PHYTOCHEMISTRY AND ANTI-MICROBIAL PROPERTY OF "PANIKKOORKA" (*Coleus amboinicus* Lour.)

By

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THESIS

submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled **"Phytochemistry and anti-microbial property of "Panikkoorka" (*Coleus amboinicus* Lour.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"Phytochemistry and anti-microbial property of "Panikkoorka" (*Coleus amboinicus* Lour.)"** is a record of research work done independently by **Mr. H. R. Shankar** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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INTRODUCTION

INTRODUCTION

The plant kingdom has been providing the therapeutic needs of human beings since many millennia. Indeed the vegetal kingdom has been therapeutic arsenal of all the documented traditional system of medicine, which made possible the modern discipline of "ethno-medicine". i.e. those beliefs and practices relating to diseases which are the products of indigenous cultural development and are not derived from the conceptual frame work of modern medicine (Hughes, 1968).

For centuries, people from all over the world have been using plants for medicinal and healing purposes. The medicinal plant utilization are mainly by three sectors namely the traditional, indigenous and folk medicine, over the counter (OTC) non prescription items involving plant parts, extracts and galenicals and phyto-pharmaceuticals used in modern system of medicine. Among these three, the direct utilization of plant material is not only a feature of Indian System of Medicines (ISM) in the developing countries but also in developed countries like USA, UK, Germany etc. Various herbal formulations, tinctures, herbal teas, galenicals and total extracts of plants also form part of many pharmacopoeias of the world. The current trend of medicinal plant based industry is to procure standard plant extracts of plant as raw material (Singh *et al.*, 2000). The purity of raw drugs used in the preparation and processing methods have significant role in determining quality of the finished product. Therefore, there is need to select appropriate technologies for the industrial production of traditional medicines such that effectiveness of the preparations is maintained. Observing Good Manufacturing Practices (GMP) would enhance the marketing prospects of these products (Chomchalow and Henk, 1995). These OTC medicines can be modified and improved using technologies available today to make them more effective, stable, reproducible and in dosage forms that can be easily transported.

China and India are the major sources for medicinal plants. India is exporting herbal materials and medicines to the tune of Rs 550 crores whereas China, besides meeting its domestic demand, is earning US$ 5 billion per year from herbal trade. India has the capability and potential to earn by export of herbs and herbal products

worth Rs 5000 crores by the year 200[illegible] (Tewari, 2000). In this scenario, harvesting and post harvesting processes acquire much importance.

The plant *Coleus amboinicus* Lour. of Lamiaceae is an accepted source of "*Karpuravalli*" drug in Kerala Many authors have equated this plant with "*Pasanabhedah*", (Kartikar and Basu, 1918; Vaidya, 1936; Nadkarni, 1954; Chopra *et al.*, 1956; Kapoor and Mitra, 1979; Chunekar, 1982; Vaidya, 1982) may be because of its reported property to have specific action on the bladder.

Coleus amboinicus (Malayalam: Panikkoorka), with its distinctive smelling leaves (Gandhaparnika) is a common home remedy for infantile cough, cold and fever. In the folk medicine, leaf juice of this plant is extracted after steaming and boiling which is administered either singly or in combination. Fevers of various etiologies are wide spread now. Great possibility exists for the development of a stable, reproducible and effective extract from this plant with antipyretic properties. Very little information is available on the stage of harvest, method of extraction, storage and anti-microbial properties of this plant. In this background, the present study was proposed with the following objectives:

1) To study the influence of stage of harvest on phytochemistry of the plant
2) To study the effect of different drying treatments on the phytochemistry of the plant
3) To study the method of extraction and storage on phytochemistry of the plant and
4) To study the anti-microbial property of the plant

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Coleus amboinicus Lour. (also known as *C. aromaticus* Benth. and *Plectranthus amboinicus* (Lour.) Spreng.) belongs to the family Lamiaceae. It is a spreading, branched, rather course, strongly aromatic herb, stems are green and fleshy, rounded or obtuse, base decurrent with many floral whorls (Merrill, 1912). It is found in varied habitats of Asia and West Indies (Rehm and Espig, 1991), where the leaves are used for seasoning fish, meat, and as a vegetable too. The leaves of *Coleus amboinicus* is used in Indo-china, Indonesia and the Philippines to treat bronchitis, asthma, chronic coughs, sores, burns and insect stings (Uphof, 1968). In this chapter, literatures pertaining to stage of harvest, method of drying and storage studies conducted on various medicinal and aromatic crops are presented under different headings, since the literature in *Coleus amboinicus* on these aspects were less. Phytochemical and antimicrobial studies pertaining to *Coleus amboinicus* is given under separate headings.

2.1. Stage of harvest

Harvesting stage is the important factor that influences the quality of the herb. Douglas (1969) reported that yield of essential oil crops will be more under hotter and drier conditions.

Field experiment on *Ocimum sanctum* revealed that increase in herbage yield was in the ratio of 1:7 between vegetative (12.9 t ha^{-1}) and seed maturity stages (19.1 t/ha). The essential oil content was highest at vegetative stage (1.73% DWB), which decreased to 0.62 percent at maturity stage. However, harvesting the crop at full bloom possessed maximum eugenol content (53.5%) in oil (Pareek *et al.*, 1982).

Pareek *et al.* (1991) found that the alkaloid content was negatively correlated with herbage production, which was highest at initiation of bloom and gradually declined with the age and reached its minimum acceptable limit at 30 days after flowering. Balancing the alkaloid content with yield was worked out and found that harvesting at 20 days after commencement of flowering (210 days after planting) yielded higher total alkaloid yield in *H. muticus* and *H.niger*.

Singh *et al.* (1995) studied the seven critical growth stages of *Tagetus minuta*. The full blooming stage was found optimum for harvesting when herb and oil yield were 37 t ha^{-1}, 73 kg/ha respectively. The oil content increased from 0.16 to 0.50 percentage.

Chalchat *et al.* (1997) reported that harvesting of peppermint at the end of flowering stage afforded a higher conversion of menthone to menthol and yield of oil was richer in menthol portion, which was more valuable commercially.

The essential oil of *Coleus aromaticus* (*Plectranthus amboinicus*) produced in September contained higher concentrations of carvacrol and beta-caryophyllene and oxygenated constituents than the essential oil produced in May (Mallavarapu *et al.*, 1999).

In Japanese mint Singh and Singh (1999) found that the growth attributes i.e. plant height, leaf stem ratio, oil content and fresh herbage, oil and menthol yield were significantly increased when the crop was harvested at 110 days after planting and 70 days after planting and 70 days after first harvest, during first and second harvests respectively (110+70 days harvesting schedules).

The shoot oil distilled from *Ocimum basilicum* cv Kusumohok at full flowering stage(s) was richer in linalool (44.6%) and methyl chavicol (24.89%). For better oil yield and quality, the full flowering stage of crop offered the most profitable time of harvest (Bahl *et al., 2000*).

Srivastava *et al.* (2000) analysed the field crop of menthol mint (*Mentha arvensis*) cv Kosi in Uttar Pradesh. The analyses showed that crops aged 3 to 4 months gave higher yields of high quality oil. The harvest of immature or over mature crops gave lower yields of oil, which had higher percentages of inferior terpenoides.

Joy *et al.* (2001) revealed that harvesting of *Alpinia galanga* at 42 months after planting gave the maximum rhizome (45 t / ha) and oil (124.4 l / ha) yields and oil of

good quality (27.1% cineole). A substantial quantity of oil (127.4 l / ha) could also be obtained from the roots (19.5 t / ha) at 39 months after planting.

Singh and Ramesh (2001) obtained maximum oil content in *Ocimum basilicum* L. at full bloom stage. Methyl chavicol content was also high at the full bloom stage. In an another study by same workers in *Plumeria alba* revealed that the maximum concrete yield of 0.339 percent was obtained when flowers were harvested at full bloom stage.

Ram *et al.* (2001) analysed the essential oil quality and yield parameters of two geranium cultivars (Algerian and Bourbon) under different harvesting schedules (136, 146, 156 and 166 days after planting). Results revealed that the cultivar Bourbon was significantly superior to the cultivar Algerian in oil production. The crop of former cultivar, when harvested at 156 days after planting gave significantly higher yield than crop harvested at 136, 146 and 166 days after planting. The quality of essential oil of cultivar Bourbon in terms of its major constituent's citronellal and geraniol were on-par of its acceptable international standards.

Field experiments were conducted by Kattimani and Reddy (2001) to investigate the effect of harvesting intervals on growth, biomass, oil yield and nutrient uptake by, Japanese mint (*Mentha arvensis*) cv. MAS-1 grown in red soil. Observations were recorded at 90, 100, 110 and 120 days after planting (DAP) in the first harvest, and 40, 50 and 60 days after the first harvest (DAFH). Harvesting of Japanese mint at 100 DAP in the first and 60 DAFH recorded the highest growth attributes, biomass and oil yield. This combination of harvest interval was more economical on the basis of biomass, oil yield and nutrient uptake compared to other combinations.

Usually leaves are gathered throughout the growing period of the plant, the aerial or top parts of the plant are harvested at the beginning or before flowering stage. Fruits and seeds are harvested when they are in maturing stage (Nambiar, 2002).

Singh *et al.* (2002) revealed that the herb yield, essential oil content and artemesia ketones content in the *Artemesia annua* oil increased with the advancement of crop age.

The maximum fresh herb (78.3 t ha^{-1}) oil (49.4 kg ha^{-1}) yield with highest content of artemesia ketone (62.5%) (the most abundant constituent) and lowest 9.8 percent artemesia alcohol content were obtained from *A. annua* harvested at full flowering stage.

Mohamed *et al.* (2002) revealed that herbage yield of *Catharantus roseus* was increased significantly with the increase in the age of crop, except that the dry weight of leaves of six month old plant gave significantly the highest leaf dry weight and alkaloid content than the three and nine months old plants.

In *Withania somnifera*, neither the crop duration nor the seed rate influenced plant height, length of the root or girth of root. However, there was an increase in yield of both thick and thin roots along with the total dry root yield when harvesting was delayed up to seven months after planting (Patel *et al* . 2003).

The oil of virgin olive contains palmetic acid (11.9%), oleic acid (79.3%) and linolinic acid (29.5%). The content of palmetic acid and saturated fatty acid decreased during fruit ripening, while oleic and linolinic acids increased. The amount of saturated acids (palmetic and stearic) and the poly unsaturated acids (linoleic and linolenic) were dependant on the crop year (Beltran *et al.*, 2004).

The seasonal variation of the essential oil content and composition of a *Mentha spicata* population grown wild in Greece was examined by Kofid *et al.* (2004). The oil content ranged from 0.1-0.8 percent with the maximum values in late summer/early autumn. The essential oil obtained from leaves was characterized by a very high content in linalool, i.e. 85.0 – 93.9 percent of total oil.

Studies were undertaken by Rao *et al.* (2005) to find out the best harvesting time for two varieties of *Ocimum basilicum* grown in semi-arid tropics of South India. The variety Vikar Sudha having 0.40 to 0.55 percent oil containing, 26 percent linalool and 69 percent methyl chavicol was best harvested at 80 days after planting when crop was in full bloom and the herb yield was maximum. The local variety having 0.1 percent oil containing, 31 percent linalool and 24 percent eugenol was best harvested at 100 days after planting when the crop was in full bloom and the herb yield highest.

2.2. Drying

Drying is one of the most expensive operations in medicinal plant processing due to its high investment and energy requirement. Special emphasis should be given to drying temperature because of its strong influence on economic parameters such as drying capacity, energy requirement and drug quality (Heindal and Muller, 1997).

2.2.1. Sun drying

Sun drying has been practiced since ancient times as it is the cheapest method but the process is slow and the products will develop dark color and may often contain deposited dust which may reduce their market value. The rate of drying depends on the temperature during the season. It is slower during winter when the day temperature ranges from 20-25°C than in summer with 35-42 ^{0}C (Gupta and Pareek, 1993). Even though, it has certain drawbacks, it is the widely used drying technique by most of the cultivators and traders of medicinal herbs.

For many medicinal plants like *Acorus calamus*, *Adhatoda zeylanica*, *Aristolochia indica*, *Atropa accuminata*, *Centella asiatica*, *Cinchona* spp., *Datura stromonium*, *Digitalis purpurea*., *Ephedra gerardiana*, *Glycyrrhiza glabra*, *Holarrhina antidysenterica*, *Hyoscyamus niger*, *Mentha* spp., *Rauvolfia serpentina*, *Tinospora cordifolia* and *Tylophora indica*, dried plant parts constitute the raw materials for medicinal preparations and in most of the cases sun drying is the resorted method of drying (Jain, 1999).

2.2.2. Shade drying

It is the commercially resorted drying technique for herbs. Many of the herbs are dried under shade especially those which cannot be sun dried. In *Melissa officinalis* L., flowering tops are dried in shade in order to preserve its natural color (Gulati and Tajuddin, 1979). Leela and Angadi (1992) recommended shade drying of mentha herbage for three days at 30 ^{0}C for obtaining maximum yield of good quality essential oil.

The effect of drying on essential oil composition up to 20 days in the shade and yield was investigated by Tonzibo *et al.* (1998) in *Eucalyptus citriodora*. Results revealed that essential oil yield increased from 1.5 to 3.6 percent on prolonged drying. GCMS (Gas Chromatographic Mass Spectrometry) analyses of the obtained essential oil indicated that the content of hydrocarbons decreased while that of oxygenated constituents increased with drying.

All the samples of *Origanum vulgare* showed a minor decrease in essential oil yield after drying when compared with fresh plants. Jerkovic *et al.* (2001) reported that drying at room temperature had no effect on the qualitative composition of oregano oil.

Meisheri (2001) suggested that the best eco-friendly method for dehydration of medicinal herbs that can keep its chemical composition, colour, flavor and taste intact at very economically viable scale is the dehydration of herbs at room temperature (27 – 34 ^{0}C).

2.2.3. Oven drying

To overcome the problems of sun drying (unhygienic surroundings and long duration of drying), the mechanical drier appeared into the drying scenario. In the air circulating type of electric dehydrators, temperature inside the chamber can be controlled to obtain desired quality of product with out spoilage and dependence on weather in a short period of time. But, higher energy consumption and initial investment hinders its wide spread use.

Correct drying is done to reduce the moisture content up to 5-10 percent to minimize the spoilage. Drying temperature has a vital influence on quality. In artificial drying, temperature should not exceed 40 ^{0}C, as the essential oil and flavor are lost at high temperature (Atal and Kapoor, 1982).

For *Hyoscyamus* (a source of tropane alkaloid), 52 ^{0}C temperature appears to be optimum for drying the leaves, which may be raised to 60 ^{0}C with out damage. Drying

can be accomplished in 30 hours with out any damage to alkaloid contents (Sievers and Lowman, 1994).

Roots of *Rauvolfia serpentina* could be dried in a mechanical drier to reduce the moisture content to about eight percent or less for safe storage (Gauniyal *et al.*, 1988). Stoffert (1997) reported that a drying temperature of 38 ^{0}C is ideal for American ginseng (*Panax quinquefolius*). Below 30 ^{0}C causes mildew while above 40 ^{0}C causes caramelisation.

Buggle *et al.* (1999) conducted studies to show the effect of drying temperature on the amount and quality of essential oil extracted from *Cymbopogan citratus*. Higher amount of oil was collected following the lower drying temperature but at 30 ^{0}C, leaves were affected by fungal (*Aspergillus* spp., *Penicillium* spp., *Rhizophus* spp., *Cladosporium* spp., *Trichoderma* spp. and *Alternaria* spp.) growth. Analyses of the oils by GCMS showed variations in citral concentration with drying temperature of 50 ^{0}C with 1.43 percent oil content.

Thin layer drying of taxus clipping by Hansen *et al.* (1999) revealed that drying rates increased 3, 15 and 28 fold as drying temperature increased from 30, 40 and 60^0 C respectively. Also, stems dried at a faster rate than needles, and needles dried at a faster rate than whole clippings. Taxol yields from stems were nearly constant for the four temperatures tested. However, yields from needles increased finally as drying temperature increased from 30 to 60^0 C. The highest taxol yield was obtained from clippings. Nearly constant yields were obtained for drying temperatures of 40, 50 and 60^0 C. The lowest yields for all three plant components occurred when the drying temperature was set at 30 ^{0}C. The results suggested that 60 ^{0}C is the best temperature set point for drying taxus.

