemia to be an important and independent risk factor for cardiovascular diseases (**Hackman & Anand, 2003; Spiller, 1998; Refsum *et al*., 1998 and Boushey, 1995**).

Carotenoids

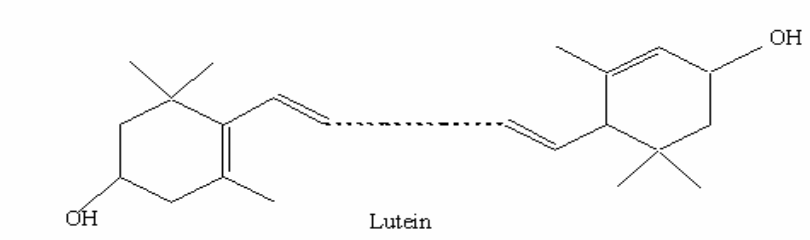
Carotenoids are yellow or red pigments which are widely distributed in plants and animals. Chemically, carotenoids are polyenes and almost all the carotenoid hydrocarbons have the molecular formula C_{40} H_{56}.

Carotenes : Carotene was first isolated from carrots. There are three isomers of carotene. α carotene, violet crystals, m.p.187-187.5 ^{0}C; optically active (dextrorotatory), β carotene, red crystals m.p.183 ^{0}C ; optically inactive, γ carotene, dark red crystals, m.p. 152-154 ^{0}C ; optically inactive.

It appears that all the three carotenes occur together in nature but their relative proportions may vary with the source e.g. carrots contain 15% α 85% β and 0.1% γ carotene.



Xanthophylls : These are naturally occurring carotenoids which contain an oxygen function. Lutein, mol. Formula $C_{40}H_{56}O_2$, m.p. 193^oC, formerly known as xanthophyll is 3, 3` dihydroxy -α- carotene.



Wide range of carotenoids have been identified in human ocular tissues. The major carotenoid constituents in human macula are lutein and zeaxanthin (**Bone et al., 1993 and Bernstein et al., 2001**). Epidemilogical studies have shown that concentration of macular carotenoids can be manipulated by dietary intake of lutein and zeaxanthin and that these carotenoids may play an important role in the prevention of age related macular degeneration (AMD) (**Landrum *et al.*, 1997 and Gerster, 1991**). Carotenoids have proved to be effective radical scavengers at low partial pressure encountered in the eye (**Gerster, 1991 and Schalch & Weber, 1994**). This has been proposed as one of the mechanisms by which lutein and zeaxanthin may protect the eyes.

β-Carotene has been proved to prevent peroxidation caused by singlet oxygen and also by scavenging free radicals (**Masio *et al.*, 1991 and Krinskys, 1989**). Carotenoids are mainly responsible for the prevention of harmful effects of singlet oxygen (**Enrique, 1989**).

MATERIALS AND METHODS

Biochemical Analysis

The plants were collected from Pithoragarh District, Uttarakhand, India in the month of Feb, 2005. The leaves were dried in shade after collection. The dried leaves were powdered separately in electric mill to 60-mesh size. The fine powder obtained was used for further nutraceutical antioxidant and mineral analysis.

Moisture

Approximately 10.0 g of fresh sample was taken in a dried weighed dish and it was kept in an air circulating oven first at 40 ^{0}C for two hours, then followed at 60 ^{0}C and 80 ^{0}C respectively. Finally, the sample was kept between 110 to150 ^{0}C for another two hours. Thereafter, the samples were cooled and again weighed using electronic balance. The loss in weight of the sample was reported as moisture percentage and calculated following the **(A.O.A.C., 1990**) method.

% Moisture = w X 100/W

Where: w = loss in weight of fresh sample and W = weight of fresh sample taken.

Crude protein

Crude protein was calculated as N x 6.25 (based on assumption that nitrogen constitutes 16.0% of a protein). Total nitrogen was estimated by (**Snell & Snell, 1995**) method. Triple acid (perchloric acid, hydrochloric acid and hydrogen peroxide (2.5: 2.5:1.0) digestion sample was used for the analysis of nitrogen. 1 ml of prepared acid solution of plant material was pipette out into 50 ml

volumetric flask. To this aliquot 1 ml of 10% sodium hydroxide and 1 ml of 10% sodium silicate was added, then solution was diluted to 35 ml. To this mixture, 1 ml of Nesseler's Reagent was added and made the volume up to the mark. The colour intensity was measured by spectrophotometer HITACHI, model U-2001 after 15 min at 420 nm using a reagent as blank, with the help of standard curve and the amount of nitrogen in sample was calculated. Ammonium chloride was used as a standard for nitrogen. The crude protein was calculated by the following formula.

Crude protein % = Nitrogen % X 6.25

Crude fat

The content of crude fat was estimated by (**A.O.A.C, 1970**) method. Dry powdered (1 g) sample was taken in an extraction thimble and placed in a soxhlet extractor fitted with condenser and flask containing petroleum ether (40 to 60 ^{o}C BP). Thereafter the flask was put on heating mantle especially designed for this purpose and fixed at 40 ^{o}C. The extraction was carried out for 6 hours. The thimble was then removed and flask was again heated so that the extractor was filled up to about two third with petroleum ether and only a small quantity of it was left in the flask. The residual petroleum ether containing crude fat was filtered. Flask and filter paper were washed carefully. Filtrate was evaporated and the beaker was again weighed after cooling. Weight of residue was calculated on the fresh weight basis and reported as crude fat.

Crude fat (%) = (w/W) x 100

where: w = Weight of residue, W = Weight of sample

Carbohydrate

Total carbohydrate content in plant leaves was estimated by the Phenol sulphuric acid method (**Dubois *et al.*, 1956**). The carbohydrate present in powered leaves was first hydrolyzed into simple sugar by acid hydrolysis (5 ml of 2.5 N HCl) for 3 hours. It was then neutralized with solid sodium carbonate until the effervescence ceases and then centrifuged. Finally the volume was made upto 100 ml. 0.5 to 1 ml supernatant were pipetted for analysis. 1 ml of 5 % phenol solution and 5 ml of 96 % sulphuric acid was added to 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard concentration of 100 mg/lit in a test tube. The absorbance was measured at 490nm after 10min. Total carbohydrate percent in the sample solution was calculated using the standard graph.

Absorbance corresponds to 0.1ml of test = x mg of glucose

Total carbohydrate (%) = X / 0.1 x 100 mg of glucose

Where; x = Spectrophotometer reading

Crude fiber

Crude fiber was estimated in dried powdered sample by the method of (**Maynard, 1970**). This method is based on the imitation of gastric and intestinal action. In a 250 ml beaker, 2.0 g of powdered sample was taken. previously marked at 100 ml level. To the beaker, 200 ml of 1.25 % Sulphuric acid (25 ml of 10 % Sulphuric acid in 175 ml water) was added and digested on a hot plate for two hours and stirred regularly. The solution was filtered through a muslin cloth by giving suction for filtration. The residue was washed several times by distilled water till it became acid free. Again the solution was transferred to original beaker and 200 ml of 1.25 % NaOH were added to it (25 ml of 10 % NaOH +175 ml water). This was again digested on hot plate, filtered and washed to make it free from alkali. The residue thereafter was washed three times with rectified spirit to remove the moisture. The residue was washed three

times with acetone to make it free from fatty compounds. The residue was dried and weighed and ignited to ash in a muffle furnace and weight of ash was determined. This weight of ash was subtracted from the weight of residue and crude fiber and calculated as following:

Crude fiber (w) = weight of residue – weight of ash

Crude fiber (%) = (w / W) X 100

Where: w = Weight of crude fiber, W = Weight of sample taken

Vitamin –C (Ascorbic Acid)

Ascorbic acid content was estimated by (**Ranganna, 1976**) method with little modification. Dried leaves powder (2.0 g) was extracted with 4 % oxalic acid, made up to 100 ml, and centrifuged at 10,000 rpm for 10 minutes. 5 ml supernatant liquid was transferred in a conical flask, 10 ml of 4 % oxalic acid was added and finally titrated against standard dye solution (2, 6-dichlorophenol indophenol). The procedure was repeated with a blank solution omitting the sample. 5 ml ascorbic acid with concentration (100 ppm) was used as standard.

$$\text{Ascorbic Acid mg/100g} = 0.5 \text{ mg} \times V_2 \times 100 \times 100 / V_1 \times 5 \times W$$

Where V_1 = Dye used for titration of standard, V_2 = Dye used for titration of sample, W = Weight of samples

Ash and Silica

Ash content was estimated by (**A.O.A.C., 1985**) method and ash insoluble content was estimated by (**Peach *et al.*, 1956 and Mishra, 1968**) method. The samples were burned in a muffle furnace at 550 ^{0}C and then oxidized. Ash was

dissolved in acidic water and insoluble content was reported as silica. Acid soluble part is reported as acid soluble ash.

Ash

The total ash was determined as described by (**A.O.A.C., 1985 and Peach *et al.*, 1956**). Dried sample (2 g) was taken in a silica crucible and burnt in muffle furnace at 550 ^{0}C until it was free from carbon. Muffle furnace was allowed to cool. Crucible was taken out of furnace, cooled in a desiccator and weighted. Total ash was calculated by following formula:

%Total ash= w X 100/ W

Where w - Weight of ash, W - Weight of sample

Silica

Silica was determined as described by (**Peach *et al.*, 1956 and Mishra, 1968**) method. Conc. HCl (10 ml) was added to the ash in the crucible and covered with a clock glass. The solution was heated for 30 minutes and clock glass was removed by rinsing and heated again to dryness. Dilute HCl was added to the residue and heated for dissolving the soluble ash and contents were filtered through Whatmann No. 44 paper in a 100 ml volumetric flask. The crucible as well as residue on filter paper were rinsed with water and volume was made up to 100ml. The residue left on paper was burnt, weighed and percentage of silica was calculated by following formula:

%Silica= w /W X 100

Where w - Weight of silica, W - Weight of sample

Chlorophyll

The chlorophyll content in dried leaves powder was estimated (**Witham *et al.*, 1971**) methods. Dried leaves powder (0.5 g) was extracted with 20 ml 80 %

acetone (prechilled), centrifuged at 5000 rpm for 5 min and transferred the supernatant to a 100 ml volumetric flask. The residue was ground with 20 ml of 80 % acetone, centrifuged and the supernatant was collected. This procedure was repeated until the residue was colourless. The volume was made up to 100 ml with 80% acetone and the absorbance was measured at 645 and 663 nm. The amount of chlorophyll a and b present in sample was calculated by following formula:

Chlorophyll a (mg /100g) = 12.7 (A_{663}) – 2.69 (A_{645}) X V/10 x W

Chlorophyll b (mg /100g) = 22.9 (A_{645}) – 2.69 (A_{663}) X V/10 x W

Where A = Absorbance of specific wavelengths, V = Final volume of chlorophyll extract in 80% acetone, W = Weight of sample.