2.2.4. Effect of drying techniques on quality

For *Digitalis lanata*, which is the source of cardiac glycoside, drying in sun lowered the quantity of active constituents (Monteverde and Ordovaskaria, 1928). Drying in shade or artificial drying at 30 to 40 ^{0}C proved to be most appropriate treatments (Silva

and Constantinescus, 1986). On large scale drying of *D. lanata* leaves in an air drier, the maximum content of lanotoside C was obtained while drying at 40 ^{0}C. The maximum content of total condenolide was reached while drying the crude drug at elevated temperature of 80 ^{0}C, which however caused the decomposition of lanotoside C (Elbanwska and Kaczmarick, 1966).

Croundell *et al.* (1983) repored that beta-carotene of orange oil was decreased to 6.6, 1.84 and 2.46 percentage upon concentrating to 5, 10 and 25 fold from an initial value of 7.2 mg of beta-carotene per 100g of oil. 25-fold sample lost 6 percentage of its carotene at 18 ^{0}C and 90 at 32 ^{0}C during 12 months storage.

The essential oil obtained from Indian spearmint subjected to different drying procedures were analysed employing GC and GC-MS technique. Forty nine flavor components were identified. Carvone to limonene ratios were 2.2:1.0 and 2.3:1.0 in the oil from fresh and shade dried spearmint respectively. Shade drying leaves resulted in a product with a good green color of volatile oil compared to other drying methods (Raghavan *et al.* 1994).

In *Drosera medagascarensis, D. peltata and D.rotundifolia*, the napthoquinone content with regard to drying method were investigated by Krenn *et al.* (1998). HPLC analyses showed that in species with methyl juglone as the main compound, lyophilisation was superior to air drying at room temperature. The napthoquinone content in these samples was between 6 and 67 percentage higher than in air dried material. In contrast to these results in *D.peltata* with plumbagin as the main napthoquinone, the level was 25 percent lower after lyophilisation.

The effect of drying on the chemical constitution of volatile oil of fresh *Rosmarinus officinalis* leaves were studied by Fadel and El-massry (2000). They reported that the monoterpene hydrocarbons exhibited the highest level after drying. The monoterpene esters decreased from 24.76 percent in fresh leaves to 1.79 percent in dried leaves.

Mikania glomerata leaves dried in a green house at an ambient temperature of 35 ^{0}C for 15 days had the lowest coumarin content (4.02 ± 0.16 mg/g DW) as compared with leaves dried in an oven with circulating air at 50 ^{0}C for 24 hours (7.31 ± 0.20 mg/g DW) in an oven without circulating air at 50 ^{0}C for 24 hours (6.73 mg/g DW) or in an air-conditioned room at 25 ^{0}Cfor 7 days (7.32 ± 0.35 mg/g DW) (Pereira *et al.* 2000).

Recent research work done at CFTRI, Mysore by Ramalakshmi *et al.* (2000) in curry leaf showed that vacuum shelf dried leaves retained a better green color than the sun dried or mechanical dried samples. There was complete darkening in color in all these cases. However, the yield of volatile oil was higher in the vacuum shelf dried leaves. Another interesting finding was that dried leaves yielded more oil than fresh leaves, which indicated that excess of moisture content played a role in the release of the oil from the leaf.

The rhizomes of *Alpinia galanga* were dried by three methods, convective, microwave-convection and infrared drying. The amount of essential oils in dried samples was increased from 67 percent to 77 percent in comparison with the fresh sample. The 1-8-cineole was the major compound in the essential oil of *Alpinia galanga* and the drying method affected its concentration. The oil yield was same in all the methods but the products of microwave-convection drying and infrared drying had a less brown colour than those of convective drying (Quyanh and Reinhard, 2002).

2.3. Storage

2.3.1. Effect of storage on phytochemistry

In a trial conducted by Elsohly *et al.* (1994), the taxol contents of stored, fresh and dried taxus clippings and that of intact or finely ground, dried clippings of taxus stored at room temperature (22 to 24 ^{0}C) or under refrigeration (2 to 4 ^{0}C) was monitored monthly for 15 months, it was found that the taxol contents of fresh intact clippings were stable for 10 weeks when stored under refrigeration.

The changes of cardiac glycoside complex in leaves of *Digitalis pupurea* during storage at different air humidity were studied and reports showed at higher RH, rapid decomposition of glycosides occurred (Rada, 1963; Kucera, 1971).

Dried chamomile were stored in plastic containers and cellophene bags at 0-20 ^{0}C and 20-23 ^{0}C in ambient condition up to two years. The content of essential oil declined from 0.8-0.5 percent during the first year and to 0.3 percent in second year. The composition of oil was not changed markedly during the course of the storage period and the length of drying period had little effect on oil content and composition (Dragbund and Aoliksu, 1997).

Krenn *et al.* (1998) reported that longterm storage of sundew herb (*Drosera* spp.) resulted in a considerable loss of napthoquinones (Plumbagin as well as 7 – methyl juglone). The decrease after 2.5 years was about 60 percent on an average. A similar decrease in the napthoquinone content was observed in commercial samples within one year.

Tsuchiya *et al.* (1999) found that beta carboline alkaloids may be responsible for the pharmacological effects of certain medicinal plants and of the plant materials tested no significant amounts of tetrahydrobetacorbolines were detected, suggesting that it may have got oxidised to betacarbolines during the drying and/or in the storing process.

During storage at 24 ^{0}C for 64 weeks, the levels of all alkamides fell by 80 percent in *Echinacea purpurea*. Alkamide levels also dropped significantly during storage at 18 ^{0}C (Perry *et al.*, 2000).

Lime-aonla (*Emblica officinalis*) spiced beverages were prepared from 'Desi' variety of aonla and kagzi lime and stored in white and amber colored bottles for six months at ambient temperature (10 – 29.6^0 C) and low temperature (4+/- 1^0 C) showed a gradual decrease in sensory quality, acidity, ascorbic acid and tannin contents. An increasing trend was found in total soluble solids, reducing sugars, total sugars, and non enzymatic browning (Deka *et al.* 2004).

Negi and Roy (2004) found that leaves of amaranth and fenugreek showed that the loss of beta-carotene ranged from 46.5-85.0 percent and 24-73 percent for fenugreek. Similarly, ascorbic acid retention ranged from 9 to 32 percent for amaranth and 23-80 percent for fenugreek and also there was a faster degradation of quality parameters at ambient condition.

2.3.2. Storage and microbial load

Medicinal plants are normally associated with a wide variety of microbial contaminants represented by bacteria, fungi (moulds), actinomycetes. Inevitably, this microbial growth depends on harvesting, handling and production techniques. Several environmental factors exert impact on the overall quality of herbal products and preparations (Kneifel *et al.* 2002).

Hitokoto *et al.* (1978) showed that moulds like *Pencillium, Aspergillus, Rhizopus, Mucor, Cladosporium* and *Aureobasidium* spp. could be found quite often in association with herbal drugs but mycotoxin produced were only around two percent.

Microbiological quality of different samples of *Mentha spicata* was determined by Lenoble *et al.* (1980). Of the 12 samples analysed, six were contaminated with mesophilic aerobic bacteria (10^6 g^{-1}) and the other six samples were contaminated with fungi of different groups.

Analytical studies on the microbial load of the crude root drugs from roots of *Achyranthus aspera, Acorus calamus, Adhatoda vascica, Clerodendron serratum* and *Picrorhiza kurroa* collected from different store houses in India showed *Aspergillus flavus* group, *A. niger, A. ochracerus, Pencillium citrinum,* and *Pencillium* spp. as the dominant microbes associated (Roy and Chourasia, 1990).

Phillipson (1993) reported that hundreds of commercial samples of medicinal plants were investigated for their microbial content and the aerobic bacterial count was found to be 10^2 to 10^5 colony forming units (CFU)/g and moulds and yeasts were found present at 10^2 - 10^6 CFU/g. Pathogenic organisms found in samples of medicinal plants

include species of *Streptococcus, Clostridium, Salmonella, Pseudomonas, Shigella* and *Escherichia coli*. He also reported that even when bacteria are killed by sterilization process, it is possible that endotoxins (liposaccharides from bacterial cell walls) may be present in dried medicinal plants. Presence of *Aspergillus* species which results in mycotoxin contamination and highly potent carcinogenic aflatoxins and sterigmatocystin were also found in dried medicinal plants. Kumar and Roy (1993) also detected considerable risk levels of aflatoxins in several medicinal samples of different taxa.

In an aflatoxin B1 analyses of 31 Egyptian herbs and medicinal plants, it was reported by Selim *et al.* (1996) that in 29 percent of the samples analysed, aflatoxin B1 was prevalent and the highest mean concentration of aflatoxin B1 was in herbs and medicinal plants (49 ppb) when compared to food samples, nuts and seeds, spices, dried vegetables and cereal grains.

The ability of fungi isolated from stored herbal drug plants to produce mycotoxins in semi-synthetic media were studied by Efuntoye (1999). Herbal plants screened included *Azadirachta indica, Plumbago zeylanica, Jatropha curcas, Vernonia amygdalina, Xylopia aethiopica and Moringa indica*. Results showed that aflatoxins and ochratoxin were produced by *Aspergillus flavus, A. parasiticus and A. ochraceus isolates*. The time production courses of aflatoxins B1, B2, G1 and ochratoxin A in crude herbal drug preparations showed that more of these toxins were produced with increase in time of storage of the drug raw material.

Cezech *et al.* (2001) screened 138 medicinal herbal drugs for microbial contaminants and for detecting pathogenic microorganisms. The results revealed that the microbial load of the samples varied considerably and nine herbal drugs contained a potentially afla-toxigenic mould flora.

Esimone *et al.* (2002) suggested that it is very necessary that herbalists should be enlightened about Good Manufacturing Practice (GMP) because the ten solid and ten liquid herbal preparations they assayed were heavily contaminated with bacteria and fungi at levels far above the officially stipulated limit for oral pharmaceutical preparations.

2.6. Phytochemistry of *Coleus amboinicus*

The phytochemical studies on *Coleus amboinicus* have made possible the identification of several active compounds in the essential oil of the crop, among them thymol and carvacrol are the major components.

The *Coleus aromaticus* leaf oil obtained by steam distillation (0.04-0.05%) was found to contain terpenolene (3.75%), alpha-pinene (3.20%), beta-pinene (2.50%), beta-caryophillene (4.20%), methyl eugenol (2.10%), thymol (41.30%), 1-8-cineole (5.45%), eugenol (4.4%), cavacrol (13.25%) and beta-phellandrene (1.90%) (Baslas and Kumar, 1981)

Essential oil of *Coleus aromaticus* was found to contain (in percent) alpha-pinene (0.465), beta-pinene (0.115), myrcene (0.172), limonene (10.888), gamma-terpene (3.589), p-cymene (1.150), beta-caryophillene (0.437), beta-selinene (17.389), ethyl salicylate (5.505), thymol (8.128), carvacrol (40.402), eugenol (7.358) and an unidentified phenolic fraction (4.25) (Malik *et al.*, 1985).

Using GLC and GLC-MS, the essential oil composition of the leaves and stems of *Plectranthus amboinicus* were analysed by Vera *et al.* (1993). The objective was to identify volatile compounds responsible for the characteristic fragrance of the plant. Of the identified compounds, 53 percent were monoterpene hydrocarbons; the remaining compounds included oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes (45%). Less than 5 percent of the compounds remained unidentified. The major components were delta-3-carene (16.3%), carvacrol (13.4%), camphor (12.3%) and gamma-terpinene(11.9%).

The essential oils, hydrodisulled from the leaves of *Coleus aromaticus* (*Plectranthus amboinicus*) was analysed by GC and GC-MS. The main constituents of the oil obtained from *C. aromaticus* were carvacrol (41.3%) and camphor (39%) (Fakim *et al.*, 1995).

Volatile compounds were isolated from leaves of *Coleus aromaticus* (*Plectranthus amboinicus*) by steam distillation, hexane extraction or supercritical CO_2 extraction (0.55, 6.52 and 1.4%, respectively). Twenty-six components were identified by GC-MS, for the first time. The main constituent was carvacrol (51.04-90.41%). Differences in the aroma of the extracts were observed which were attributed to qualitative and quantitative differences in extract composition (Pino *et al.*, 1996).

The essential oils, hydro-distilled from leaves of *Coleus aromaticus* (*Plectranthus amboinicus*), were analysed by capillary GC and GC-MS. The essential oils contained carvacrol (53-67%), p-cymene (6.5-12.6%) and gamma-terpinene (5.9-15.5%) as major constituents. The essential oil produced in September contained higher concentrations of carvacrol and beta-caryophyllene and oxygenated constituents than the essential oil produced in May (Mallavarapu *et al.*, 1999).

The air-dried leaves of *Coleus amboinicus* afforded three flavones: salvigenin, cirsimaritin and chrysoeriol by silica gel chromatography. Their structures were elucidated by extensive 1D and 2D NMR and UV spectroscopy (Ragasa *et al.*, 1999).

Chemical investigation of the leaf essential oil of *Coleus amboinicus* by GC and GC/MS technique indicated the presence of six components accounting for 97 percent of the total oil. The major component was thymol (94.3%) followed by carvacrol (1.2%),1.8-cineole (0.8%), P-cymene (0.3%), spathulenol (0.2%), terpen-4-ol (0.2%) and an unidentified component (1.4%) (Singh *et al.*, 2002).

2.7. Anti-microbial properties of *Coleus amboinicus*

The essential oil of the *Coleus amboinicus* is reported to have several pharmacological activities, the oil and extracts are having potential effect against some of the bacteria and fungi, which are having ability to cause diseases in human beings, animals and plants.

Anti-microbial and anti-fungal properties of the essential oil from wild *Coleus aromaticus* were reported and the essential oil inhibited the growth of six bacteria and six fungi (Prudent *et al.*, 1995).

The essential oil obtained from *Coleus aromaticus* was tested against 20 strains of bacteria. The bacteria comprised of nine strains of *Escherichia coli*, eight strains of *Salmonella*, and one strain each of *Proteus morganii*, *Staphylococcus aureus* and *Shigella sonnei*. *Coleus aromaticus* leaf oil was found effective on most bacteria tested (Nyein *et al.*, 1996).

Anti-microbial assay on salvigenin and cirsimaritin obtained from the air-dried leaves of *Coleus amboinicus* indicated that they have low anti-microbial activities against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger* (Ragasa *et al.*, 1999).

The ambient dried leaf extract of *Coleus amboinicus* was tested against *Xanthomonas campestris* pv. *malvacearum* from cotton (*Gossypium herbaceum* L.), *Xanthomonas campestris* pv. *phaseoli* from French bean (*Phaseolus vulgaris* L.) and *Xanthomonas campestris* pv. *vasicatoria* from tomato (*Lycopersicon esculentum* Mill.). The study revealed that the ambient dried leaf extract did not show any inhibition against all the tested bacteria (Satish *et al.*, 1999).

In vitro microbial activity of essential oils of *Coleus aromaticus* and *C. zeylanicus* were tested against seven bacteria (*Bacillus meganerium, B. subtilis, Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa and Xanthomonas campestris*) and eight fungi (*Aspergillus niger, A. parasiticus, Rhizopus oryzae, Rhizoctonia oryzae-sativae, Colletotrichum musae, Fusarium solani, Candida albicans, and Alternaria brassicicola*). The oil of *C. zeylanicus* had slightly higher inhibitory activity against a wide spectrum of bacteria and fungi than *Coleus aromaticus* (Deena *et al.*, 2002).

MATERIALS AND METHODS

MATERIALS AND METHODS

The investigation on the phytochemistry and antimicrobial properties of "panikkoorka" (*Coleus amboinicus*. Lour) were carried out at the Department of Plantation Crops and Spices and the Biochemistry and Microbiology laboratories of the College of Horticulture, Kerala Agricultural University. Vellanikkara, Thrissur, Kerala during 2003-2005.

The experimental material and methodology of the study are presented in this chapter. The whole programme was divided into five sets of experiments as follows:

A) Stage of harvest
B) Method of drying
C) Method of extraction
D) Storage
E) *in-vitro* antimicrobial activity

3.1 STAGE OF HARVEST

This experiment was carried out to find out the optimum stage of harvest of *Coleus amboinicus*.

3.1.1 Raising of crop

Coleus amboinicus was raised as a field crop under fully open conditions. The land was cleared and raised beds of 6 m x 1 m x 0.30 m were taken, powdered FYM (Farm Yard Manure) was applied and mixed well with the top soil. Softwood cuttings of *Coleus amboinicus* were planted at a spacing of 30 cm x 30 cm on August 10, 2004. The crop was weeded and watered whenever necessary. Two top dressings of dried and powdered FYM were given at third and fifth months after planting followed by earthing up. Periodic nippings were given after each harvest to facilitate the production of secondary and tertiary branches (Plate 1). The weather data during growth period of crop is given in Appendix I.

Plate 1: Field crop of *Coleus amboinicus* Lour.

3.1.2 Treatments

T_1: *3 months after planting*
T_2: 4 months after planting
T_3: 5 months after planting
T_4: 6 months after planting
T_5: *7 months after planting*

Fully matured healthy leaves were harvested by nipping in the early morning hours. The harvested leaves were immediately transported to the laboratory for various analyses.

3.1.2.1 Observations

Observations on biochemical changes at each stage of harvest are given.

3.1.2.1.1 Biochemical analyses

Chemical analyses were carried out with the plant sample collected from the field. Literatures on the phytoconstituents of *C. amboinicus* were scanty and all the extraction procedures and estimation methods were standardised.

3.1.2.1.1.1 Estimation of Total soluble sugars

The total sugars were estimated with a modification of Phenol sulphuric acid method suggested by Sadasivam and Manickham (1992). One gram of the fresh sample was homogenised with pestle and mortar and exhaustively extracted with methanol and the supernatant was evaporated and made up to 100 ml with distilled water. One ml of aliquot was taken in a test tube, added one ml of five percent phenol and five ml of concentrated H_2SO_4 were added and the colour was read at 490 nm.

3.1.2.1.1.2 Estimation of Total amino acids

The total amino acids content was estimated by the method suggested by Sadasivam and Manickham (1992). Ten percent isopropyl alcohol was used as the extraction medium of the sample.

3.1.2.1.1.3 Total soxhlet extractables

Two grams of the finely powdered sample was taken in a filter paper thimble and soxhletted using 140 ml of ethyl acetate until the solvent became colourless. The solvent was evaporated in the evaporating chamber and the percent recovery of total extractable matter was calculated.

3.1.2.1.1.4 Essential oil content.

Fresh leaves of *Coleus amboinicus* collected at monthly intervals were washed with tap water and cut into small pieces. The volatile oil was recovered by hydrodistillation (3h) using Clevenger's apparatus. The light yellow oil thus obtained was subjected to GC analyses.

3.1.2.1.1.5 GC Profile of the Essential oil

A GC-MS analysis of the essential oil was carried out at the Sophisticated Analytical Instrument Facility (SAIF), Cochin University of Science and Technology, Cochin.

The GC profile of the essential oil was obtained by using a Varian – CP 3800 model with 2m x 3mm SS column packed with five percent carbowax. 20 m on 100/120 chromosorb WAW. The oven temperature was programmed from 100-200°C @ 4°C/min with initial hold of two minutes. The injector and detector temperature were 220°C and 250°C respectively. The flow rate of carrier gas Helium was 1.0 ml min^{-1}.

3.1.2.1.1.6 Thin Layer Chromatography (TLC) of Sugars

Thin Layer Chromatographic method suggested by Daniel (1991) was followed for sugar analyses.

3.1.2.1.1.6.1 Preparation of Gel Plate

Sixty grams of Silica Gel G of 160 to 250 mesh size was taken in a round-bottomed flask, mixed with 120 ml of distilled water. The slurry was spread on gel plate 20cm x 10cm size with an applicator to provide 0.25 mm thick gel layer. The plates were allowed to set for ten minutes at room temperature and then placed in a chromatographic chamber at 150^0 C for an hour to activate the silica gel.

Leaf samples were extracted with methanol and five micro litres applied by using a capillary tube on the Silica Gel plate.

After the application of the sample, the silica gel plate was transferred to a chromatographic glass chamber, saturated with the solvent system (Table 1) and the chamber was closed with a lid and the solvent was allowed to run up to two-third portion of the plate. Afterwards the plate was taken out and sprayed with the reagent (Table 1), air dried and then plate was kept in a chromatographic oven at 120 to 130 ^{0}C for 20 minutes. The spots were marked, noted the colour and the Rf values were worked out.

Table 1: Details of TLC analysis of sugars

Phytochemical constituent	Absorbent	Solvent System	Spray Reagent
Sugars	Silica Gel G	Ethyl acetate + mixture of iso propanol – water (2 : 1)	Anisaldehide-sulphuric acid

3.1.2.1.1.7 Thin Layer Chromatography (TLC) of amino acid analysis

Thin Layer Chromatographic method suggested by Harborne (1973) was adopted for amino acids identification

Extraction of leaf sample was carried out with ten percent isopropyl alcohol. Five micro litres of the sample was applied using a capillary tube on the Silica Gel plate.

The plate was transferred to a chromatographic glass chamber, saturated with the solvent system (Table 2), then the chamber was closed with a lid and the solvent was allowed to run up to two-third portion of the plate. Afterwards the plate was taken out and sprayed with the reagent (Table 2) and then plate was kept in a chromatographic oven at 120 to 130 ^{0}C for 20 minutes. The spots were marked, colour noted and the Rf values were recorded.

Table 2: Details of TLC analysis of amino acids.

Phytochemical constituent	Absorbent	Solvent System	Spray Reagent
Amino Acids	Silica Gel G	n – Butanol : Acetic acid : Water (4 : 1 : 1)	0.1 % Ninhydrin in acetone

3.2. DRYING METHOD OF SAMPLES

The experiment was carried out with an objective to find out the best method for drying of *Coleus amboinicus* leaf sample.

3.2.1 Treatments.

T_1: Ambient temperature drying

T_2: Sun drying

T_3: Oven drying (60^0 C)

3.2.1.1 Ambient Temperature Drying

Samples were dried in open condition inside a well ventilated room till they attained constant weight for two consecutive days. Temperature inside the room ranged from 21 to 31^0 C.

3.2.1.2 Sun drying

Samples were sun dried till a constant weight for two consecutive days were recorded. Temperature during the drying period ranged 23 to 33 ^{0} C and in the night hours samples were kept in the plastic covers to prevent re-absorption of moisture.

3.2.1.3 Oven Drying (60 ^{0}C)

Samples were dried in hot air oven at 60 ^{0}C till a constant weight for two consecutive days were recorded.

3.2.2 Observations

Observations on both physical and biochemical changes after drying were taken as detailed below:

3.2.2.1 Physical observations

3.2.2.1.1 Residual moisture

Moisture content was estimated by drying ten grams of the samples in hot air oven at 70 ± 2^0 C till the samples attained constant weight. The moisture content was expressed in percentage (Ranganna, 1986).

3.2.2.1.2 Colour value

Colour changes due to various treatments were assayed by using Spectronic 20 Genesis. The wavelength at which dried samples of *Coleus amboinicus* gave peak

absorbance at 598 nm was found by using Spectronic genesis.

Five gram of dry powdered sample was soaked in 25 ml of water and 25 ml of methanol for two hours and absorbance was read at 598 nm, using spectronic 20 genesis (Vijayanand *et al.*, 2000).

3.2.2.1.3 Days taken for drying

Number of days taken by each treatment for drying was recorded.

3.2.2.1.4 Drying rate

Drying rate was found out for all samples using the method described by Narasimhan and John (1995). Samples kept for drying were taken at definite intervals and their weight as percentage to original weight was worked out.

The temperature ranged from 23 to 33^0 C during the period of sun drying, 21 to 31 ^{0}C in ambient temperature drying and in oven drying it was maintained at 60 ^{0}C.

3.2.2.2 Biochemical analysis

The total sugars, total amino acids and the soxhlet extractables in dried leaves subjected to different drying treatments were estimated as mentioned in 3.1.2.1.

3.2.2.2.1 Essential oil content and GC profile of the essential oil

The leaves, which are subjected to different drying treatments, were taken for the estimation of essential oil by Clevenger's apparatus. The light yellow coloured oil was then subjected to GC study.

Table 3: The extraction procedures of *Coleus amboinicus* leaves.

Treatments	Extraction procedure
T_1 - Water extract of fresh leaves	25 grams of fresh leaf sample was extracted with 100 ml water. Volume of the extract reduced to 25 ml by heating at boiling temperature.
T_2 – Water extract of leaves dried at ambient temperature.	25 grams of dry powder was extracted with 100 ml water and reduced the volume to 25 ml by heating at boiling temperature.
T_3 – Alcohol extract of fresh leaves	25 grams of fresh leaf sample was extracted with 100 ml of ethyl alcohol and reduced the volume to 25 ml by heating at boiling temperature.
T_4 - Alcohol extract of leaves dried at ambient temperature.	25 grams of dry powder was ground with 100 ml of ethyl alcohol and reduced the volume to 25 ml by heating at boiling temperature.
T_5 – Water extract residue (fresh leaves)	25 grams of fresh leaf sample was extracted with 100 ml of water and subjected to vacuum drying to get final residue without degradation at high temperature.
T_6 – Water extract residue (dried leaves)	25 grams of dry leaf powder was extracted with 100 ml of water and subjected to vacuum drying to get final residue without degradation at high temperature.
T_7 – Alcohol extract residue (fresh leaves)	25 grams of fresh leaf sample was extracted with 100 ml of ethyl alcohol and subjected to vacuum drying to get final residue without degradation at high temperature.
T_8 - Alcohol extract residue (dried leaves)	25 grams of dry leaf powder was extracted with 100 ml of ethyl alcohol and subjected to vacuum drying to get final residue without degradation at high temperature.
T_9 – Dry powder	The powder was obtained from the leaves dried at ambient temperature

The oil obtained from different drying treatments were subjected to GC study as mentioned in 3.1.2.1.1.5.

3.3 METHOD OF EXTRACTIONS

The extracts of fresh and dried leaves were prepared by using water and alcohol. The extraction procedures for various treatments are given in table 3.

3.4 STORAGE STUDIES

Extracts of *Coleus amboinicus* were stored in air tight amber colored glass bottles to study the changes during storage

3.4.1. Treatments.

T_1: 1 month after extraction
T_2: 2 months after extraction
T_3: 3 months after extraction
T_4: 4 months after extraction
T_5: 5 Months after extraction

3.4.2. Observations

Observations on biochemical parameters and microbial load in the stored samples were taken at monthly intervals.

3.4.2.1 Biochemical analysis

Total sugars and total amino acids were determined every month after storage as mentioned in 3.1.2.1.

3.4.2.1.1 GC profile of Essential oil

GC profile of essential oil samples were taken as per the procedure mentioned in 3.2.2.2.5.

3.4.2 Total microbial load

The quantitative estimation of micro-flora of stored *Coleus amboinicus* extracts were carried out by serial dilution plate technique (Johnson and Curl, 1972). The sample [1g (Residues/powder) or 1 ml (Liquid sample)] was added to 99 ml sterile distilled water in 250 ml of conical flasks and serial dilutions were prepared up to 10^{-5}.

3.4.2.1 Enumeration of fungi

A one ml of 10^{-5} dilution was pipetted out into sterile petri dish using a micropipette and 20 ml of melted and cooled Potato Dextrose Agar (PDA) medium (Appendix II) was poured to the petriplates and swirled for uniform spreading. Three Petridishes were kept as replicate for each sample. The petriplates were incubated at 28^0C ± 2 for 5 to 7 days. The fungal colonies developed at the end of fourth day were counted and expressed as CFU/ ml of sample.

3.4.2.2 Enumeration of total bacteria

The bacterial populations were estimated using 10^{-5} dilution in Nutrient Agar (NA) medium (Appendix II). The same method as employed for the estimation of fungal population was followed. The bacterial colonies developed were counted and expressed as CFU/ml of sample.

3.4.2.3 Enumeration of total actinomycetes

The estimation of actinomycetes population was carried out at 10^{-4} dilution using Ken knights Agar medium (Appendix II) and followed the same method as in the estimation

of fungal population. The actinomycetes colonies developed were counted and expressed as CFU/ml of sample.

3.5. *IN VITRO* ANTI MICROBIAL ACTIVITY

This experiment was carried out to find out the effectiveness of leaf extracts of *Coleus amboinicus* against selected human and plant pathogens. The following bacteria and fungi were used for the study.

Bacteria

- *Escherichia coli*
- *Staphylococcus* spp.
- *Salmonella* spp.
- *Pseudomonas* spp.
- *Ralstonia* spp.

Fungi

- *Alternaria* spp.
- *Colletotrichum* spp.
- *Fusarium* spp.
- *Aspergillus niger*
- *Candida* spp.

3.5.1 Preparation of water extract

The water extract of *Coleus amboinicus* was prepared as method suggested by Satish *et al.* (1999) and the concentrations of five, ten and fifteen percent were prepared and used in the study.

3.5.2. In-vitro evaluation for antimicrobial activity

The *in vitro* evaluation of water extract against above mentioned bacteria and fungi was carried out as method suggested by Satish *et al.* (1999). The percent inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$ (Dennis and Webster, 1971)

Where C = Growth in control plate
T = Growth in extract treated plate

3.6. TABULATION AND STATISTICAL ANALYSES

Observation on biochemical studies were tabulated by taking means, where as for microbial load the data were tabulated and analysed statistically in Completely Randomised Design (CRD) as proposed by Panse and Sukhatme (1976). The treatments were ranked according to Duncans' Multiple Range Test (DMRT) as suggested by Duncan (1955). For in-vitro evaluation of fungi and bacteria, T - test and Factorial CRD test was performed respectively.

RESULTS

The results of the study "Phytochemistry and anti-microbial property of 'Panikkoorka' (*Coleus amboinicus* Lour)"carried out at the Department of Plantation Crops and Spices and the Biochemistry and Microbiology laboratories of the College of Horticulture, Vellanikkara during 2003-2005 are presented in this chapter under the following sub titles:

1. Stage of harvest
2. Drying
3. Method of extraction and storage
4. Anti-microbial activity

4.1 STAGE OF HARVEST

The *Coleus amboinicus* plant was harvested at monthly intervals to know the ideal stage of harvest. The total sugars, free amino acids, soxhlet extractables and essential oil content were evaluated at different stages of harvest. Results are presented in table 4. Thin Layer Chromatography (TLC) was done for sugars and amino acids and Gas Chromatography (GC) of the essential oil was carried out at all stages (Table 5, 6 and 7).

4.1.1 Total sugars

Maximum quantity of sugars was recorded in the samples harvested at fourth month after planting (2.80 mg100g^{-1}) and minimum at fifth month after planting (2.09 mg100g $^{-1}$). During third, sixth and seventh month after planting 2.28 mg100g $^{-1}$, 2.19 mg100g $^{-1}$ and 2.42 mg100g $^{-1}$ were recorded respectively (Table 4).

4.1.2 TLC of Sugars

The Thin Layer Chromatogram of the sample expressed three spots at all stages of harvest with Rf values 31.39, 60.94 and 91.55. The colour of the spots were light

Table 4: Quantity of total sugars, free amino acids, soxhlet extractables and essential oil content at different stages of harvest.

Stage of Harvest	Total soluble sugars (mg $100g^{-1}$)	Total amino acids (mg $100g^{-1}$)	Soxhlet extractables (%)	Essential oil content (%)
3 MAP	2.28	5.19	09.12	0.12
4 MAP	2.80	5.50	09.13	0.12
5 MAP	2.09	5.55	10.32	0.12
6 MAP	2.19	5.24	10.31	0.20
7 MAP	2.42	5.12	10.05	0.20

MAP: Months after planting

Table 5: TLC of sugars at different stages of harvest

Stage of Harvest	T L C of Sugars	
	Rf values	Colour of the spot
3 MAP	31.39	Light Yellow
	60.94	Light Blue
	91.55	Violet
4 MAP	31.39	Light Yellow
	60.94	Light Blue
	91.55	Violet
5 MAP	31.39	Light green
	60.94	Light Blue
	91.55	Violet
6 MAP	31.39	Light Yellow
	60.94	Light Blue
	91.55	Violet
7 MAP	31.39	Light green
	60.94	Light Blue
	91.55	Violet

MAP: Months after planting

Table 6: TLC of free amino acids at different stages of harvest

Stage of Harvest	T L C of amino acids	
	Rf values	Colour of the spot
3 MAP	22.23	Violet
	36.12	Violet
	44.37	Violet
4 MAP	22.23	Violet
	36.12	Violet
	44.37	Violet
5 MAP	22.23	Violet
	36.12	Violet
	44.37	Violet
6 MAP	22.23	Violet
	36.12	Violet
	44.37	Violet
7 MAP	22.23	Violet
	36.12	Violet
	44.37	Violet

MAP: Months after planting

yellow/green, light blue and violet respectively, which indicated the presence of three different sugars in the leaves of *Coleus amboinicus* at all harvesting stages (Table 5).

4.1.3 Total free amino acids

Results of the total amino acids at different stage of harvest are presented in the table 4. The quantity of total free amino acid was highest at fifth month after planting (5.55 mg100g $^{-1}$) and the lowest value was recorded at seventh month after planting (5.12 mg100g $^{-1}$). The total amino acids during third, fourth and sixth months after planting were 5.19 mg100g $^{-1}$, 5.50 mg100g $^{-1}$ and 5.24 mg100g $^{-1}$.