Phenolics

Total phenolic content was estimated by (**Singleton, 1999**) method with minor modification. Dried leaves powder (0.5 g) was extracted with 10 time volume of sample with 80 % ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes and the supernatant collected. The residue was re-extracted three times with 80 % ethanol, centrifuged and supernatant was collected. The supernatant was evaporated to dryness. The residue was dissolved in 5 ml distilled water. 1 ml aliquot was taken in a test tube and 0.5 ml Folin-Ciocalteau reagent was added to it. After three minutes, 2 ml of 20 % sodium carbonate solution was added to test tube and the absorbance was measured at 650 nm. Catechol was used as a standard. The concentration of phenolics in test sample was calculated and expressed as mg phenolics/100g samples.

Mineral

Mineral content in plant was estimated by wet digestion method. 2 g of plant material was first digested with two successive aliquots of conc. HNO_3 (5 ml each). After burning, the organic matter was slowly digested with 15 ml of triple acid mixture (HCl, $HClO_4$, H_2O 2:2:1 v/v) at 200^0C and reduced to about 1 ml. The residue after digestion was dissolved in double distilled water, filtered and diluted to 100 ml. This solution was used for the estimation of minerals. Macro minerals viz., Na, K, Ca and Li were estimated by SYSTRONICS Flame photometer 128 model. Phosphorous content was estimated by (**Allen, 1974**) method. Micro elements were analyzed by AAS.

Estimation of Sodium, Potassium, Calcium and Lithium

The samples were digested in triple acid for the estimation of sodium, potassium, calcium and lithium content. Sodium was determined by using flame photometer. Standard, blank and sample solutions of each were passed through flame photometer and concentration of sodium was determined in ppm. Blank was prepared in a similar manner to that of standard except that only distilled water was used in place of sodium chloride, potassium chloride, calcium carbonate, lithium chloride. Following formula was used to calculate mg of these in 100g of leaves sample:

Sodium (mg/100g) = (Y / W) X 10

Where Y = flame photometer readings, W = weight of sample

Potassium (mg/100g) = (Y / W) X 10

Where Y = flame photometer readings, W = weight of sample

Calcium (mg/100g) = (Y / w) X 10

Where Y = flame photometer readings, W = weight of sample

Lithium (mg/100g) = (Y / W) X 10

Where Y = flame photometer riding, W = weight of sample

Estimation of Phosphorus

Phosphorus was estimated by (**Allens, 1974**) method. Sample was digested in triple acid and taken for the estimation of phosphorus. 1ml of 4% ascorbic acid and 4 ml of molybadate reagent were added into 1 ml of digested sample, mixed well, and left for 1h for colour developing. The standard curve was made by different concentration of standard 0-20 µg phosphorus per liter. The concentration of phosphorus was calculated by using the following formula:

Phosphorus (mg/100g) = (X / W) x 10

Where X = ppm Phosphorus in 1.0 ml, W = weight of sample

The absorption spectra of micro mineral estimated by Atomic Absorption spectroscopy, model - 4129, Electronic Corporation of India Ltd., Hyderabad.

Amino Acid Analysis

Amino acid analysis was performed using the Waters Associates PICO-TAG method (**Bidlingmeyer *et al.*, 1984**), an integrated technique for precolumn derivatization of amino acids using phenylisothiocyanate (PITC). The PICO-TAG technique comprises of three steps: (i) Hydrolysis of protein or peptide samples to yield free amino acids, (ii) pre-column derivatization of the samples with PITC and (iii) analysis by reverse phase HPLC. The chromatographic separation on the hydrolyzates was performed using a reverse phase Pico-Tag column (3.9 x 300 mm) C_{18} at 40^0C and a UV detector at 254 nm. The solvent system consisted of two eluents, (A) an aqueous buffer and (B) 60% acetonitrile in water. Gradient elutions were employed using two pumps, programmed to

deliver the mobile phases eluents A and B. A gradient which was run for the separation consisted of 10% B traversing to 51% B in 10 min using a convex curve (number 5). A set of amino acid standards (Merck Germany) was analyzed with each set of three experimental samples. Identification of the amino acids in the samples was carried out by comparison with the retention times of the standards.

Isolation and Extraction of Carotenoid and Tocopherol

The plant material was dried in shade and powdered using electrical grinder (Philips-HL1616). Dried plant material (1.0g of each) was extracted with light petroleum ether/methanol/ethyl acetate (1:1:1, V/V/V, 4 x 30 ml) until the extracts were colourless. The extracts were mixed in a 250 ml separating funnel and shaken vigorously and allowed to stand for phase separation. Upper layer was collected and lower layer was shaken with 50 ml water and 50ml petroleum ether for phase separation. Again upper layer was mixed with the first extract. The organic extract was dried over anhydrous sodium sulphate (10 g), filtered and evaporated to dryness in a Rotary Vacuum Evaporator under reduced pressure. The residue was dissolved in light Petroleum ether (5 ml) and filtered by 0.2 μm membrane filter before HPLC analysis (**Dietmar *et al.*, 2002**).

HPLC Analysis

The samples were analyzed by Shimadzu HPLC system, column used was C_{18} phenomenex$^{(R)}$ (5 μ, 150 x 4.6 mm analytical column) with solvent system 8:2:40:50 (methanol, ethyl acetate, acetonitrile and acetone) flow rate 0.7 ml/min, run time 20 minutes and detector wavelength was 450 nm. The HPLC condition of the estimation DL-α-tocopherol as described by**(Kurilich *et al.*, 1999**) methods at 291nm.

RESULTS AND DISCUSSION

Biochemical Composition of *Pouzolzia hirta*

The amount of certain nutrients in *Pouzolzia* rhizomes is presented in Table-3.2. Crude lipid, protein and total carbohydrate content in *Pouzolzia* rhizomes were found 6.02 ± 0.59, 4.24 ± 0.02 and 36.52 ± 0.38g.100g^{-1} respectively on dry weight basis. The ranges were 5.33-6.78, 12.86 – 12.87 and 12.42 – 14.34 mg.100g^{-1} of crude lipid, protein and total carbohydrate respectively. Starch, amylose and amylopectin content in *Pouzolzia* were found 33.05 ± 0.64, 4.86 ± 0.21 and 28.19 ± 0.49 g.100g^{-1} respectively.

The cellulose, crude fiber and moisture content were found 4.10 ±0.39, 12.35 ± 0.25 and 66.09 ± 0.21 g.100g^{-1} respectively. The ash content was found 5.79 ± 0.09 g.100 g^{-1} on dry weight basis. Acid insoluble ash was found 1.17 ± 0.41 g.100g^{-1} and acid soluble ash was found 4.58 ± 0.30 g.100g^{-1}. The energy content of plant rhizome was determined by multiplying the crude protein, crude lipid and total carbohydrate content by the factor 4, 9 and 4 respectively (**Osborne & Voogt, 1978**). Calorific value of the plant rhizomes was found 217.22 K.Cal.100 g^{-1}. The content of tannins in plant was found 1663.84 ± 0.45 mg.100g^{-1}. The range of tannin was 1663.33 - 1664.43 mg.100 g^{-1}.

The mineral content of *Pouzolzia* rhizomes are presented in Table 3.3. The contents of sodium, potassium, calcium and lithium in *Pouzolzia* rhizome were found 157.69 ± 0.47, 4106.36 ± 0.43, 178.42 ± 0.41 and 42.28 ± 0.13 mg.100g^{-1} respectively on dry weight basis.

The present study was carried out to assess the levels of some macro and micro elements in medicinal plants in order to focus the contribution of these Ayurvedic plants to the mineral intake of consumers. The importance of this study becomes evident when considered against the claims by manufacturers that the mineral element content of their products have been considerably enhanced.

Table 3.2. Nutrients composition investigated in *Pouzolzia hirta* rhizomes.

Biochemical Parameter	Composition ($g.100g^{-1}$)	Range ($g.100g^{-1}$)
1. Moisture	66.09± 0.21	65.89-66.38
2. Crude protein (Kjeldahl N x 6.25)	4.24±0.02	4.22-4.26
3. Crude fat	6.02±0.59	5.33-6.78
4. Total carbohydrate	36.52±0.38	36.04-36.97
5. Starch	33.05±0.64	32.47-33.94
6. Amylose	4.86±0.21	4.58-5.06
7. Amylopctin	28.19±0.49	27.79-28.88
8. Cellulose	4.10±0.39	3.69-4.62
9. Crude Fiber	12.35±0.25	12.14-12.40
10. Ash	5.79±0.09	5.69-5.90
11. Acid soluble ash	4.58±0.30	4.17-4.89
12. Acid insoluble ash	1.17±0.41	0.80-1.63
13. Tannins	1663.84 ± 0.45	1663.33-1664.43
14. Calorific value (Kcal 100 g^{-1} DM)	217.22	

All values are mean of triplicate determinations expressed on dry weight basis.

± Denotes the standard error.

Table 3.3. Mineral composition investigated in *Pouzolzia hirta* rhizomes.

Mineral	Composition ($mg.100g^{-1}$)	Range ($mg.100g^{-1}$)
Sodium – Na	157.69 ± 0.47	157.04 -158.11
Potassium – K	4106.36 ± 0.43	4105.99 - 4106.97
Calcium - Ca	178.42 ± 0.41	178.04 - 178.99
Lithium – Li	42.28 ± 0.13	42.11 - 42.42
Nitrogen – N	678.55 ± 2.15	676.04 - 681.30
Phosphorus – P	216.32 ± 0.64	215.49 - 217.07
Sulphur – S	464.06 ± 1.00	462.64 - 464.82
Iron – Fe	83.04 ± 0.53	82.44 - 83.72
Copper - Cu	3.61 ± 0.32	3.18 - 3.96
Manganese – Mn	8.49 ± 0.32	8.21 - 8.93
Zinc – Zn	16.68 ± 0.49	16.00 - 17.12
Cobalt - Co	0.00	-

All values are mean of triplicate determinations expressed on dry weight basis.

± Denotes the standard error.

Ranges were 157.04 -158.11, 4105.99 - 4106.97, 178.04 - 178.99 and 42.11 - 42.42 $mg.100g^{-1}$ of Na, K, Ca and Li respectively. The contents of nitrogen, phosphorus and sulphur were found 678.55 ± 2.15, 216.32 ± 0.64 and 464.06 ± 1.00 $mg.100g^{-1}$ respectively on dry weight basis. The contents of iron, copper, manganese, zinc and cobalt in *Pouzolzia* rhizomes were found 83.04 ± 0.53, 3.61 ± 0.32, 8.49 ± 0.32, 16.68 ± 0.49 and 0 respectively on dry weight basis. The ranges were 82.44 - 83.72, 3.18 - 3.96, 8.21 - 8.93, 16.00 - 17.12and 0.0 $mg.100g^{-1}$ of Fe, Cu, Mn, Zn and Co respectively. This is the first work of analysis of nutraceutical antioxidants and minerals in P. hirta, rhizomes.

Deficiency of copper may cause hypertension, antibiotic sensitivity, hyperactivity, hyperglycemia, manic disorders, insomnia, allergies and osteoporosis (**Watts, 1997**). Calcium plays a major role in CNS function. It is essential for nerve impulse conduction and activates some enzymes, which generate neurotransmitters (**Watts, 1997**). Phosphorous is tied to calcium in bone structure and plays a significant role in CNS function. Many enzymes contain phosphoproteins as a base. Phospholipids are involved in nerve conduction. Phosphate is the primary ion in extra and intracellular fluid. It aids absorption of dietary constituents, helps to maintain the blood at a slightly alkaline level regulates enzyme activity and is involved in the transmission of nerve impulses (**Watts, 1997**).

Potassium plays very important role in various activities in the human body such as activation of many enzymes, stimulation of the movement of intestinal tract and also used for protein synthesis. Excess of potassium can produce neurological disturbances such as numbness of hand and feet (**Watts, 1997**). Zinc is extremely important for numerous body functions. Zinc deficiency is associated with mental impairments, mental lethargy, emotional disorder and irritability (**Watts, 1997**). Iron plays a significant role in oxygen transport in the body. Disturbance in mental function can be caused by flows in the metabolic pathways that require iron. This is because of too little oxygen reaching the brain. Iron is required for DNA synthesis. It is also necessary for the activation of enzymes involved in brain neurotransmitters (**Watts, 1997**).

Thus the study concludes that the rhizomes of *P. hirta* are excellent source of nutrients, antioxidants and minerals. The distribution of these compounds in common wild plants has an important application for the health of people in addition to the basic need of developing countries. There is a great need to further research. The composition knowledge of these materials could help in developing technological processes to destroy, eliminate or inactivate toxic

antinutritional factors to make the plant rhizomes safe for eating and more digestible.

Biochemical Analysis in *Euphorbia thymifolia*

Biochemical and certain nutrient contents in aerial parts of *Euphorbia* plants is presented in Table 3.4. The amount of chlorophyll-a and chlorophyll-b in aerial parts of plants were found 115.37 ± 1.05 and 72.98 ± 0.51 mg.100 g^{-1} on dry weight basis. The content of tannins in plant was found 2465.74 ± 0.72 mg.100 g^{-1}. The range of tannin was 2464.92 – 2466.67 mg.100 g^{-1}.

Table 3.4. Phytochemical composition in aerial parts of *Euphorbia thymifolia* Linn.

Phytochemical	**Composition (mg.100g^{-1})**	**Range (mg. 100 g^{-1})**
1) Chlorophyll-a	115.37 ± 1.05	114.16 - 116.73
2) Chlorophyll-b	72.98 ± 0.51	72.32 - 73.57
3) Tannins	2465.74 ± 0.72	2464.92 - 2466.67

All values are mean of triplicate determinations expressed on dry weight basis.

± Denotes the standard error.

The amount of certain nutrients in aerial parts of plants are presented in Table 3.5. Crude lipid, protein and total carbohydrate content in aerial parts of plants were found 4.63 ± 0.44, 13.42 ± 0.37 and 11.99 ± 0.35 g.100g^{-1} respectively on dry weight basis. The range were, 4.01- 4.98, 12.96 – 13.87 and 11.58 – 12.44 mg.100g^{-1} of crude lipid, protein and total carbohydrate respectively. Starch, amylose and amylopectin content in *Euphorbia* were found 22.31 ± 0.48, 1.39 ± 0.28 and 20.93 ± 0.58 g.100g^{-1} respectively.

The cellulose, crude fiber and moisture content were found 4.35 ± 0.69, 24.34 ± 0.64 and 78.60 ± 0.59 g.100g^{-1} respectively. The ash content was found

10.29±0.08 g.100 g^{-1} on dry weight basis. Acid insoluble ash was found 3.24 ± 2.9 g.100 g^{-1} and acid soluble ash was found 7.07 ± 0.38 g.100g^{-1}. The energy content of plant aerial parts of plants was determined by multiplying the crude protein, crude lipid and total carbohydrate content by the factor 4, 9 and 4 respectively (**Osborne & Voogt, 1978**). The content of vitamin C in aerial parts of plants was found 88.48 ± 0.95 mg.100 g^{-1} on dry weight basis. The range of vitamin C was 87.37 – 89.68 mg.100 g^{-1}. Calorific value of the aerial parts of plant was found 143.32 K.Cal.100 g^{-1}.

Table 3.5 Nutrients composition in aerial parts of *Euphorbia thymifolia* Linn.

Biochemical Parameter	**Composition ($g.100g^{-1}$)**	**Range ($g.100g^{-1}$)**
1. Moisture	78.60 ± 0.59	77.96 - 78.99
2. Crude protein (Kjeldahl N x 6.25)	13.42 ± 0.37	12.96 -13.87
3. Crude fat	4.63 ± 0.44	4.01 - 4.98
4. Total carbohydrate	11.99 ± 0.35	11.58 -12.44
5. Starch	22.31 ± 0.48	21.78 -22.95
6. Amylose	1.39 ± 0.28	1.14 -1.79
7. Amylopectin	20.93 ± 0.58	20.42 - 21.74
8. Cellulose	4.35 ± 0.69	3.38 - 4.96
9. Crude fiber	24.34 ± 0.64	23.47 -24.98
10. Ash	10.29 ± 0.08	10.18 -10.34
11. Acid soluble ash	7.07 ± 0.38	6.53 - 7.37
12. Acid insoluble ash	3.24 ± 2.9	3.03 - 3.65
13. Vitamin C	88.48 ± 0.95	87.37 - 89.68
14. Calorific value (Kcal 100 g^{-1} DM)	143.31	–

All values are mean of triplicate determinations expressed on dry weight basis.

± Denotes the standard error.

Minerals are called as “spark plugs of life” because they are required to activate hundreds of enzyme reactions within the body. Life depends upon the body’s ability to maintain balance between the minerals (**Watts, 1997**). The mineral content of aerial parts of plant is presented in Table 3.6. The amount of sodium, potassium, calcium and lithium in aerial parts of plants were found 75.40 ± 0.74, 4786.48 ± 0.81, 242.46 ± 0.56 and 46.64 ± 1.36 $mg.100g^{-1}$ respectively on dry weight basis. Ranges were 74.36 -76.05, 4785.34 - 4787.14, 241.68 - 242.96 and 45.06 - 48.37 $mg.100g^{-1}$ of Na, K, Ca and Li respectively.

Table 3.6 - Mineral composition in aerial parts of *Euphorbia thymifolia* Linn.

Mineral	**Composition ($mg.100g^{-1}$)**	**Range ($mg.100g^{-1}$)**
Sodium – Na	75.40 ± 0.74	74.36 -76.05
Potassium – K	4786.48 ± 0.81	4785.34 - 4787.14
Calcium - Ca	242.46 ± 0.56	241.68 - 242.96
Lithium – Li	46.64 ± 1.36	45.06 - 48.37
Nitrogen – N	2151.85 ± 1.35	2150.49 - 2153.69
Phosphorus – P	226.81 ± 0.47	226.15 - 226.32
Sulphur – S	325.24 ± 0.76	324.19 - 325.96
Iron – Fe	121.04 ± 0.38	120.63 -121.55
Copper - Cu	3.45 ± 0.29	3.04 -3.68
Manganese – Mn	9.28 ± 0.30	8.96 - 9.69
Zinc – Zn	6.47 ± 0.32	6.04 - 6.78
Cobalt - Co	0.00	-

All values are mean of triplicate determinations expressed on dry weight basis.

± Denotes the standard error.

The amount of nitrogen, phosphorus and sulphur were found 2151.85 ± 1.35, 226.81 ± 0.47 and 325.24 ± 0.76 $mg.100g^{-1}$ respectively on dry weight basis.

The amount of iron, copper, manganese, zinc and cobalt in aerial parts of plant were found 121.04 ± 0.38, 3.45 ± 0.29, 9.28 ± 0.30, 6.47 ± 0.32 and 0.00 respectively on dry weight basis. The ranges were 120.63 -121.55, 3.04 -3.68, 8.96 - 9.69 and 6.04 - 6.78 mg.100g^{-1} of Fe, Cu, Mn, and Zn respectively. This is the first work of analysis of nutrients antioxidants and minerals in *Euphorbia thymifolia* Linn., aerial parts of plants.