4.1.4 TLC of amino acids

The TLC results of amino acids also expressed three different spots with Rf values (22.23, 36.12 and 44.37) at all stages of harvest. The colour of the spots was violet, which indicated the presence of three amino acids of same or similar functional groups in the leaves of *Coleus amboinicus* at all harvesting stages (Table 6).

4.1.5 Estimation of soxhlet extractable

Results of the soxhlet extraction of *Coleus amboinicus* using ethyl acetate are presented in the table 4. The percent total extractables was highest at fifth month after planting (10.32%) and the lowest was noted during third month after planting (9.12%). The percentage of extractables during fourth, sixth and seventh month after planting were 9.13, 10.31 and 10.05 percent respectively.

4.1.6 Content of essential oil

The content of essential oil was highest during sixth and seventh month after planting (0.2%) and it remained constant during third, fourth, and fifth months after planting (0.12%) (Table 4).

Table 7: The GC profile of essential oil during different stages of harvest. *

Components	Third month after planting		Fourth month after planting		Fifth month after planting		Six month after planting		Seventh month after planting	
	RT	%Area	RT	%Area	RT	%Area	RT	%Area	RT	%Area
Component A	10.142	8.78	10.220	24.22	10.350	17.02	10.193	32.21	10.106	30.99
Component B	25.766	12.30	25.869	15.79	26.233	14.40	26.073	16.26	25.865	14.49
Component C	28.203	7.35	28.497	9.62	27.468	5.18	27.331	6.14	27.171	5.16
Component D	28.584	3.13	28.687	2.10	28.791	8.96	28.709	10.94	28.516	8.28
Component E	32.018	13.00	32.132	13.22	32.421	11.75	32.439	15.64	32.200	12.91
Component F	32.972	2.27	33.062	2.46	33.313	2.96	33.307	4.31	33.130	2.52

* Data derived from the GCMS data presented in Appendix III

4.1.7 GC profile of essential oil

From the Gas Chromatogram of the essential oil six peaks of high percent area were selected for comparative studies (Appendix III) and presented in table 7.

A graphical representation of the same is presented in fig 2.

The samples of third and fourth month after planting (Table 7) had maximum and minimum Retention Time Index (RTI) values of 32.972, 33.062 and 10.142, 10.220 respectively. The maximum and minimum percent area values of the sample recorded during third and fourth month after planting were 13.00 percent, 15.79 percent and 2.27 percent, 2.46 percent, respectively.

The samples of fifth, sixth and seventh months after planting recorded the maximum RTI values 33.313, 33.307 and 33.130, while minimum RTI values were 10.350, 10.193 and 10.106. Corresponding maximum percent area values were 17.02 percent, 32.21 percent and 30.99 percent, while minimum percent area values were recorded as 2.96 percent, 4.31 percent and 2.52 percent (Table 7).

4.2 DRYING

The effect of different drying methods on various parameters was evaluated and the results are presented in the table 8, 9 and 10.

4.2.1 Residual moisture, colour value and number of days taken for drying

Residual moisture, colour value and number of days taken for drying of *Coleus amboinicus* were recorded after drying to a constant weight and the data are presented in table 8.

Table 8: Qualitative analyses of *Coleus amboinicus* leaves subjected to different drying treatments.

Treatments	Total Sugars (mg $100g^{-1}$)	Total amino acids (mg $100g^{-1}$)	Soxhalet extractables (%)	No. of days taken for drying	Essential oil content (%)	Color value (A)	Residual moisture (%)
Sun drying	1.24	3.70	13.55	5 (120 hr)	1.20	0.172	12.99
Ambient temperature drying	1.68	3.90	10.85	14 (336 hr)	0.90	0.159	11.38
Oven drying	1.05	3.73	12.03	9 (216 hr)	1.10	0.202	10.57

Table 9: Drying rate of *Coleus amboinicus* leaves subjected to sun, shade and oven drying.

Days	Sun drying (percent by original weight)	Ambient temperature drying (percent by original weight)	Oven drying (60 °C) (percent by original weight)
1	100.00	100.00	100.00
2	56.33	80.00	70.00
3	28.73	46.93	42.06
4	4.60	39.86	23.33
5	4.46	30.00	16.00
6	4.40	23.33	7.00
7	-	19.86	5.60
8	-	17.00	4.96
9	-	13.66	4.90
10	-	11.86	-
11	-	8.93	-
12	-	7.00	-
13	-	6.00	-
14	-	5.60	-
15	-	5.56	-

4.2.1.1 Residual Moisture (RM) and Drying Rate

Analysis of residual moisture revealed that the lowest percent was in oven dried samples at 60 °C (10.57%) and the higher values were recorded in samples dried in ambient condition (12.99%) followed by sun drying (11.38%) (Table 8).

Rate of drying has profound influence on the quality of the materials and it may depend on several factors like temperature, RH around the drying material, surface area and the very nature of the material. The results of drying rate of the treatments are presented in table 9.

Among the three methods of drying, sun drying was faster compared to oven and ambient temperature drying. Sun drying took only five days (120 hr) to attain a constant weight. At the same time oven drying at 60 °C took nine days (216 hr) while ambient temperature drying took maximum of 14 days (336 hr) to attain the constant weight.

4.2.1.2 Number of days taken for drying

It varied from five days to 14 days. Under ambient temperature it was 14 days, in sun drying five days and in oven drying it took nine days. Oven dried leaves looked attractive and retained the green colour to some extent (Table 8).

4.2.1.3 Colour value

Colour retention after drying is one of the quality attributes of the sample. Therefore an attempt has been made to assess the colour of the dried material and the results are given in table 8.

The results revealed that the leaves dried under ambient temperature condition recorded the lowest colour value (0.159 A) while oven (60°C) dried leaves recorded the highest colour value (0.202 A) followed by sun dried leaves (0.172 A).

4.2.2 Quality parameters of *Coleus amboinicus* subjected to different drying treatments

The estimation of total sugars, total free amino acids, crude extract, essential oil content and GC profile of the essential oil under different drying methods are presented in table 8 and 11.

4.2.2.1. Total sugar

The total sugar content was comparatively high (1.68 mg100g $^{-1}$) at ambient temperature and it was low in sun-dried samples (1.24 mg100g^{-1}) and oven-dried (1.05 mg100g $^{-1}$) (Table 8).

4.2.2.2. Total free amino acids

Total amino acid was high (3.90 mg100g^{-1}) at ambient temperature and low (3.70 mg100g^{-1}) in sun drying and oven drying (3.73 mg100g $^{-1}$). (Table 8)

4.2.2.3. *Crude extractables*

Results of the soxhlet extraction of all the three methods of drying are presented in table 8. The percent of crude extractables was highest in sun-dried leaves (13.55%) followed by oven-dried leaves (12.03%) and the lowest (10.85%) in leaves dried under ambient temperature condition.

4.2.2.4. Essential oil

The content of essential oil was found highest in ambient dried leaves (1.20%) followed by oven dried leaves (1.10 %) and lowest percent (0.90%) was recorded in sun dried leaves (Table 8).

Table 10: The GC profile of essential oil during different method of drying. *

Components	Sun drying		Ambient temperature drying		Oven drying	
	RT	%Area	RT	%Area	RT	%Area
Component A	10.256	10.85	10.037	10.78	10.073	10.66
Component B	26.259	3.10	26.166	21.79	26.120	18.50
Component C	27.463	8.40	27.4858	7.98	27.353	7.29
Component D	28.792	12.02	28.833	11.20	28.698	13.09
Component E	32.326	9.35	32.264	10.45	32.307	6.02
Component F	33.223	2.10	33.180	1.38	33.197	2.38

* Data derived from the GCMS data presented in Appendix III

4.2.2.5. GC profile of essential oil

Effect of different drying metho 's on essential oil content is presented in table 10. In sun drying method the maximum RTI value was 33.223 and minimum RTI value was 10.256. In oven drying method the maximum and minimum RT values were recorded as 33.197and 10.073. The ambient temperature drying showed RTI values 33.180 and 10.037.

The maximum percent area was recorded in ambient temperature dried sample (21.79%), followed by oven drying (18.50%) and sun drying (12.02%), while minimum percent area values were recoded in ambient temperature drying (1.38%) followed by sun drying (2.10%) and oven drying (2.38%)(Table 10).

4.3. METHOD OF EXTRACTION AND STORAGE

The extracts of selected methods were stored for five months and the values regarding total amino acids, total sugars, microbial load and the GC profile of essential oil were presented in tables 11 to 18.

4.3.1. Total sugars

The changes in the sugar content during storage period are presented in table11. Among the various extracts, sugar content was highest in the alcohol extract of fresh leaves during the entire storage period. It was 2.30 mg $100g^{-1}$at one month after storage and 2.38 mg $100g^{-1}$ at the fifth month after storage, followed by water extract of fresh leaves (2.09 mg $100g^{-1}$ at first month after storage and 2.20 mg $100g^{-1}$ at fifth month after storage) and water extract residue of fresh leaves (1.70 mg $100g^{-1}$ at first month after storage and 1.78 mg $100g^{-1}$ at fifth month after storage).

The lowest content of sugar was recorded for the dry powder (1.19 mg $100g^{-1}$ at first month after storage and 1.30 mg $100g^{-1}$ at fifth month after storage) followed by water extract residue of leaves dried at ambient temperature (1.30 mg $100g^{-1}$ at first

Table 11: Content of total sugars during storage

Treatments		Total sugars (mg $100g^{-1}$)				
		1 MAS	2 MAS	3 MAS	4 MAS	5 MAS
Water extract	Fresh leaves	2.09	2.13	2.15	2.18	2.20
	Ambient dried leaves	1.53	1.83	1.89	1.92	1.93
Alcohol extract	Fresh leaves	2.30	2.34	2.36	2.37	2.38
	Ambient dried leaves	1.35	1.45	1.54	1.58	1.60
Water extract residue	Fresh leaves	1.70	1.75	1.76	1.77	1.78
	Ambient dried leaves	1.30	1.35	1.37	1.38	1.39
Alcohol extract residue	Fresh leaves	1.40	1.42	1.41	1.45	1.46
	Ambient dried leaves	1.69	1.75	1.78	1.76	1.80
Dry powder		1.19	1.26	1.27	1.29	1.30

MAS: Months after storage

Table 12: Content of total free amino acids during storage

Treatments		Total free amino acids (mg $100g^{-1}$)				
		1 MAS	2 MAS	3 MAS	4 MAS	5 MAS
Water extract	Fresh leaves	4.32	4.29	4.18	4.17	4.15
	Ambient dried leaves	3.88	3.79	3.73	3.67	3.60
Alcohol extract	Fresh leaves	5.27	5.21	5.18	5.15	5.09
	Ambient dried leaves	3.13	3.09	3.04	3.01	2.95
Water extract residue	Fresh leaves	2.57	2.33	2.29	2.28	2.26
	Ambient dried leaves	1.14	1.12	1.09	1.07	1.04
Alcohol Extract residue	Fresh leaves	3.01	2.98	2.95	2.93	2.88
	Ambient dried leaves	1.89	1.84	1.80	1.78	1.75
Dry powder		1.90	1.85	1.83	1.78	1.72

MAS: Months after storage

Table 13: The GC profile of essential oil during different months of storage.*

Components	First month after storage		Second month after storage		Third month after storage		Fourth month after storage		Fifth month after storage	
	RT	%Area	RT	%Area	RT	%Area	RT	%Area	RT	%Area
Component A	10.251	3.98	10.253	4.64	10.239	4.46	10.234	4.36	10.235	4.24
Component B	25.998	15.51	25.998	15.71	25.908	15.66	25.900	15.60	25.891	15.50
Component C	27.513	4.89	27.713	4.60	27.814	4.45	27.970	4.31	27.913	4.11
Component D	28.605	7.17	28.605	7.24	28.656	7.16	28.655	7.10	28.651	7.08
Component E	32.283	9.27	32.281	9.60	32.314	9.40	32.313	9.38	32.312	9.36
Component F	33.00	1.92	33.012	1.97	33.112	1.86	33.111	1.84	33.110	1.81

* Data derived from the GCMS data presented in Appendix III

month after storage and 1.39 mg $100g^{-1}$ at fifth month after storage). It was also observed that the sugar content was high in extracts of fresh leaves and there was an increasing trend in sugar content during storage irrespective of extracts.

4.3.2. Total free amino acids

The content of total free amino acids recorded during the storage period is presented in table12. Among the various extracts, the amino acids were maximum in the alcohol extract of fresh leaves during the entire storage period. It was 5.27 mg $100g^{-1}$ after one month of storage and 5.09 mg $100g^{-1}$ after five month of storage respectively. It was closely followed by water extract of fresh leaves (4.32 mg $100g^{-1}$ after one month of storage and 4.15 mg $100g^{-1}$ after five month of storage) and water extract of dried leaves at ambient temperature (3.88 mg $100g^{-1}$ after one month of storage and 3.60 mg $100g^{-1}$ after five month of storage).

The lowest amino acid content was recorded for the water extract residue of dried leaves at ambient temperature (1.14 mg $100g^{-1}$ after one month of storage and 1.04 mg $100g^{-1}$ after five month of storage) followed by alcohol extract residue of dried leaves at ambient temperature (1.89 mg $100g^{-1}$ after one month of storage and 1.75 mg $100g^{-1}$ after five month of storage). It was also observed that the amino acid content was higher in extract of fresh leaves compared to dried leaves and there was a general decline in the amino acid content during storage irrespective of extraction method.

4.3.3 GC profile of essential oil

The GC data of stored essential oil are presented in table 13. The GC profile of oil for the first and second month of storage recorded the maximum RTI values of 33.00 and 33.012 minimum RTI values of 10.251 and 10.253 respectively. The GC profile of third, fourth and six month storage showed the maximum RTI values 33.112, 33.111 and 33.110 and minimum RTI values 10.239, 10.234 and 10.235 respectively.

During first, second and third month after storage of oil, the maximum percent area were recoded as 15.51 percent, 15.71 percent and 15.66 percent. Accordingly the

minimum percent area during first, second and third month after storage of oil were recorded as 1.92 percent, 1.97 percent and 1.86 percent respectively. In fourth and fifth month of storage of oil the maximum and minimum area percent were recorded as 15.60 percent, 15.50 percent and 1.84 percent, 1.81 percent. (Table 13)

4.3.4 Microbial load of stored samples

The microbial population of different stored samples of *Coleus amboinicus* estimated at monthly intervals are presented in tables 14 to 18.

The initial microbial status of the sample was evaluated before storing and it was found to contain bacteria (6 x 10^5 CFU/ml), fungi (8 x 10^5 CFU/ml) and actinomycetes was absent.

In the first month after storage, the maximum and minimum bacterial populations were recorded in T_1 (4.33 x 10^5 CFU/ml) and T_3 (1.66 x 10^5 CFU/ml). The highest and lowest fungal counts were recorded in T_5 (9.66 x 10^5 CFU/ml) and T_3 (3.00 x 10^5 CFU/ml) respectively (Table 14).

During second month after storage, the maximum bacterial populations were recorded in T_1 (5.33 x 10^5 CFU/ml); T_8 (5.33 x 10^5 CFU/ml) and minimum bacterial populations were recorded in T_3 (2.33 X10^5 CFU/ml) and T_4 (2.33 x 10^5 CFU/ml). The maximum fungal count was found in T_5 (11.67 x 10^5 CFU/ml) while T_3 (4.00 x 10^5 CFU/ml) recorded minimum level of fungal population (Table 15).

The highest and lowest bacterial count after third month of storage are recorded as follows, the maximum count of bacterial population was found in T_9 (6.66 x 10^5 CFU/ml) and while T_7 (3.33 x 10^5 CFU/ml) recorded minimum counts of bacteria for the same period. Fungal count for the same period are recorded as follows,

Table 14: Total microbial population during first month after storage of *Coleus amboinicus* extracts.