The minerals present in this medicinal plant may play an important role in human nutrition. Magnesium, calcium, and potassium are required in the human body for building red blood cells and for body mechanism (**WHO, 1996**). Deficiency of copper may cause hypertension, antibiotic sensitivity, hyperactivity, hyperglycemia, maniac disorders, insomnia, allergies and osteoporosis (**Watts, 1997**). Calcium plays a major role in CNS function. Calcium is essential for nerve impulse conduction and activates some enzymes, which generate neurotransmitters (**WHO, 1996**). Phosphorous is tied to calcium in bone structure and plays a significant role in CNS function. Many enzymes contain phosphoproteins as a base. Phospholipids are involved in nerve conduction. Phosphate is the primary ion in extra and intracellular fluid. It aids absorption of dietary constituents, helps to maintain the blood at a slightly alkaline level, regulates enzyme activity and is involved in the transmission of nerve impulses **(Katade, 2004**). Potassium has many functions for protein synthesis, activation of many enzymes, stimulation of the movement of the intestinal tract etc. excess of potassium can produce neurological disturbances such as numbness of hand and feet (**Watts, 1997**). Zinc is extremely important for numerous body functions. Zinc deficiency is associated with mental impairments, mental lethargy, emotional disorder and irritability (**WHO, 1996**). Iron plays a significant role in oxygen transport in the body. Disturbance in mental function can be caused by flows in the metabolic pathways that require iron. This is because of too little oxygen reaching the brain. Iron is required for

DNA synthesis. It is also necessary for the activation of enzymes involved in brain neurotransmitters (**WHO, 1996**). Thus this plant could serve as a good source of minerals when consumed. This conformed the observation of some researchers who concluded that green vegetables are good source of iron, copper and zinc **(Barasi and Mottram, 1987**).

We conclude that the aerial parts of plant contain good amount of antioxidants, nutrients and minerals. The distribution of these components in common medicinal plants has an important application for the health of people in addition to the basic need of developing countries. There is a great need to further research.

Biochemical Analysis of *Pavetta indica* Leaves

Biochemical and certain nutrient content in *Pavetta* leaves are presented in Table 3.7. The amount of vitamin C in *Pavetta* leaves was found 77.49 ± 1.83 mg.100 g^{-1} on dry weight basis. The range of vitamin C was 74.90 – 78.90 mg.100 g^{-1}. The amount of chlorophyll-a and chlorophyll-b in *Pavetta* leaves were found 91.88 ± 0.63 and 64.50 ± 0.54 mg.100 g^{-1} on dry weight basis. The amount of tannin in plant was found 2390.18 ± 0.34mg.100 g^{-1}. The range of tannin was 2390.85 – 2391.65 mg.100 g^{-1}.

The amount of certain nutraceuticals in *Pavetta* leaves are presented in Table 3.8. Crude lipid, protein and total carbohydrate content in *Pavetta* leaves were found 2.82 ± 0.07, 12.87 ± 0.00 and 13.36 ± 0.78 g.100g^{-1} respectively on dry weight basis. The range were 2.75 – 2.92, 12.86 – 12.87 and 12.42 – 14.34 mg.100g^{-1} of crude lipid, protein and total carbohydrate respectively. The amount of starch, amylose and amylopectin in *Pavetta* were found 17.19 ± 1.30, 2.70 ± 0.11 and 14.49 ± 1.40 g.100g^{-1} respectively.

The cellulose, crude fiber and moisture content were found 3.90 ± 0.33, 39.94 ± 0.63 and 64.42 ± 0.38 g.100g^{-1} respectively. The ash content was found 9.12 ± 0.03 g.100 g^{-1} on dry weight basis. Acid insoluble ash was found 1.46 ± 0.27 g.100 g^{-1} and acid soluble ash was found 7.66 ± 0.27g.100g^{-1}. The energy content of plant leaves was determined by multiplying the crude protein, crude lipid and total carbohydrate content by the factor 4, 9 and 4 respectively (**Osborne & Voogt, 1978**). Calorific value of the plant leaves was found 141.0 K.Cal.100 g^{-1}.

The mineral content of *Pavetta* leaves is presented in Table 3.8. The amount of sodium, potassium, calcium and lithium in *Pavetta* leaves were found 112.9 ± 1.22, 8049.7 ± 0.43, 231.88 ± 0.67 and 54.6 ± 0.87 mg.100g^{-1} respectively on

dry weight basis. Ranges were 111.68 – 114.56, 8049.20 – 8050.25, 231.02 – 232.66 and 53.95-55.84 mg.100g^{-1} of Na, K, Ca and Li respectively.

Table 3.7. Biochemical and nutrient composition of *Pavetta indica* Linn leaves.

Biochemical Parameter	**Composition (g.100g^{-1})**	**Range (g.100g^{-1})**
1. Moisture	64.42 ± 0.38	64.12 – 64.95
2. Crude protein (Kjeldahl N x 6.25)	12.87 ± 0.00	12.86 – 12.87
3. Crude fat	2.82 ± 0.07	2.75 – 2.92
4. Total carbohydrate	13.36 ± 0.78	12.42 – 14.34
5. Starch	17.19 ± 1.30	15.70 – 18.87
6. Amylose	2.70 ± 0.11	2.60 – 2.86
7. Amylopectin	14.49 ± 1.40	12.84 – 16.27
8. Cellulose	3.90 ± 0.33	3.45 – 4.24
9. Crude fiber	39.94 ± 0.63	39.11 – 40.62
10. Ash	9.12 ± 0.03	9.09 – 9.16
11. Acid soluble ash	7.66 ± 0.27	7.33 – 7.98
12. Acid insoluble ash	1.46 ± 0.27	1.11 – 1.78
13. β-Carotene	277.64 ± 0.88	276.98 – 278.88
14. Vitamin C	77.49 ± 1.83	74.90 – 78.90
15. Chlorophyll-a	91.88 ± 0.63	91.23 – 92.73
16. Chlorophyll-b	64.50 ± 0.54	63.73 – 64.92
17. Tannins	2390.18 ± 0.34	2390.85 – 2391.65
18. Calorific value (Kcal 100 g^{-1} DM)	141.00	–

Table 3.8 Mineral Composition of *Pavetta indica* Linn. leaves.

Mineral	**Composition** (**mg.100g^{-1}**)	**Range** (**mg.100g^{-1}**)
Sodium – Na	112.9 ± 1.22	111.68 – 114.56
Potassium – K	8049.7 ± 0.43	8049.20 – 8050.25
Calcium - Ca	231.88 ± 0.67	231.02 – 232.66
Lithium – Li	54.60 ± 0.87	53.95 – 55.84
Nitrogen – N	2058.4 ± 0.55	2057.73 – 2058.93
Phosphorus – P	143.25 ± 1.07	142.05 – 144.64
Sulphur – S	1975.00 ± 1.04	1973.63 – 1976.15
Iron – Fe	74.93 ± 0.69	74.06 – 75.76
Copper - Cu	2.27 ± 0.44	1.92 – 2.80
Manganese – Mn	11.17 ± 0.80	10.05 – 11.80
Zinc – Zn	2.67 ± 0.33	2.29 – 3.10
Cobalt - Co	0.00	-

The amount of nitrogen, phosphorus and sulphur were found 2058.4 ± 0.55, 143.25 ± 1.07 and 1975.00 ±1.04 mg.100g^{-1} respectively on dry weight basis. The contents of iron, copper, manganese, zinc and cobalt in *Pavetta* leaves were found 74.93 ± 0.69, 2.27 ± 0.44, 11.17 ± 0.80, 2.67 ± 0.33 and 0 respectively on dry weight basis. The ranges were 74.06 – 75.76, 1.92 – 2.80, 10.05 – 11.80, 2.29 – 3.10 and 0.0 mg.100g^{-1} of Fe, Cu, Mn, Zn and Co respectively. This is the first work of analysis of nutraceutical antioxidants and minerals in *Pavetta indica* Linn., leaves.

Amino Acid Composition

The amino acid content of each of the three plants viz., *P. hirta, E. thymifolia* and *P. indica* is summarized in Table 3.9. Quantitative determination of amino acid concentration was conducted by HPLC and the amino acid profile is shown in the chromatogram (Fig. 3.1 to 3.4). Seventeen amino acids were detected and the separation of these amino acids in the sample is reasonably resolved. All the essential amino acids i.e. methionine, leucine, lysine, cysteine, phenylalanine, tyrosine, arginine, isoleucine, threonine and valine and seven non-essential amino acids were found to be present in the three plants. The total amino acid content in *P. indica* was 58.80 mg amino acid/g sample (dry weight), *E. thymifolia* was 123.92 mg amino acid/g sample (dry weight) and *P. hirta* was 225.73 mg amino acid/g sample (dry weight). The total essential amino acids in *P. indica*, *E. thymifolia* and *P. hirta* were 33.58, 57.99 and 145.82 mg amino acid/g respectively.

The ratio of essential amino acids to total amino acid is 0.57 i.e. more then half of the amino acid in *P. indica*. The results also indicated that the ratio of essential amino acids to non-essential amino acids is 1.33. *P. indica* is rich in alanine, lysine, valine, arginine, alanine, glutamic acid, proline, and aspartic acid. Glycine, cysteine, methionine and phenylalanine are present in lower amount compared to the other amino acids. Data on threonine is not included in this work since this amino acid may be destroyed during acid hydrolysis.

The ratio of essential amino acids to total amino acid is 0.47 i.e. almost half of the amino acid in *E. thymifolia* consist of essential amino acids. The results also indicated that the ratio of essential amino acids to non-essential amino acids is 0.88.

Table- 3.9 Amino acid content of three plants in mg/g dry weight basis.

Amino	*P. indica*	% of total AA	*E. thymifolia*	% of total AA	*P. hirta*	% of total AA
Aspartic acid[n]	1.34±0.15	2.28	5.86±0.09	4.73	12.75±0.60	5.65
Glutamic acid[n]	1.66±0.06	2.82	7.31±0.05	5.90	14.00±0.09	6.20
Serine[n]	1.19±0.08	2.02	3.47±0.58	2.80	8.03±0.06	3.56
Glycine[n]	0.58±0.01	0.99	1.80±0.04	1.45	8.16±0.05	3.61
Histidine[n]	1.23±0.01	2.09	3.65±0.04	2.95	-	-
Alanine[n]	17.96±0.01	30.54	43.84±0.37	35.38	36.59±0.05	16.21
Proline[n]	1.26±0.07	2.14	-	-	0.38±0.03	0.17
Lysine[a]	3.03±1.34	5.15	-	-	1.44±0.04	0.64
Threonine[a]	-	-	5.99±0.04	4.83	40.74±0.08	18.05
Tyrosine[a]	2.20±0.06	3.74	4.51±0.13	3.64	10.06±0.10	4.46
Valine[a]	3.61±0.07	6.14	11.69±0.13	9.43	25.04±0.03	11.09
Methionine[a]	0.55±0.13	0.94	2.46±0.07	1.99	4.24±0.02	1.88
Cysteine[a]	0.60±0.33	1.02	0.90±0.10	0.73	1.95±0.03	0.86
Isoleucine[a]	1.97±0.33	3.35	8.66±0.48	6.99	17.50±0.08	7.75
Leucine[a]	1.93±0.09	3.28	11.71±0.14	9.45	27.90±0.01	12.36
Phenylalanine[a]	0.62±0.07	1.05	4.38±0.01	3.53	12.06±0.02	5.34
Arginine[a]	19.07±0.09	32.43	7.69±0.05	6.21	4.89±0.04	2.17
TEAA	33.58	51.96	57.99	46.80	145.82	63.96
TNEAA	25.22	48.04	65.93	53.20	79.91	36.04
TAA	58.80		123.92		225.73	

All values are mean of triplicate determinations expressed on dry weight basis.

±, Denotes the standard error; [a]-TEAA, total essential amino acid; [b]TNEAA, total non essential amino acid; AA, amino acid

Table 3.10 Comparison of the content of selected essential amino acid of 3 plants with that of the WHO Ideal pattern.

Plant specimen	ILE	LEU	VAL	PHE+ TYR	LYS	THR	MET+ CYS	SCORE*
WHO standard	4.0	7.0	5.0	6.0	5.5	4.0	3.5	---
P. indica	3.35	3.28	6.14	4.79	5.15	-	1.96	1/7
E. thymifolia	6.99	9.45	9.43	7.17	-	4.83	2.72	5/7
P. hirta	7.75	12.36	11.09	9.80	0.64	18.05	2.74	5/7

*this pattern is based on the essential amino acid need for the preschool child; WHO/FAO. Energy and Protein Requirements. WHO Technical Report Series, No. 522. Geneva, World Health Organization, 1973.

E. thymifolia is rich in alanine, methionine, phenylalanine, valine, glycine,arginine, alanine, glutamic acid, and aspartic acid. Cysteine is present in lower amount as compared to the other amino acids. Data on proline and lysine are not included in this work since this amino acid may be destroyed during acid hydrolysis.

The ratio of essential amino acids to total amino acid is 0.65 i.e. more then half of the amino acid in *P. hirta* consist of essential amino acids. The results also indicated that the ratio of essential amino acids to non-essential amino acids is 1.82. *P. hirta* is rich in alanine, glycine, phenylalanine, threonine, valine, methionine,arginine, alanine, glutamic acid, proline, and aspartic acid. Lysine and cysteine are present in lower amount as compared to the other amino acids. Data on histidine is not included in this work since this amino acid may be destroyed during acid hydrolysis.

In this study we compared the amino acid composition of each of three specimens to that of a World Health Organization standard protein (**WHO,**

1973). According to the WHO reference protein, the highest quality plant proteins were found in *Euphorbia thymifolia*, and *Pouzolzia hirta* (Table 3.10) each of these scored at or above the score of the WHO standard for 5 of 7 amino acids or amino acid pairs.

The nutritional analysis of the indigenous edible and fodder plants of the Uttarakhand region by chemical means gives the potential values of these foods to those populations who rely upon them as staples or supplements to their diet. The next step is to assess the bioavailability of the essential nutrients in these plants, such studies must be contemplate. These studies will focus on the composition of the biochemical, mineral, amino acid present in these plants and on the possible presence of antinutrients, such as metal chelators (e.g., phytates, oxalates) and protease inhibitors.