Treatments	Total microbial count (CFU/ml sample)		
	Fungi ($x10^5$)	Bacteria ($x10^5$)	Actinomycetes ($x10^4$)
T_1	9.00^a(0.9542)	4.33^a (0.6367)	nd
T_2	9.00^a(0.9542)	3.66^{ab}(0.5643)	nd
T_3	3.00^b(0.4771)	1.66^c(0.2219)	nd
T_4	3.00^b (0.4771)	2.00^c(0.3010)	nd
T_5	9.66^a(0.9852)	4.00^a(0.6020)	nd
T_6	7.66^a(0.8846)	3.66^{ab}(0.5643)	nd
T_7	4.33^b(0.6367)	2.00^c (0.3010)	nd
T_8	3.66^b(0.5643)	$2.33b^c$ (0.3679)	nd
T_9	8.00^a(0.9030)	4.00^a (0.6020)	nd
CD at 5% level	1.952	1.362	

The values with different superscripts differ significantly ($p<0.05$)
The values in parentheses represent log transformed values
The values represent average of three replication
nd - not detected

Microbial load before storage
Bacteria: 6×10^5 CFU/ml
Fungi : 8×10^5 CFU/ml
Actinomycetes: Absent

T_1: Water extract of fresh leaves.
T_2: Water extract of ambient temperature dried leaves
T_3: Alcohol extract of fresh leaves.
T_4: Alcohol extract of ambient temperature dried leaves.
T_5: Water extract residue of fresh leaves
T_6: Water extract residue of ambient temperature dried leaves
T_7: Alcohol extract residue of fresh leaves
T_8: Alcohol extract residue of ambient temperature dried leaves
T_9: Dry powder

Table 15: Total microbial population during second month after storage of *Coleus amboinicus* extracts.

Treatments	Total microbial count (CFU/ml sample)		
	Fungi ($x10^5$)	Bacteria ($x10^5$)	Actinomycetes ($x10^4$)
T_1	11.00^a(1.0413)	5.33^a(0.7269)	nd
T_2	9.66^a(0.9852	3.66^{abc}(0.5643)	nd
T_3	4.00^b (0.6020)	2.33^c(0.3679)	nd
T_4	4.33^b(0.6367)	2.33^c(0.3679)	nd
T_5	11.67^a(1.0670)	4.66^b(0.6689)	nd
T_6	10.67^a(1.0281)	4.33^{bc}(0.6367)	nd
T_7	5.00^b(0.6989)	3.00^{bc}(0.4771)	nd
T_8	4.33^b(0.6020)	5.33^a(0.7269)	nd
T_9	11.00^a(1.0431)	3.66^{abc}(0.5643)	nd
CD at 5% level	2.035	2.088	

The values with different superscripts differ significantly ($p<0.05$)
The values in parentheses represent log transformed values
The values represent average of three replication
nd - not detected

Microbial load before storage
Bacteria: 6×10^5 CFU/ml
Fungi : 8×10^5 CFU/ml
Actinomycetes: Absent

T_1: Water extract of fresh leaves.
T_2: Water extract of ambient temperature dried leaves
T_3: Alcohol extract of fresh leaves.
T_4: Alcohol extract of ambient temperature dried leaves.
T_5: Water extract residue of fresh leaves
T_6: Water extract residue of ambient temperature dried leaves
T_7: Alcohol extract residue of fresh leaves
T_8: Alcohol extract residue of ambient temperature dried leaves
T_9: Dry powder

Table 16: Total microbial population during third month after storage of *Coleus amboinicus* extracts.

Treatments	Total microbial count (CFU/ml sample)		
	Fungi ($x10^5$)	Bacteria ($x10^5$)	Actinomycetes ($x10^4$)
T_1	12.33^a(1.0910)	6.00^{ab}(0.6989)	nd
T_2	11.00^a(1.0413)	4.66^{ab}(0.6690)	nd
T_3	6.00^b(0.7781)	3.66^{ab}(0.4643)	nd
T_4	5.00^b(0.6989)	4.66^{ab}(0.6690)	nd
T_5	12.33^a(1.0910)	6.00^{ab}(0.7781)	nd
T_6	12.00^a(1.0791)	4.66^{ab}(0.6690)	nd
T_7	5.66^b(0.7533)	3.33^b(0.5228)	nd
T_8	6.33^b(0.8016)	3.66^{ab}(0.5643)	nd
T_9	12.33^a(1.0910)	6.66^a(0.8239)	nd
CD at 5% level	1.867	2.878	

The values with different superscripts differ significantly ($p<0.05$)
The values in parentheses represent log transformed values
The values represent average of three replication
nd - not detected

Microbial load before storage
Bacteria: 6×10^5 CFU/ml
Fungi : 8×10^5 CFU/ml
Actinomycetes: Absent

T_1: Water extract of fresh leaves.
T_2: Water extract of ambient temperature dried leaves
T_3: Alcohol extract of fresh leaves.
T_4: Alcohol extract of ambient temperature dried leaves.
T_5: Water extract residue of fresh leaves
T_6: Water extract residue of ambient temperature dried leaves
T_7: Alcohol extract residue of fresh leaves
T_8: Alcohol extract residue of ambient temperature dried leaves
T_9: Dry powder

Table 17: Total microbial population during fourth month after storage of *Coleus amboinicus* extracts.

Treatments	Total microbial count (CFU/ml sample)		
	Fungi ($x10^5$)	Bacteria ($x10^5$)	Actinomycetes ($x10^4$)
T_1	12.67^{a}(1.1027)	6.66^{ab}(0.8239)	nd
T_2	11.67^{a}(1.0670)	6.00^{abc}(0.7781)	nd
T_3	6.667^{b}(0.8239)	4.33^{bc}(0.6367)	nd
T_4	6.00^{b}(0.7781)	4.66^{abc}(0.6689)	nd
T_5	13.33^{a}(1.1248)	7.00^{a}(0.8450)	nd
T_6	12.67^{a}(1.1027)	5.00^{abc}(0.6989)	nd
T_7	7.00^{b}(0.8450)	3.66^{c}(0.5643)	nd
T_8	7.33^{b}(0.8652)	6.66^{ab}(0.8239)	nd
T_9	14.33^{a}(3.1566)	6.00^{abc}(0.7781)	nd
CD at 5% level	2.762	2.334	

The values with different superscripts differ significantly ($p<0.05$)
The values in parentheses represent log transformed values
The values represent average of three replication
nd - not detected

Microbial load before storage
Bacteria: 6×10^5 CFU/ml
Fungi : 8×10^5 CFU/ml
Actinomycetes: Absent

T_1: Water extract of fresh leaves.
T_2: Water extract of ambient temperature dried leaves
T_3: Alcohol extract of fresh leaves.
T_4: Alcohol extract of ambient temperature dried leaves.
T_5: Water extract residue of fresh leaves
T_6: Water extract residue of ambient temperature dried leaves
T_7: Alcohol extract residue of fresh leaves
T_8: Alcohol extract residue of ambient temperature dried leaves
T_9: Dry powder

Table 18: Total microbial population during fifth month after storage of *Coleus amboinicus* extracts.

Treatments	Total microbial count (CFU/ml sample)		
	Fungi ($x10^5$)	Bacteria ($x10^5$)	Actinomycetes ($x10^4$)
T_1	13.67^{a}(1.1359)	8.33^{a}(0.9208)	nd
T_2	12.67^{a}(1.1030)	7.00^{abc}(0.8450)	nd
T_3	7.66^{b}(0.8846)	5.33^{bc}(0.7269)	nd
T_4	6.66^{b}(0.8239)	5.33^{bc}(0.7269)	nd
T_5	14.00^{a}(1.1461)	7.66^{ab}(0.8846)	nd
T_6	14.00^{a}(1.1461)	5.33^{bc}(0.7690)	nd
T_7	7.66^{b}(0.8846)	4.66^{c}(0.6690)	nd
T_8	8.00^{b}(0.9030)	5.66^{bc}(0.7533)	nd
T_9	15.33^{a}(1.1855)	6.66^{abc}(0.8239)	nd
CD at 5% level	2.682	2.334	

The values with different superscripts differ significantly ($p<0.05$)
The values in parentheses represent log transformed values
The values represent average of three replication
nd - not detected

Microbial load before storage
Bacteria: 6×10^5 CFU/ml
Fungi : 8×10^5 CFU/ml
Actinomycetes: Absent

T_1: Water extract of fresh leaves.
T_2: Water extract of ambient temperature dried leaves
T_3: Alcohol extract of fresh leaves.
T_4: Alcohol extract of ambient temperature dried leaves.
T_5: Water extract residue of fresh leaves
T_6: Water extract residue of ambient temperature dried leaves
T_7: Alcohol extract residue of fresh leaves
T_8: Alcohol extract residue of ambient temperature dried leaves
T_9: Dry powder

the maximum fungal counts were found in T_5 (12.33 x 10^5 CFU/ml), T_1 (12.33 x 10^5 CFU/ml) and T_9 (12.33 x 10^5 CFU/ml) while T_4 (5.00 x 10^5 CFU/ml) recorded least count of fungal population (Table 16).

In fourth month of storage, T_5 (7.00 x 10^5 CFU/ml) recorded maximum bacterial population while T_7 (3.66 x 10^5 CFU/ml) recorded least bacterial population. The maximum and minimum fungal count was found in T_9 (14.33 x 10^5 CFU/ml) and T_4 (6.00 x 10^5 CFU/ml) respectively (Table 17).

During fifth month after storage, treatments T_1 (8.33 x 10^5 CFU/ml) and T_7 (4.66 x 10^5 CFU/ml) recorded highest and lowest bacterial population. Treatments T_9 (15.33 x 10^5 CFU/ml) and T_4 (6.66 x 10^5 CFU/ml) recorded maximum and minimum fungal counts respectively (Table 18) (Plate 2).

Actinomycetes population was found absent in all the stored samples during the entire period of storage (Table 14 to 18).

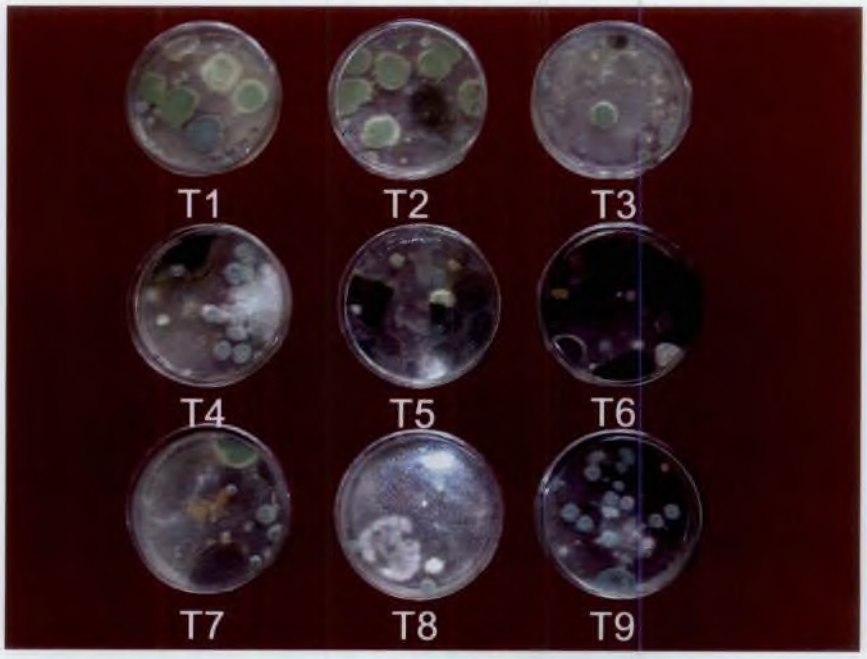
In brief, alcohol extract and its residue recorded lower population of bacteria and fungi when compared to water extract residues and dry powder.

4.4 *IN VITRO* ANTIMICROBIAL ACTIVITY

The study regarding effectiveness of *Coleus amboinicus* leaf water extract against different human and plant bacteria and fungi are presented in table 19.

The water extract of *Coleus amboinicus* leaves was found to inhibit the growth of *Escherichia coli* and *Staphylococcus* spp. at 15 per cent concentration giving inhibition percentage of 7.7 per cent and 6.6 per cent respectively (Table 19) (Plate 3).

Among fungi, the water extract of leaves was found to inhibit the growth of plant pathogens *Colletotrichum* spp. and *Fusarium* spp. at five, ten and fifteen percent concentrations giving growth inhibition percentage of 1.78 per cent, 3.5 per cent and 5.35 per cent, and 4.5 per cent, 9.0 per cent and 13.03 per cent respectively (Plate 3).



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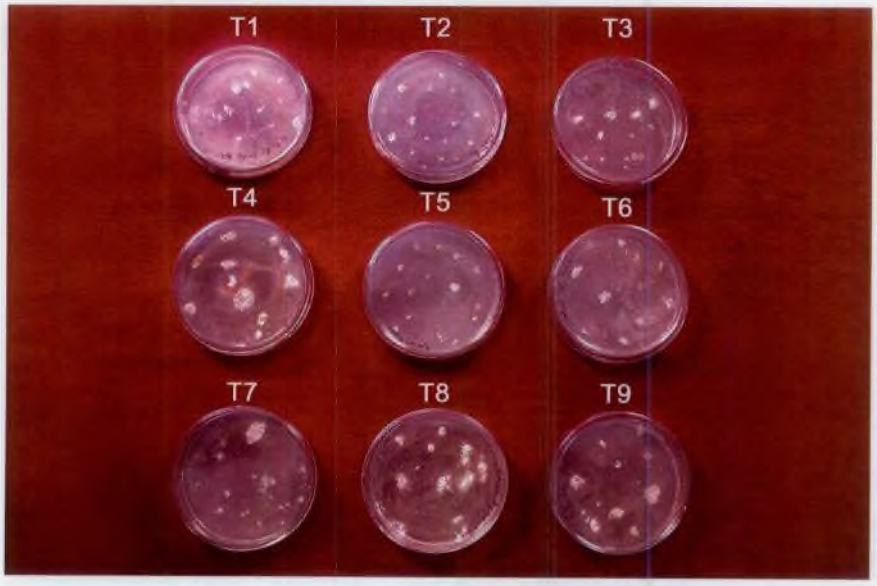
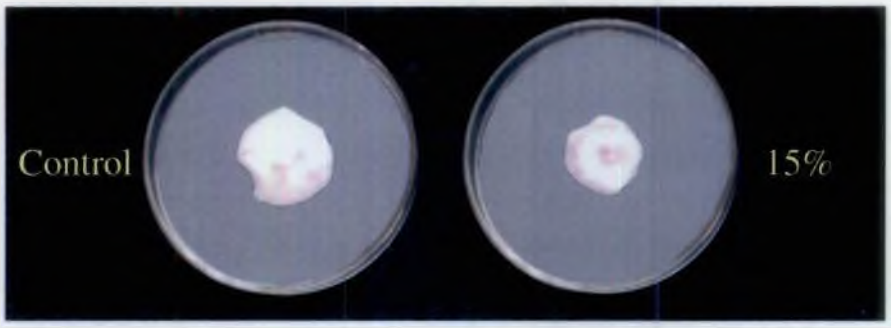


b

Plate 2: Microbial growth in *Coleus amboinicus* extracts after five months of storage

a. **Fungal growth**
b. **Bacterial growth**



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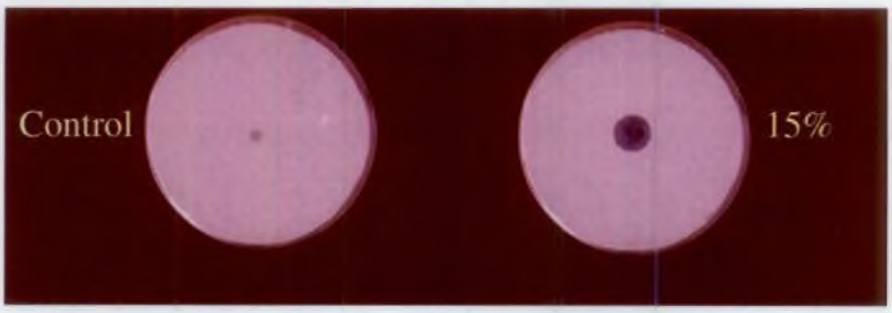

b

Plate 3: ***In vitro*** **anti-microbial activity of** ***Coleus amboinicus*** **against**

a. ***Fusarium*** **spp.**
b. ***Staphylococcus*** **spp.**

Table 19: Antimicrobial assay of water extract of *Coleus amboinicus* leaf.

Sl. No	Culture	Inhibition (%)		
		5 %	10 %	15 %
I. Bacteria				
1	*Escherichia coli*	0.0	0.0	7.7
2	*Staphylococcus* spp.	0.0	0.0	6.6
3	*Salmonella* spp.	0.0	0.0	0.0
4	*Pseudomonas* spp.	0.0	0.0	0.0
5	*Ralstonia* spp.	0.0	0.0	0.0
II. Fungi				
1	*Alternaria* spp.	0.0	0.0	0.0
2	*Colletotrichum* spp. **	1.78	3.5	5.35
3	*Fusarium* spp. **	4.5	9.0	13.63
4	*Aspergillus niger*	0.0	0.0	0.0
5	*Candida* spp.	0.0	0.0	0.0
CD at 5% level (between species)		0.243		

** Significant at 5% level
The values represent average of three replication

DISCUSSION

The results of the study entitled "Phytochemistry and anti-microbial property of 'Panikkoorka' (*Coleus amboinicus* Lour.)" are discussed under different subtitles given below.

5.1 STAGE OF HARVEST

The aim of this experiment was to determine the optimum stage of harvest of *Coleus amboinicus* in terms of quality. Total sugars, total amino acids and total soxhlet extractable matter were estimated. Essential oil content was quantified and GC profile of the oil was recorded.

Sugars are the initial product of photosynthesis. Generally plant always maintains equilibrium level of sugars in the source and the concentration of sugar source may vary with the plant growth stage. In the present study, the total soluble sugar content was high during fourth month after planting (Fig.1). It is also evident that the total sugar content did not fluctuate drastically during the entire growth stage. The plant always maintained the photosynthetic activity at a higher level. Thin Layer Chromatography (TLC) of sugars revealed that there are three sugars at all stages of harvest. At all harvesting stages, the same sugars were present but the concentration of the sugar with Rf value (30.39) was found to vary at different stages as indicated by the color and intensity of the spot (light yellow, light green). Such meager variation in sugar content may not be considered as a criterion for harvest.

Amino acids like ornithine, lysine, phenylalanine, tyrosine and tryptophan are the precursors of many secondary metabolites in plants. In the present study, amino acid content was found high during fifth month after planting. Like sugars, amino acids content also did not fluctuate drastically during the entire growth period (Fig.1). The plant must have maintained normal metabolic activity from planting till harvest. The TLC of amino acids also followed

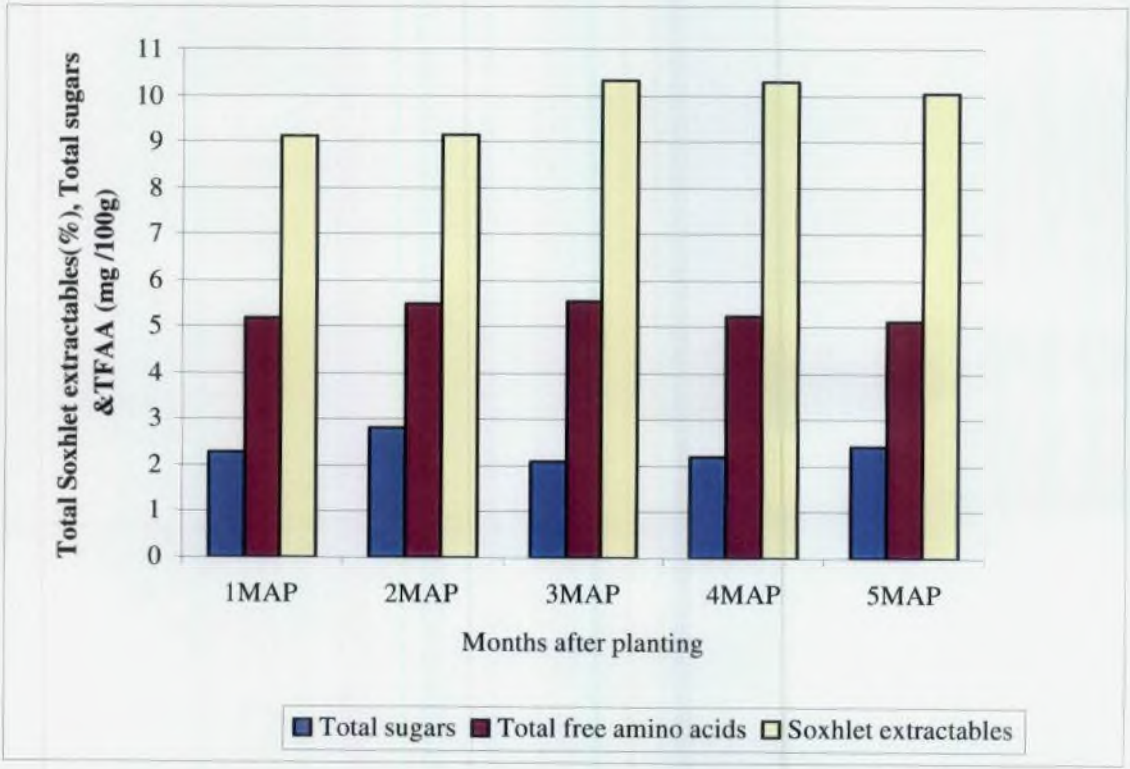

Fig.1: Content of total soluble sugars, total free amino acids and total soxhlet extractables during different harvesting stages.

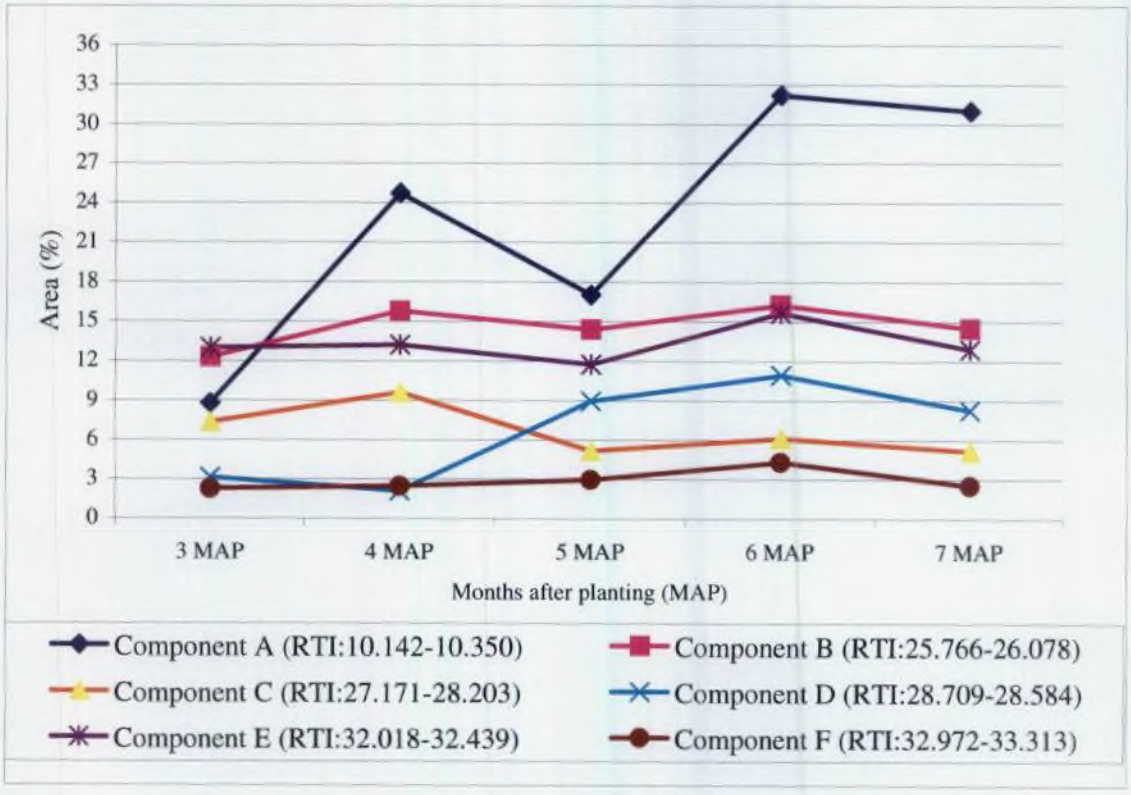

Fig. 2: GC profile of essential oil during different harvesting stages.

the same trend as sugars. The study revealed that the leaves contain three amino acids with same three Rf values at all stages of harvest. Intensity of the spot was also the same, indicating the same proportion of amino acids.

In the present study, variation in the sugar content at the initial and final stages of harvest was not having any influence on the amino acid content. This may be an indication of the metabolic stability and efficiency of *Coleus amboinicus* Lour. The presence of same type of amino acids in the initial and final stages may be a critical aspect of the biochemical status of the plant. The same trend in the amino acid content at all stages revealed that the plant can be harvested for medicinal use at any time, provided that the amino groups are the contributing factors for its property.

The total extractable matter was found highest in fifth and sixth month after planting (Fig.1). This may be due to the production of secondary metabolites at higher rate in a particular condition especially season.

At fifth month after planting the percent of soxhlet extractables was high and the sugar low. It is an indication of relation between soxhlet extractable and sugar content. This could be due to the conversion of more primary metabolites (total sugars) to secondary metabolites which may be manifested in the form of higher total extractables.

The content of essential oil in the leaves was found maximum during sixth and seventh month after planting (Table 4). High temperature might have got positive influence on the increase of oil content. The same note was given by Douglas (1969) that, the essential oil bearing plants contain maximum amount of essential oil during warmer and hotter condition. Similar findings were made by Srivastava *et al.* (2000) in *Mentha arvensis* cv Kosi and Ram *et al.* (2001) in geranium cv Bourbon.

The GC profile of essential oil of the samples revealed that the percent area covered by component A was highest in all the stages of harvest and during sixth month after planting

the concentration of component A was highest when compared to other components. Concentration of other components *viz.*, B, C, D, E and F in the essential oil remained low with very low variation during different stages (Fig.2). In general it can be inferred that, concentration of the various components in the oil of *C. amboinicus* during the growth period may be genetically controlled and not by the environment.

Analytical data of qualitative parameters revealed that the stages of harvest (from third month after planting to seventh month after planting i.e. from the month of October to February) did not have much influence on the quality of the plant *Coleus amboinicus* to a considerable extent. Even though fifth month after planting can be considered as the optimum stage of harvest with high proportion of the components, for all practical purpose, the crop can be harvested for medicinal use during its entire growth period.

5.2 DRYING

This experiment was taken up primarily to see the qualitative changes during drying and to find out the best method for use as a drug. Of the different drying methods *viz.*, sun drying, oven drying and ambient temperature drying (Table 9) the residual moisture was found low in the samples dried in oven. It may be due to increased surface area of the material. The heat transfer and mass transfer during drying will depend on the increased surface area. In oven drying, the material was exposed fully to hot air in its surrounding while drying, hence maximum escape of moisture happening leading to lower residual moisture. The findings of this experiment also support the findings of Gauniyel *et al.*(1988), who reported that the roots and whole plant samples of *Adhatoda zeylanica* and *A. beddomi* with thicker texture and less surface area exposed to drying recorded more residual moisture in case of sun and shade drying and lower residual moisture for mechanical drying. It is also in agreement with the study by Sujatha (2002) of *A. beddomi*, where in machine dried roots gave low amount of residual moisture than sun and shade dried roots.

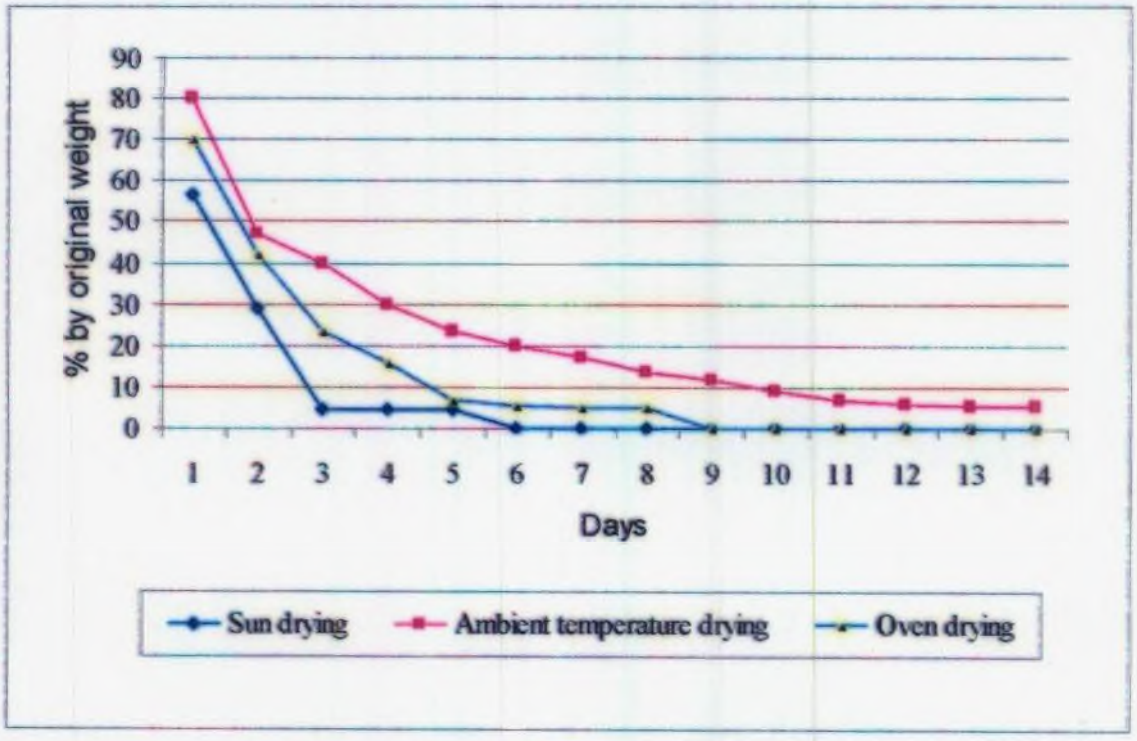

Fig. 3: Drying rate of *Coleus amboinicus* leaf samples in different methods.

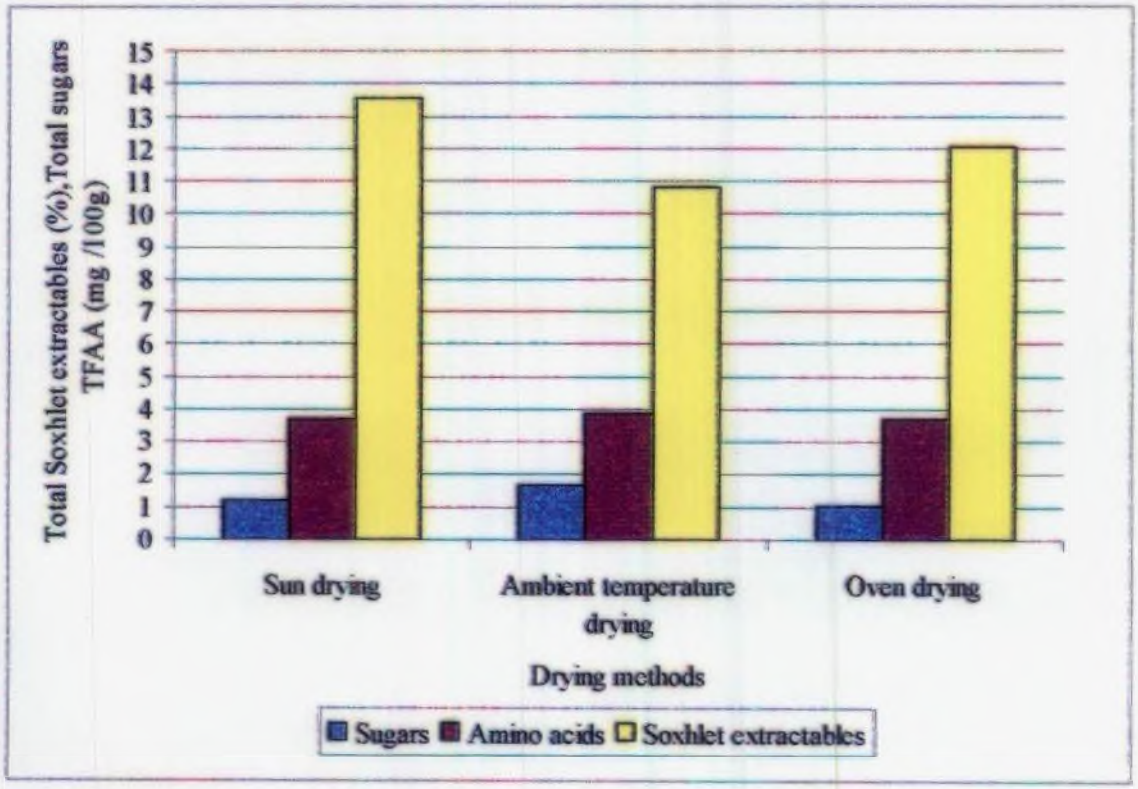

Fig. 4: Content of total soluble sugars, total free amino acids and soxhlet extractables during different drying methods.