Fig. 3.1 Amino acid profile of Standard

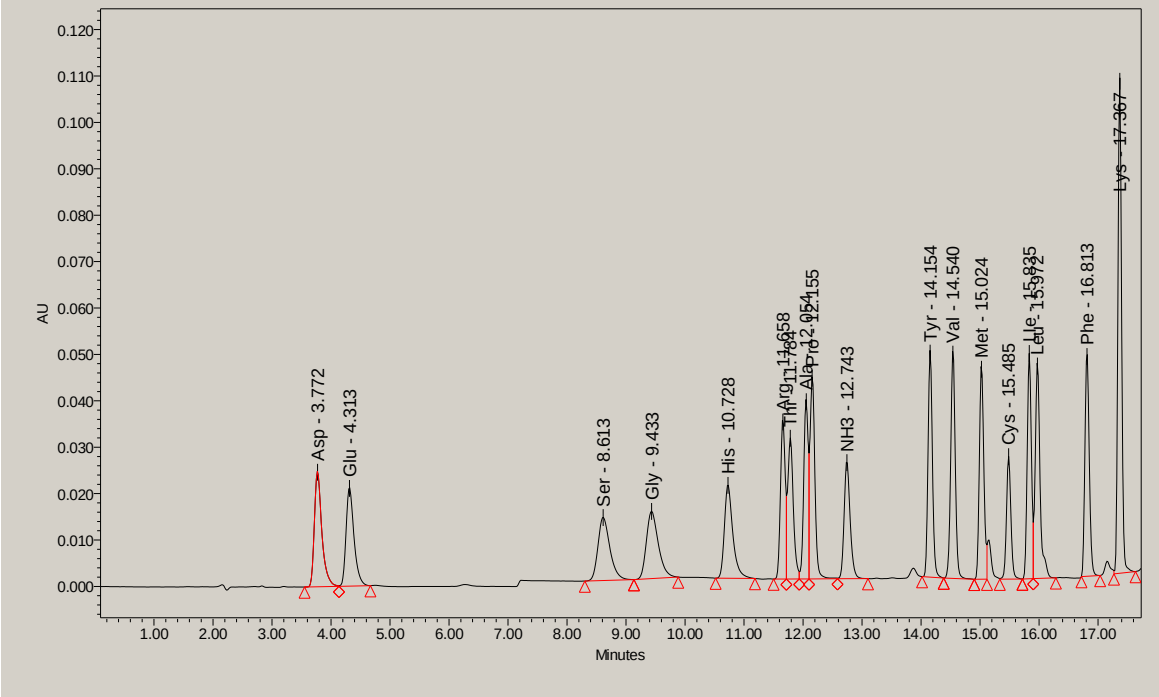


Fig. 3.2 Amino acid profile of *Pavetta indica*

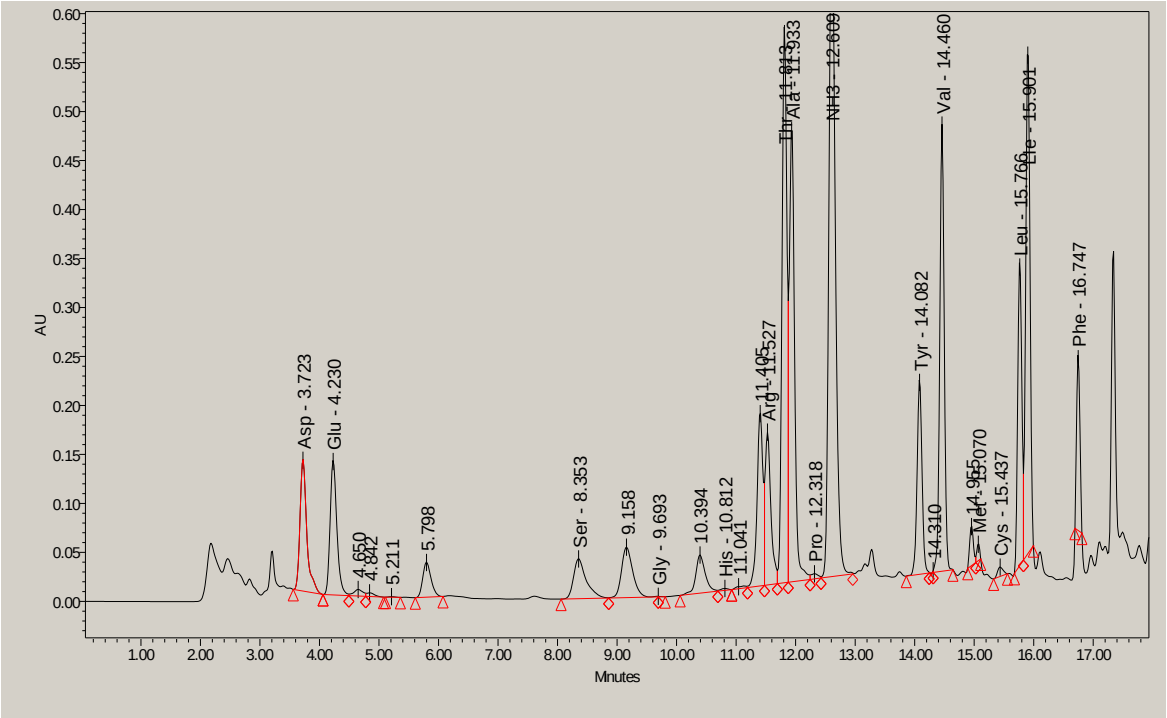


Fig.3.3 Amino acid profile of *Euphorbia thymifolia*

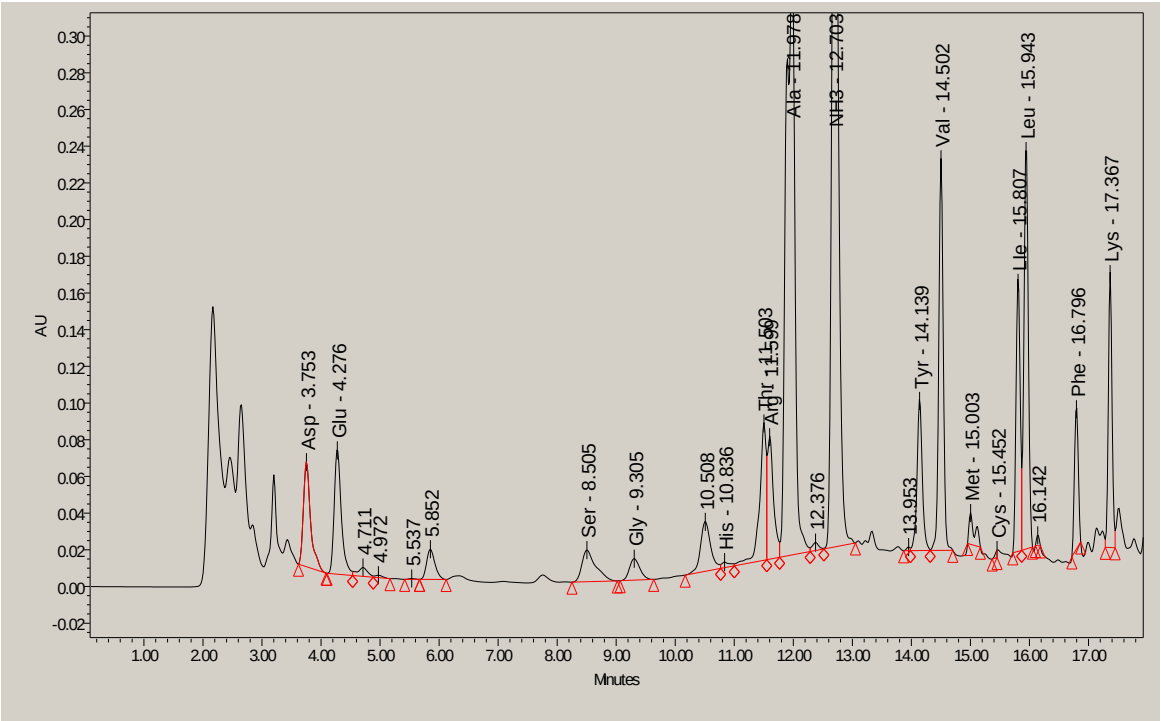
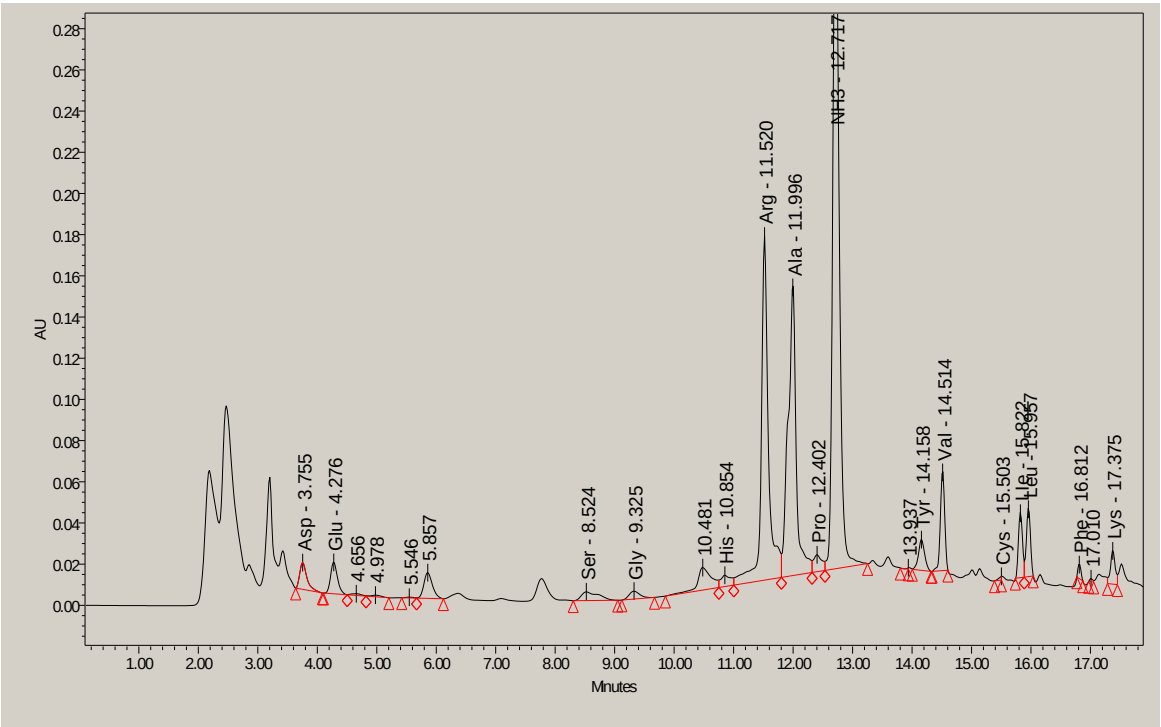


Fig.3. 4 Amino acid profile of *Pouzolzia hirta*



Antioxidant analysis

The aim of this work was to characterize the antioxidant value of the medicinal plants with particular attention to carotenoids, phenolics and vitamins. In this study we observed that xanthophyll, α-carotene, β-carotene, vitamin C, and DL-α-tocopherol contents are present in theses medicinal plants. (Table-3.11). The retention time of xanthophyll, α-carotene, β-carotene and DL-α- tocopherol were found to be 2.045, 10.947, 11.495 and 11.780 minutes respectively (Fig-3.5).

Among the three investigated plants, Carotenoids viz . xanthophyll content was found 0.13 to 151.01 mg/100g dry weight basis (Table-3.11). The maximum xanthophyll content was found in *P. indica* leaves and minimum in *P. hirta* rhizomes. The α-carotene content in *P. hirta* and *E. thymifolia* was below detection limit (BDL), but in *P. indica* it was found 1.96 mg/100g dry weight basis. The β-carotene content varies from 4.62 - 374.55 mg/100g on dry weight basis. *P. indica* contains more β-carotene content than *P. hirta* rhizomes. α-Carotene and β- carotene were found more in the leaves of *P. indica* as compared to other two plants, but DL-α-tocopherol was found more in *E. thymifolia* and the range was 3.48 to 24.14 mg/100g on the dry weight basis. This is the first study for quantitative variation of antioxidant in these three medicinal plants, so we could not correlate above data with earlier workers.

Table-3.11. Antioxidant content in medicinal plants.

S.N.	Antioxidants	*Pouzolzia hirta*		*Euphorbia thymifolia*		*Pavetta indica*	
		mg/100g	**Range**	**mg/100g**	**Range**	**mg/100g**	**Range**
1.	Total phenolics	230.59 ± 0.33	230.15-230.95	336.73 ± 0.55	336.25 - 337.50	251.52 ± 1.00	250.23 – 252.68
2.	Xanthophyll	0.13 ± 0.01	0.12 - 0.13	0.51 ± 0.04	0.48 - 0.55	151.01 ± 2.16	149.34 - 152.34
3.	α-Carotene	-	-	-	-	1.96 ± 0.07	1.96 - 2.01
4.	β-Carotene	4.62 ± 0.68	4.14 - 5.10	178.98 ± 4.62	175.45 - 181.90	374.55 ± 1.40	373.77 - 375.66
5.	DL-α-tocopherol	13.48 ± 0.83	12.81 - 13.92	24.95 ± 1.10	24.14 - 25.69	9.13 ± 0.28	8.94 - 9.33
6.	Vitamin-C	108.40 ± 0.32	108.05-108.83	88.48 ± 0.95	87.37 - 89.68	77.49 ± 1.83	74.90 – 78.90

All values are mean of triplicate determinations expressed on dry weight basis.

± Denotes the standard error.

The amount of total phenolics content varies between three plants rhizomes/leaves (Table 3.11). The phenolics content (336.73 mg/100gm) was found higher in *E. thymifolia* leaves as compared to *P. hirta* rhizomes (230.59 mg/100g), while (251.52 mg/100g) was found in *P. indica* leaves. As such phenolics are known for their antioxidant activity. Also the phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables and other plants. For example, caffeic acid, ferulic acid, and vanillic acid are widely distributed in the plant kingdom, resmarinic acid, an important phytochemical has been found to be potent active substances against human immunodeficiency virus type1 (HIV-1).

DL-α-tocopherol in these medicinal plants was found 13.48 mg/100g, 24.95 mg/100g and 9.13 mg/100g on dry weight basis in *P. hirta*, *E. thymifolia* and *P. indica* respectively. The maximum (24.95 mg/100gm) in *E. thymifolia* and minimum (9.13 mg/100gm) in the *P. indica*. DL-α-tocopherol is essential for the human body because it halts lipid oxidation and counteract the prooxidative effect of other compounds like ascorbate and combination of ascorbate and β-carotene. The amount of vitamin C content varied between three plants rhizomes/ leaves (Table 3.11). The vitamin C contents (108.40 mg/100gm) was found higher in *P. hirta* rhizomes as compared to (77.49 mg/100g) *P. indica* leaves, while (88.48 mg/100g) was found in *E. thymifolia* leaves.

Fig. 3.5 – Chromatogram of standard peak of xanthophyll.

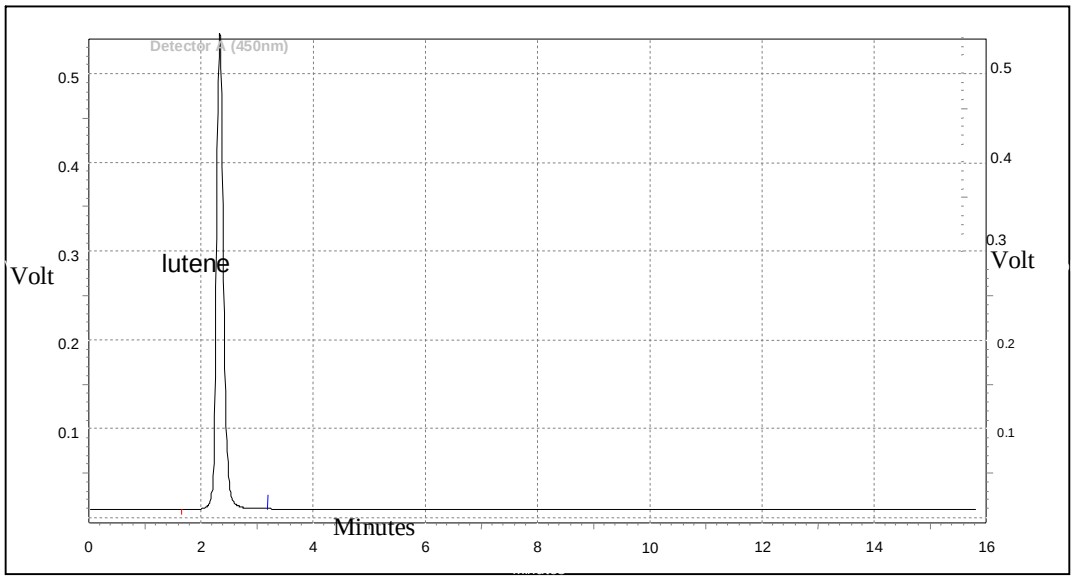


Fig. 3.6 – Chromatogram of standard peak of α-carotene and β-carotene

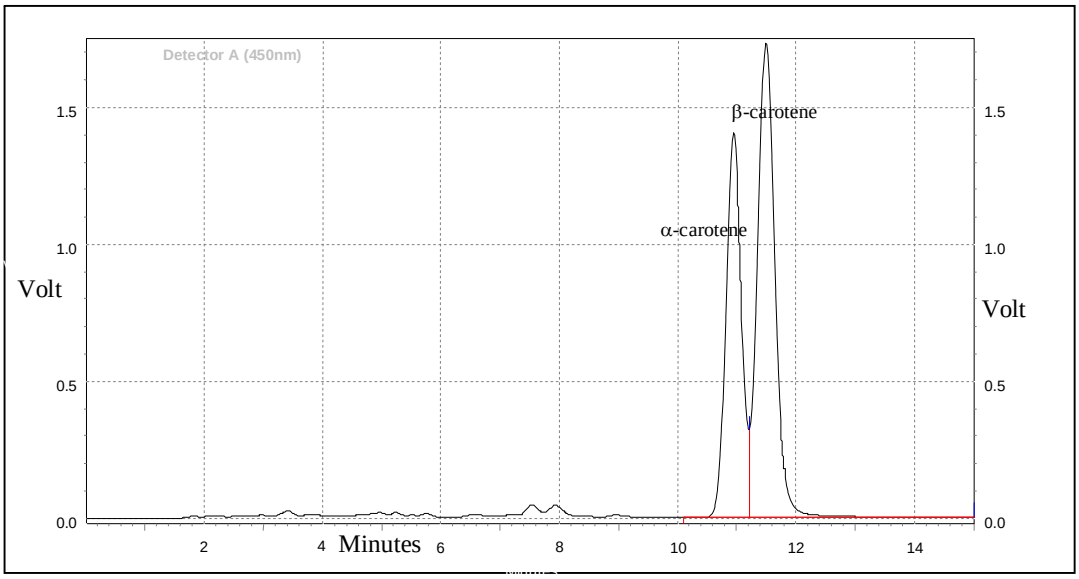


Fig. 3.7 – Chromatogram of *Pavetta indica* leaves.

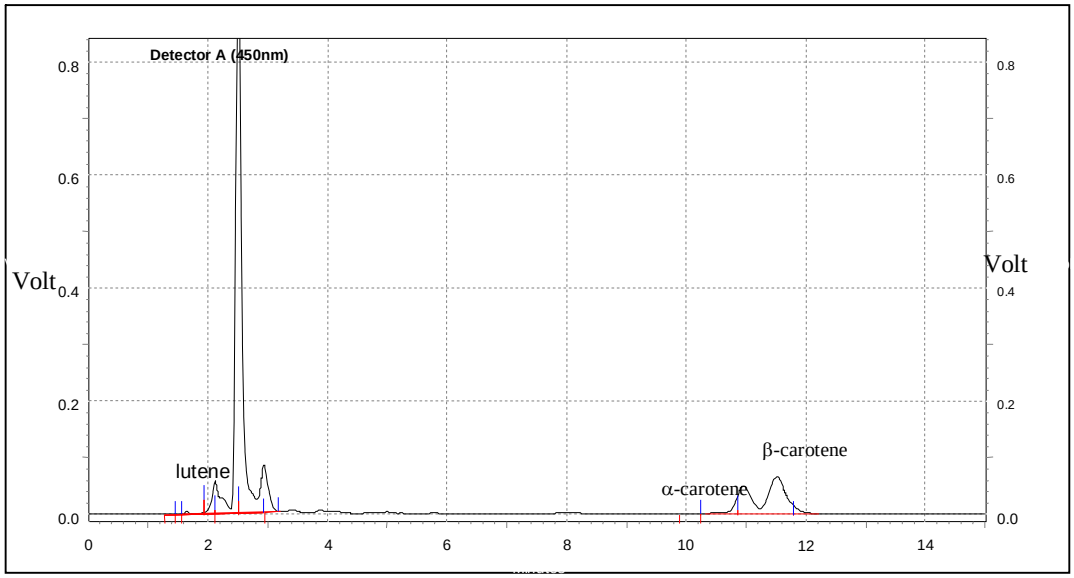


Fig. 3.8 – Chromatogram of *Euphorbia thymifolia* aerial parts .