Generally rate of drying depends on several factors like drying temperature, initial moisture content and the texture of the drying tissues. Higher the gradient of temperature, higher will be the rate of drying and *vice versa*. Drying at ambient temperature condition took maximum days for attaining constant weight in the present study (Fig.3). It could be due to higher residual moisture content of the leaves. The same result was obtained by Tonzibo *et al.* (1998) in *Eucalyptus citriodora* , it took exactly 20 days for drying under shade. Sun drying took lesser days for drying due to high temperature and air movement.

The leaves dried in oven gave maximum absorbance value (color value) compared to shade and sun dried materials (Table 9). Retention of green colour was the main reason for good colour and appearance of oven dried sample. The lower absorbance value (color value) was recorded in the sample dried at ambient temperature. This finding is in conformity with the findings of Gulati and Tajuddin (1979) in *Mellisa officinalis* and Ramalakshmi *et al.* (2000) in curry leaf and Sujatha (2002) in *Adhatoda beddomei*, where in mechnical dried roots gave higher color value and shade dried roots gave lower color value.

With respect to the qualitative parameters of dried samples, the content of total sugars (Figure 4) was low in all dry samples when compared to that of fresh sample. The percent reduction was 78 percent. It may be due to the degradation/interconversion of sugars to other components during the drying process. The maximum change was in sun drying.

Total free amino acids was also found low in sun drying (Fig. 4) when compared to fresh samples and the percent reduction from fresh to dried was 41 percent. Among different methods of drying the amino acid profile did not change at all.

The percent soxhlet extractable was found highest in sun dried material (Fig. 4). The higher value obtained may be due to short duration of drying at moderate temperature of 30 to 33 °C. The findings of present study are in agreement with Smith *et al.* (1998), where the oven dried leaf sample gave slightly lower extractable than sun dried material. Liu Zhi Jun *et al.* (1998) also got similar results where in oven drying caused degradation of campothesin.

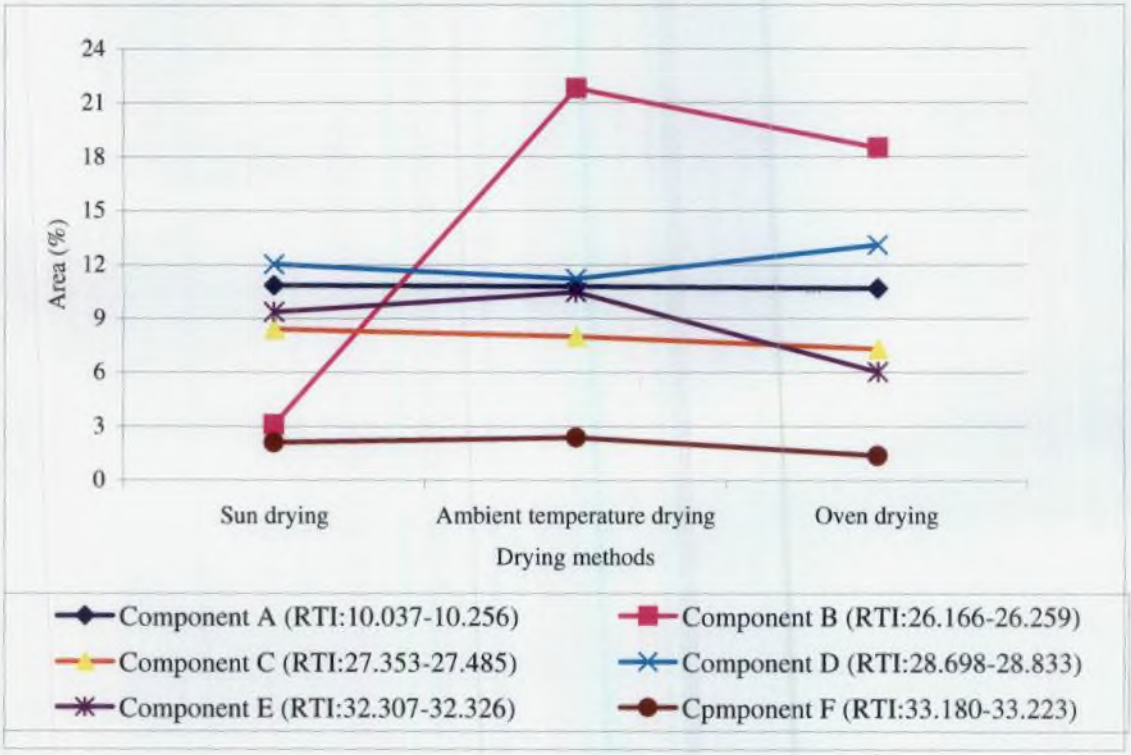

Fig 5: GC profile of essential oil during different drying methods.

The lowest extractable matter was obtained in dry sample at ambient temperature. It took longer time to accomplish the drying and this might have lead to low percent of extractables. Similar findings were reported by Elsohly *et al.* (1997), who got lower taxane content in shade dried taxus samples.

The content of essential oil in the dried leaves also varied with respect to different drying treatments. The findings of the study are in agreement with the report of Tonzibo *et al.* (1998) in *Eucalyptus citriodora*. Maximum percent increase (53.84%) was observed in samples dried at ambient temperature. It may be due to prevalence of lower temperature (23 - 33 °C) condition at ambient temperature, resulting in low volatilization of oil than the sun and oven drying methods. The finding is in agreement with the study by Leela and Angadi, (1992) in mentha herbage dried under shade.

The GC profile of the essential oil from dried leaves showed that the concentration of components in essential oil was very low in all methods of drying, except the component B (Fig.5). The present study is in agreement with the study by Monteverde and Ordovaskaria (1928) in *Digitalis lanata* where drying of *D. lanata* lowered the active constituents. All the components including component B maintained same percent area in samples dried at ambient temperature.

Based on the above facts it can be inferred that from among methods of drying, drying at ambient temperature was more efficient than sun and oven drying, even though the content of extractable matter and color value were comparatively low in leaves dried at ambient temperature.

5.3 METHOD OF EXTRACTION AND STORAGE

This experiment was carried out to find out the most suitable extract of *Coleus amboinicus* for preparing crude drug. The effectiveness of different types of extracts was

Table 20: Percent change in total sugar content of different extracts during storage

Treatments		Total sugars					Increase of total sugars during storage (%)
		1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	
Water extract	Fresh leaves	-	1.13	0.93	1.39	0.92	5.26
	Ambient dried leaves	-	5.78	3.27	1.58	0.52	11.56
Alcohol extract	Fresh leaves	-	1.73	0.85	0.84	2.58	0.86
	Ambient dried leaves	-	6.20	6.20	2.58	1.26	18.51
Water extract residue	Fresh leaves	-	3.84	1.48	0.72	0.72	6.92
	Ambient dried leaves	-	1.44	0.57	0.57	0.56	1.71
Alcohol extract residue	Fresh leaves	-	1.42	0.70	2.83	0.68	2.81
	Ambient dried leaves	-	5.91	1.71	1.13	2.27	6.50
Dry powder		-	5.82	6.79	1.57	0.77	9.24

MAS – Months after storage

Table 21: Percent change in total free amino acid content of different extracts during storage

Treatments		Total free amino acids					Decrease of total free amino acids during storage (%)
		1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	
Water extract	Fresh leaves	-	0.69	2.63	0.23	0.48	4.09
	Ambient dried leaves	-	2.37	1.60	1.63	1.94	7.77
Alcohol extract	Fresh leaves	-	1.15	0.57	0.58	1.17	3.53
	Ambient dried leaves	-	1.29	1.64	0.99	2.03	6.10
Water extract residue	Fresh leaves	-	10.30	1.71	0.43	0.88	13.71
	Ambient dried leaves	-	1.78	2.75	1.86	2.88	9.61
Alcohol extract residue	Fresh leaves	-	1.00	1.01	0.68	1.73	4.51
	Ambient dried leaves	-	2.71	2.22	1.12	1.71	8.00
Dry powder		-	2.70	1.09	2.80	3.48	10.46

MAS – Months after storage

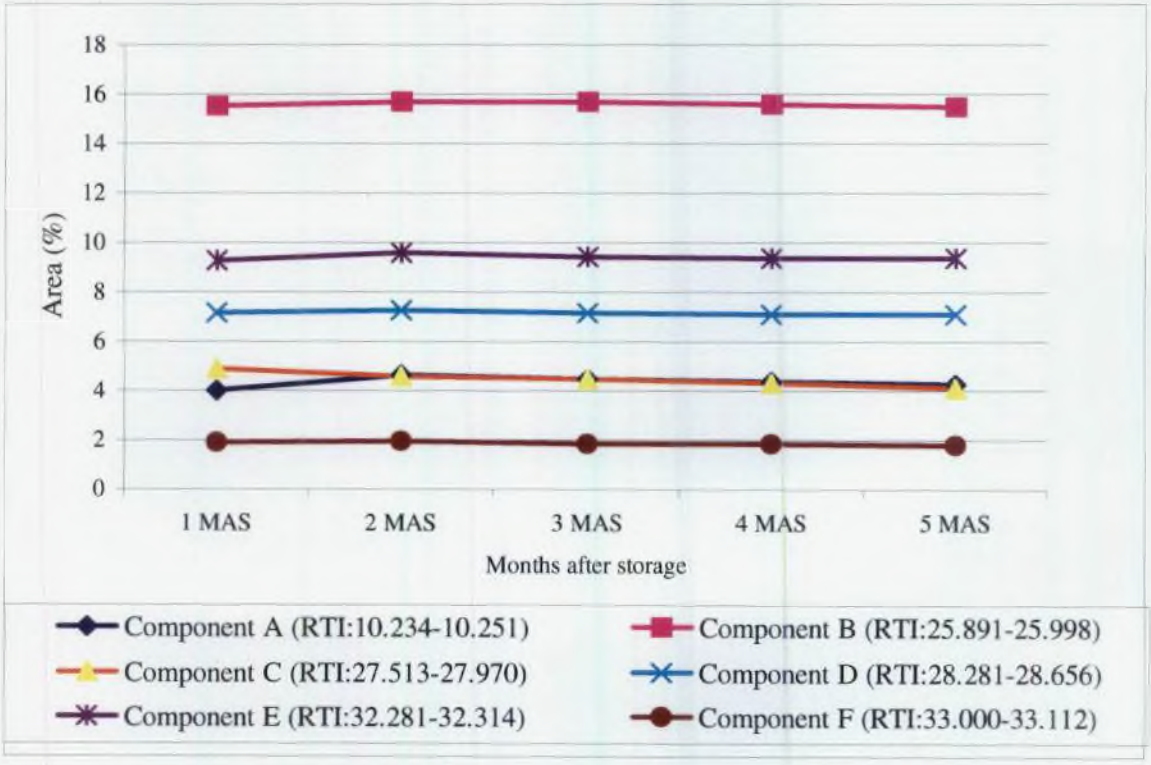

Fig 6: GC profile of essential oil during storage.

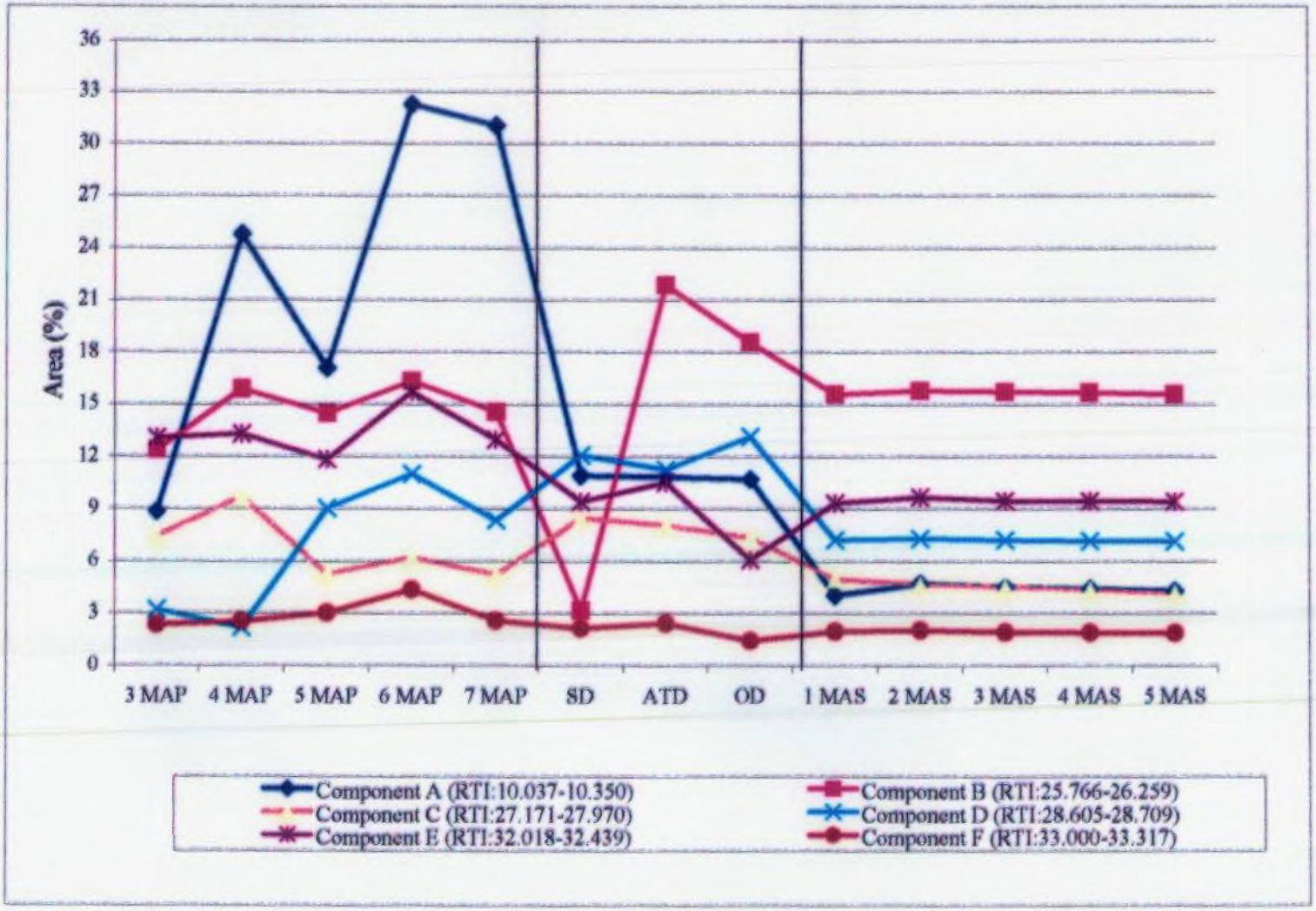

Fig 7: GC profile of essential oil of *Coleus amboinicus* leaves during different harvesting stages, methods of drying and storage.

judged by subjecting them to storage and analyzing the quality parameters during storage.

During storage qualitative parameters like total sugars and total free amino acids were estimated and the microbial load of different extracts also enumerated. The GC profile of essential oil during storage was also recorded.

Among the different extracts, the total sugar content was maximum in alcohol extract of fresh leaves (Table 11), it may be due to the partitioning property of alcohol which extracts even the insoluble components from fresh leaves having high water content. The lowest content of total sugar was recorded in dry powder. Degradation of sugar may also contribute much in the storage period. Another observation is that in all extracts other than dry powder, the sugar content was increasing during storage but the rate of increase very low (Table 20). It may be due to conversion/release of sugars from other components.

Similar to sugars, the amino acid content was also found maximum in alcohol extracts of fresh leaves (Table 12). The lowest content of total free amino acids was recorded in water extract residue of leaves dried at ambient temperature, where degradation of amino acids is anticipated. Another observation is that the content of total free amino acids was decreasing at an increasing rate during storage (Table 21). It may be an indication of the degradation of amino acids during storage period.

The GC profile of stored essential oil revealed that the percent concentration of all the components did not vary with the advancement of storage period (Fig. 6). This can be considered as a positive indication for preserving the oil for future use.

5.3.4 Microbial growth in stored samples

The medicinal plants are often associated with a wide range of microorganisms, which may be soil borne, air borne, or water borne. Among them bacteria and fungi dominate more than any other organisms. Current practices of handling, processing and production may cause

addition of microbial population and their growth. If certain precautions are not heeded during the various stages of processing and production, it may aggravate contamination and influence the quality of the herbal product. The results of the study on the quantitative analysis of microbes during storage are discussed hereunder.

The initial enumeration of the samples prior to storage itself has shown colonies of both bacteria and fungi. These organisms are either soil-borne or air-borne along with the harvested material, where it was reported that the harvested medicinal plants harbors many microbes (Phillipson, 1993; Kneifel *et al.*, 2002). The actinomycetes population of the sample before storage was zero. The results are in agreement with the studies earlier by Jha (1998).