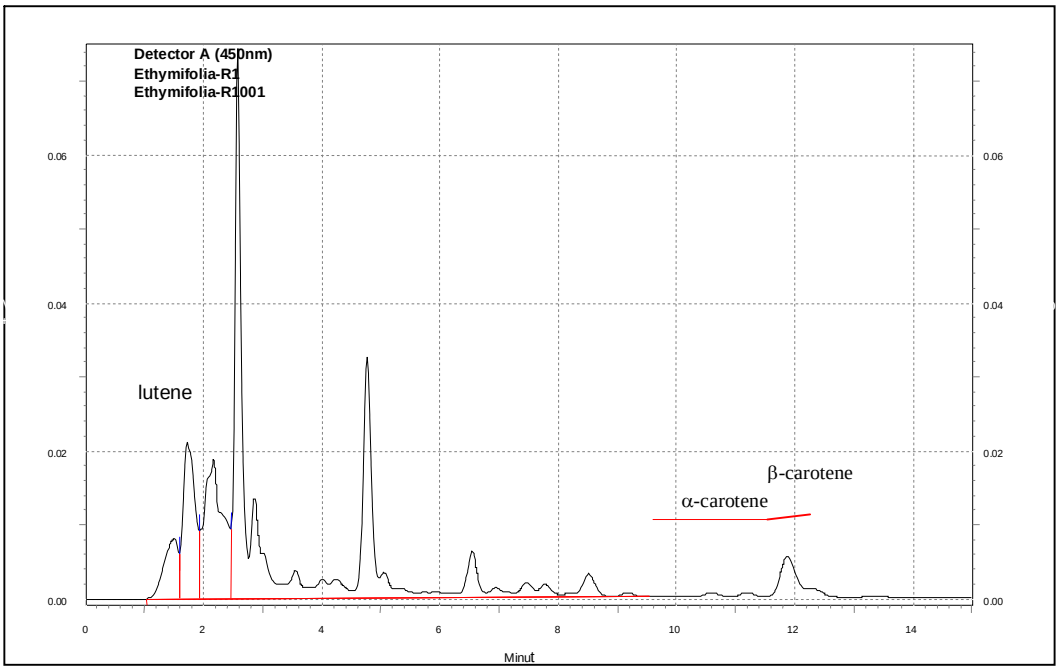


Fig. 3.9 – Chromatogram of *Pouzolzia hirta* rhizomes

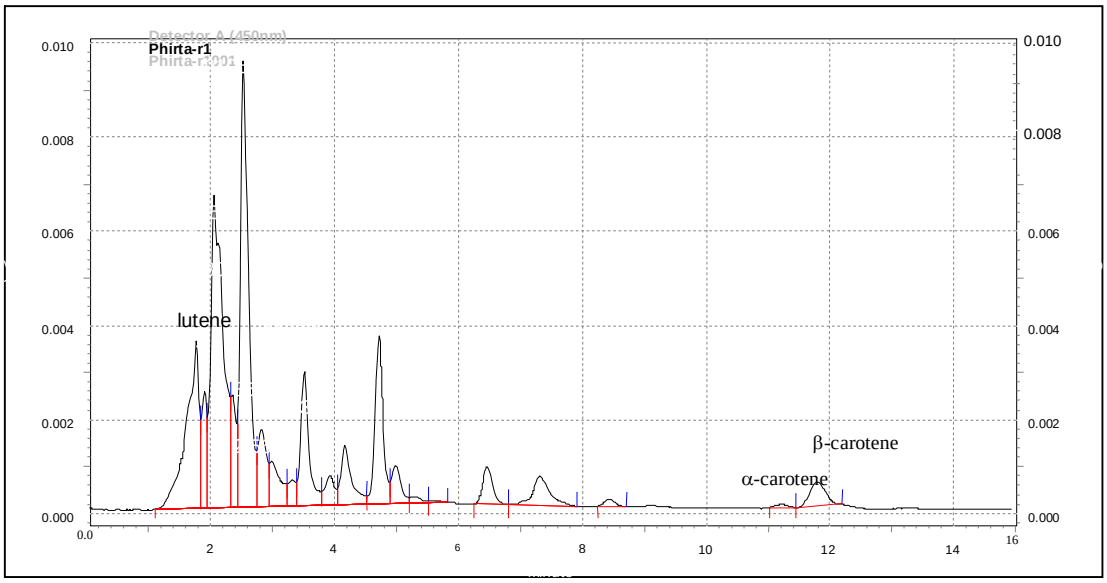


Fig. 3.10 Chromatogram of standard peak of DL-α-tocopherol

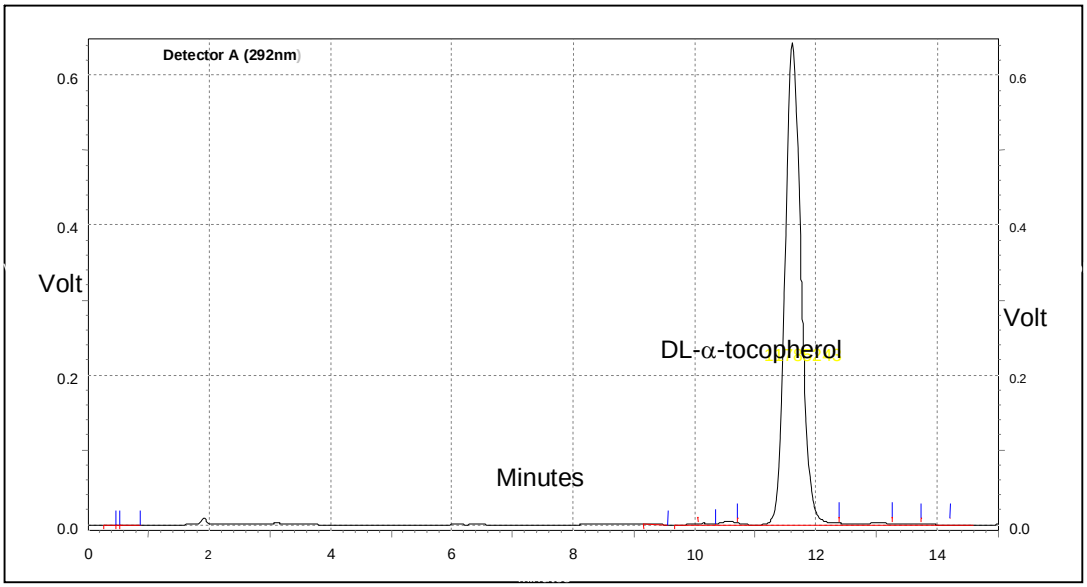


Fig. 3.11 – Chromatogram of *Pavetta indi*ca leaves

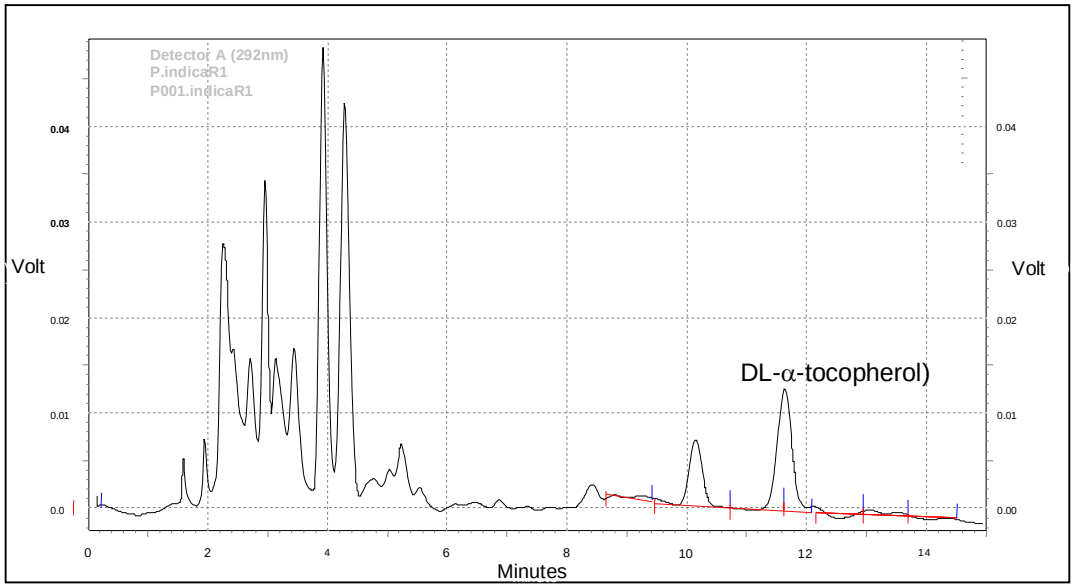


Fig. 3.12 – Chromatogram of *Euphorbia thymifolia* aerial parts.

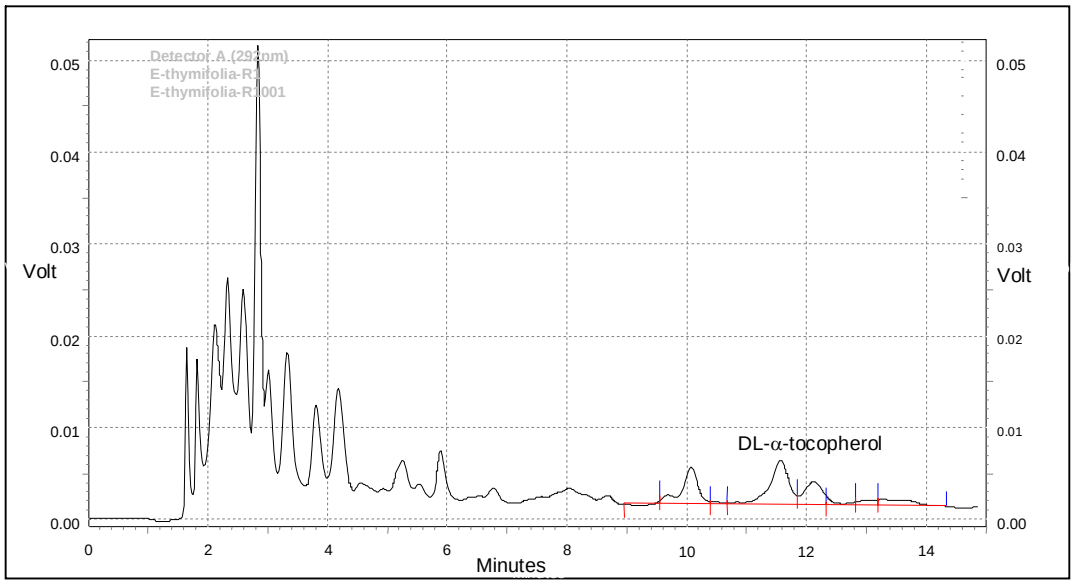
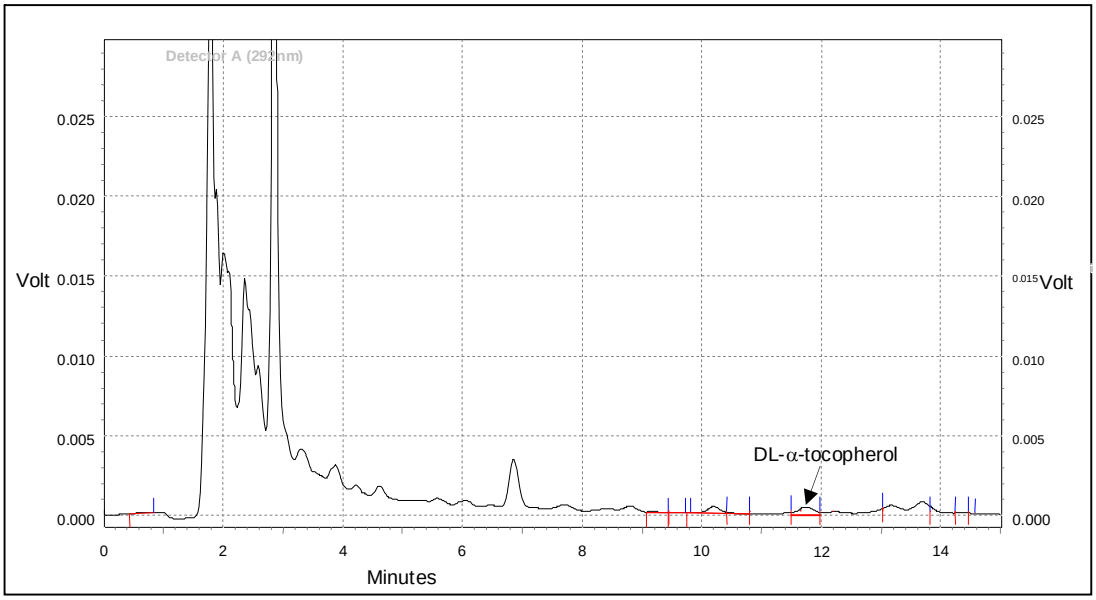


Fig. 3.13 – Chromatogram of *Pouzolzia hirta* rhizomes



The analytical data on crude protein, crude fat, gross energy and amino acid profiles of *Pavetta*, *Euphorbia* and *Pouzolzia* clearly suggested their high potentials as cheap source of alternative proteins for humans and animals. Because of the simplicity of technology involved in leaf protein concentrate production, its incorporation into local food production systems is recommended as a practicable, sustainable and ameliorative intervention strategy for the endemic protein under-nutrition in this region.

The leaves/ rhizomes of the plant from the data, reveals that it contains an appreciable amount of proteins, minerals, fats, fibres, amino acids, antioxidants, carbohydrates, caloric value and low levels of toxicants whose value can be reduced by cooking. Since it contains substantial amount of nutrients, it can therefore be concluded that these plant leaves/ rhizomes can contribute significantly to the nutrient requirements of man and should be recommended.

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