Both bacterial and fungal populations were maximum in water extract residues during the period of storage. Increase in the residual moisture with the advancement of storage period will increase the water activity and increased water activity will prompt the growth of microorganisms. In all samples the population of microorganisms gradually increased with the advancement of storage. It was also observed by Jha (1998) that relatively dry substances can even support the growth of certain organisms like *Pencillium*, *Aspergillus* and can proliferate under storage.

The build up of microbes in water extracts and residues with storage time may be due to higher water content in them; Kniefel *et al.* (2002) observed that the storage of water extracts which host a considerable amount of microbes at ambient temperature usually increases microbial population during storage.

The lower amount of microbial load was assessed in alcohol extracts and its residues in comparison to water extracts and its residues. It may be due to the selective absorption and sterilization properties of the alcohol which lead to less microbial population.

Combining together the dynamics of the various phytochemical constituents and the microbial load during storage of the extracts, it may be concluded that the alcohol extracts and

its residues which recorded higher sugar and amino acids with less number of microbes seems to be the best extracts for storing *Coleus amboinicus* for use as a drug.

5.4 ANTIMICROBIAL ASSAY OF *COLEUS AMBOINICUS* LEAF WATER EXTRACT

Leaves of *Coleus amboinicus* are a common home remedy for infantile cough, cold, skin diseases and fever. This experiment was laid out to find the effectiveness of extract against different human and plant pathogenic bacteria and fungi at the laboratory level.

The water extract of *Coleus amboinicus* inhibited the growth of human pathogenic bacteria like *Escherichia coli* (which causes gastro-intestinal diseases and diarrhoea problems in human beings) and *Staphylococcus* spp.(which causes skin diseases and certain types of fever in human beings) (Table 19). Similar findings were reported with essential oil of *Coleus amboinicus* by Nyein *et al.* (1996); Ragasa *et al.* (1999) and Deena *et al.* (2002), where the essential oil of *Coleus amboinicus* found effective against bacteria like *Escherichia coli, Salmonella, Staphylococcus aureus and Pseudomonas aeruginosa*. The water extract was not found effective against bacteria like *Solmonella* spp., *Pseudomonas* spp. and *Ralstonia* spp. These findings are in agreement with the study by Ragasa *et al.* (1999), where in the active ingredients (Salvigenin, Cirsimaritin and Chrysoeriol) present in the leaf of *Coleus amboinicus* have found low antimicrobial activity against *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Candida albicans, Trichophyton mentagrophytes* and *Aspergillus niger*.

Among fungi, the water extract inhibited the growth of *Colletotrichum* spp. (which causes red rots in plants) and *Fusarium* spp. (which causes wilts in plants) (Table 19). Effectiveness of essential oil of *Coleus amboinicus* against *Colletotrichum musae, Fusarium solani, Candida albicans* and *Alternaria brasicola* have been reported by Nyein *et al.*(1996) and Ragasa *et al.*(1999). The extract showed no effect against fungi like *Alternaria* spp., *Aspergillus niger* and human/animal fungus *Candida* spp. These findings are in agreement with the study by Ragasa *et al.* (1999), where in the active ingredients (Salvigenin, Cirsimaritin and

Chrysoeriol) present in the leaf of *Coleus amboinicus* have found low antimicrobial activity against *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Candida albicans, Trichophyton mentagrophytes* and *Aspergillus niger.*

In brief, at the laboratory level, water extract of *Coleus amboinicus* inhibited the growth of human pathogens *Escherichia coli* and *Staphylococcus* spp. and plant pathogens *viz.*, *Colletotrichum* spp. and *Fusarium* spp.

SUMMARY

SUMMARY

Salient results of the study "Phytochemistry *and anti-microbial property of* 'Panikkoorka' (*Coleus amboinicus* Lour)"carried out at the Department of Plantation Crops and Spices and the Biochemistry and Microbiology laboratories of the College of Horticulture, Vellanikkara during *2003-2005 are summarized and presented here under:*

Total sugars and total free amino acids was found maximum in the leaves during fourth and fifth month after planting and also the reserve of these primary compounds did not reduce drastically at any stage of harvest.

TLC of total sugars and total free amino acids revealed that there are three kinds of sugars and amino acids present in the leaf of the *Coleus amboinicus* which were present in all stages of harvest.

The soxhlet extractable matter was found maximum during fourth month after planting.

The essential oil content was found maximum in sixth and seventh month after planting. GC profile of essential oil revealed that the percent concentration of individual components did not vary much during stages of harvest but one component (Component A) maintained dominance in its reserve at all stages of harvest.

Among different drying methods, drying at ambient temperature was found to be the best. The total sugars, free amino acids, essential oil content and residual moisture were found to be high in the samples dried at ambient temperature.

Sun drying recorded high quantity of extractable matter than ambient temperature drying and oven drying. Oven drying of *Coleus amboinicus* leaves recorded maximum color value than ambient temperature drying and sun drying.

Essential oil content increased in dried leaves compared to fresh ones and it was found maximum in ambient temperature. GC profile of essential oil revealed that the concentration of essential oil components decreased while drying, except component B which recorded high concentration irrespective of the method of drying.

Storage study of the different extracts revealed that the sugar content was highest in alcohol extracts and its residue of fresh leaves in the fifth month after storage. The total free amino acid content was also found high in alcohol extract and its residue of fresh leaves.

Total sugar content was increasing at slow rate while content of total free amino acids was decreasing at faster rate during storage, which may be an indication of interconversion/degradation of amino groups or similar components in the sample.

GC profile of stored oil revealed that the proportion of concentration of various components in essential oil did not vary much as storage period advanced.

Alcohol extract and its residue of *C. amboinicus* leaves recorded low amount of microbial load during entire storage period, which can be exploited in preparation of crude drug extracts of *Coleus amboinicus*.

In the laboratory studies, water extract of *C. amboinicus* leaves inhibited the growth of human pathogenic bacteria like *Escherichia coli* and *Staphylococcus* spp. and plant pathogenic fungi like *Colletotrichum* spp. and *Fusarium* spp.

To conclude, the plant *Coleus amboinicus* was effective as a drug throughout its active growth stage, in the fresh form. Fresh sample was ideal with respect to quality. Drying at ambient temperature was found to be the best method of drying. Alcohol extract was found best for the preparation of crude drug extract of *C. amboinicus*. Water extract of leaves inhibited the growth of select human and plant pathogens *in vitro*, however this needs confirmation through massive clinical and field studies.

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* Originals not seen.

APPENDIX

APPENDIX-I

Weather parameters during the study period

Months	Temperature (^{0}C)		Relative humidity (%)	Total rainfall (mm)	Total sunshine (h)	Rainy days
	Maximum	Minimum				
Aug-04	31.3	21.5	83	386.9	137.1	14
Sep-04	32.8	22.6	80	208.8	154	10
Oct-04	33.8	20.8	73	493.2	185.3	11
Nov-04	32.8	21.4	65	71.7	211.9	3
Dec-04	33.6	18.6	55	0.0	279.9	0
Jan-05	35.0	19.8	56	7.6	264	1
Feb-05	33.0	23.0	53	00.0	280.7	0
Mar-05	38.2	22.0	42	00.0	193.2	0
Apr-05	36.7	22.8	74	171.4	208.2	10
May-05	35.5	21.5	72	89.2	217.5	5
Jun-05	33.2	21.8	86	711.4	94.3	23

Source: Department of Agro meteorology, College of Horticulture, K. A. U., Vellanikkara.

APPENDIX-II

Media Composition for microbial studies

1. *Potato Agar Medium (for fungi)*

   Potato – 200g

   Dextrose – 20g

   Agar – 20g

   Distilled water – 1litre

   pH – 7.00

2. Nutrient Agar Medium (for bacteria)

   Glucose – 5g

   Peptone – 5g

   Beef extract – 3g

   NaCl – 5g

   Agar – 20g

   Distilled water – 1litre

   pH– 6.5 - 7.5

3. Ken knights Agar Medium *(for actinomycetes)*

   Dextrose – 1g

   KH_2PO_4 – 0.1g

   $NaNO_3$ – 0.1g

   KCl – 0.1g

   $MgSO_4$ – 0.1g

   Agar – 20g

   Distilled water – 1litre

   pH – 7.00

APPENDIX-III

Retention time and percent area of chromatogram of *Coleus amboinicus* oil during harvesting, drying and storage stages. *

3 MAP		4 MAP		5 MAP		6 MAP		7 MAP		Sun drying	
RT	%Area	RT	%Area	RT	%Area	RT	%Area	RT	%Area	RT	%Area
4.071	0.58	4.723	1.04	4.828	1.13	4.62	0.21	4.609	0.26	6.510	0.87
5.655	1.30	6.600	1.30	6.711	1.44	6.464	1.01	6.490	1.36	6.774	0.44
5.8	0.53	6.8	0.83	6.9	1.05	6.8	1.10	6.754	0.65	7.319	0.04
6.376	0.28	7.427	0.10	7.547	1.19	7.264	0.58	7.304	0.09	8.241	0.21
7.1	0.04	8.133	0.08	8.3	3.15	8.0	1.77	8.0	0.03	8.553	1.94
7.502	2.51	8.446	5.19	8.787	2.83	8.495	2.00	8.542	2.76	9.221	0.60
8.095	0.32	8.949	0.06	9.453	0.35	9.148	0.14	9.211	0.59	9.651	1.74
8.484	1.47	9.336	0.48	9.863	3.21	9.560	2.57	9.629	4.22	9.679	2.78
8.966	0.60	9.737	2.58	10.350	9.02	10.193	19.21	10.106	17.99	10.256	1.38
9.093	0.01	10.220	12.22	10.9	8.22	10.468	13.00	10.193	13.00	10.701	0.12
9.513	0.14	10.782	12.00	11.745	8.81	11.396	7.11	10.679	0.15	11.505	9.37
10.142	8.019	11.444	8.64	12.557	0.30	12.213	0.19	11.440	14.53	11.572	1.23
11.031	0.11	12.950	1.12	12.758	0.10	12.767	0.63	12.822	0.48	11.852	0.88
11.613	0.48	16.383	0.01	13.124	1.34	25.750	0.91	25.865	14.49	12.321	0.22
15.100	0.48	25.869	15.79	24.781	0.15	26.073	16.26	27.171	5.16	12.850	0.28
24.429	0.02	26.883	0.07	25.458	0.32	27.331	6.14	28.516	8.28	25.350	0.1
24.713	1.94	27.513	0.01	26.233	14.40	28.704	10.94	32.200	12.91	26.259	3.10
25.766	12.30	28.497	9.62	27.468	5.18	32.439	15.64	33.130	2.52	27.463	8.40
26.805	3.98	28.687	2.10	28.791	8.96	33.307	4.31			28.792	12.02
28.203	7.35	30.693	0.06	28.965	2.63					32.326	9.35
28.584	3.13	32.132	13.22	30.909	0.20					33.223	2.10
30.644	1.36	33.062	2.46	32.421	11.75						
32.018	13.00			33.313	2.96						
32.972	2.27										

MAP – Months After Planting

Conti.....

Oven drying		Ambient temperature drying		1 MAS	
RT	%Area	RT	%Area	RT	%Area
6.385	0.86	6.384	0.91	4.62	0.12
6.644	0.48	6.638	0.42	6.503	1.84
7.174	0.05	7.167	0.04	6.8	0.91
8.070	0.11	8.055	0.26	7.2	0.09
8.377	2.11	8.363	2.05	8.213	1.08
9.030	0.21	9.008	0.34	8.547	3.50
9.444	3.15	9.072	0.46	9.181	0.40
9.805	3.60	9.438	4.84	9.606	4.33
10.073	1.91	10.037	0.70	10.251	2.20
10.516	0.11	11.217	6.48	10.683	0.25
11.317	8.76	11.358	3.48	11.581	1.53
11.363	1.67	11.666	1.13	12.264	0.30
11.599	0.21	12.568	0.32	12.772	0.47
12.081	0.29	12.710	0.12	25.998	15.51
12.594	0.36	25.201	0.04	28.605	7.17
25.236	0.04	26.166	18.50	32.283	9.27
26.120	21.79	26.278	7.33	33.00	1.92
27.353	7.98	27.485	7.29		
28.698	11.20	28.833	13.09		
32.307	10.45	32.264	6.02		
33.197	2.38	33.180	1.38		

MAS – Months After Storage

Conti .....

2 MAS		3 MAS		4 MAS		5 MAS	
RT Index	%Area	RT Index	%Area	RT Index	%Area	RT Index	%Area
4.601	0.26	4.620	0.19	4.612	0.18	4.610	0.19
6.501	1.80	6.601	1.60	6.607	1.66	6.602	1.64
6.751	0.81	6.741	0.98	6.742	0.99	6.740	0.99
7.304	0.08	7.201	0.18	7.221	0.16	7.225	0.16
8.213	1.07	8.313	1.08	8.312	1.06	8.314	1.06
8.547	3.67	8.532	3.70	8.531	3.71	8.541	3.70
9.181	0.50	9.281	0.60	9.281	0.60	9.279	0.60
9.606	4.77	9.616	4.59	9.616	4.49	9.616	4.31
10.253	2.80	10.239	2.60	10.234	2.56	10.235	2.50
10.683	0.24	10.681	0.30	10.683	0.30	10.681	0.29
11.581	1.60	11.580	1.56	11.578	1.50	11.570	1.49
12.261	0.40	12.201	0.50	12.211	0.50	12.210	0.50
12.772	0.45	12.782	0.46	12.712	0.46	12.701	0.45
25.998	15.71	25.908	15.66	25.900	15.60	25.891	15.56
28.605	7.24	28.656	7.16	28.655	7.10	28.651	7.08
32.281	9.60	32.314	9.40	32.313	9.38	32.312	9.36
33.012	1.97	33.112	1.86	33.111	1.84	33.110	1.81

MAS – Months After Storage

* GCMS data analyzed at Sophisticated Analytical Instrument Facility (SAIF), CUSAT, Kochi, Kerala,

PHYTOCHEMISTRY AND ANTI-MICROBIAL PROPERTY OF "PANIKKOORKA" (*Coleus amboinicus* Lour.)

By

H. R. SHANKAR

ABSTRACT OF THE THESIS

submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

An experiment was conducted at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur during 2003-2005 to study the influence of stage of harvest, drying method and method of extraction and storage on phytochemistry and antimicrobial property of *Coleus amboinicus* Lour.

Stages of harvest did not alter the quality of the plant *Coleus amboinicus* to a considerable extent. All the quality parameters *viz.*, total sugars, amino acids and crude extractables were maximum at fifth month after planting, but there was only proportionate increase. The plant also maintained the same sugar and amino acid profile through out the growth period. GC profile of the essential oil revealed that the percent concentration of individual components did not vary much, with one of the components maintaining dominance in its reserve at all stages of harvest. With these results, the study concludes that *Coleus amboinicus* could be harvested and used as a drug during its entire growth period.

Among different methods of drying, ambient temperature drying was more efficient than sun and oven drying as it recorded more sugars and amino acids. GC profile of essential from dried leaves showed that the concentration of all the components except component B was very low in all the methods of drying and all the components maintained the same per cent area in samples dried at ambient temperature. When fresh and dry samples were compared, the former was superior in all the quality parameters.

Among the various extracts made out of *Coleus amboinicus* leaves, alcohol extracts and its residues made from fresh leaves recorded highest sugar as well as amino acids contents. When the extracts were stored, the sugar content increased at a slow rate where as the amino acid content decreased at a faster rate.

In the studies on microbial growth in the extracts during storage lower microbial load was assessed in alcohol extracts and its residues in comparison to water extract and its residues.

Considering the above findings alcohol extracts and its residues seemed to be the best extracts for storing *Coleus amboinicus* for drug purpose.

GC profile of the stored essential oil revealed that the percent concentrations of components did not vary considerably during storage. This may be considered as positive indication for preserving the oil for future use.

Anti-microbial assay of *Coleus amboinicus* water extracts revealed that the extract inhibited the growth of human pathogens *viz.*, *Escherichia coli* and *Staphylococcus* species and plant pathogens *viz.*, *Collectotrichum* species and *Fusariam* species. Further field and clinical studies are required to confirm these findings.

To conclude, the plant *Coleus amboinicus* was effective as a drug throughout its active growth stage, in the fresh form. Fresh sample was ideal with respect to quality. Drying at ambient temperature was found to be the best method of drying. Alcohol extract was found best for the preparation of crude drug extract of *C. amboinicus*. Water extract of leaves inhibited the growth of select human and plant pathogens *in vitro*, however this needs confirmation through massive clinical and field studies.