DRYING AND STORAGE STUDIES IN 'KIZHARNELLI' (*Phyllanthus amarus* Schum.&Thonn.)

By

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(2010-12-108)

THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Drying and storage studies in 'Kizharnelli' (*Phyllanthus amarus* Schum and Thonn.)"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

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ABBREVIATIONS

µg	microgram
CFU	Colony Forming Units
CD	Critical difference
CV	Coefficient of Variation
EMC	Equilibrium Moisture Content
ERH	Equilibrium Relative Humidity
et al.	Co workers
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
IC	Inhibitory Concentration
KMS	Potassium meta bisulphite
MAS	Months After Storage
MD	Mechanical Drying
ppm	Parts per million
RH	Relative Humidity
SFE	Super Critical Fluid Extraction
sp. or spp.	Species
viz.	Namely

INTRODUCTION

1. INTRODUCTION

During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs

In almost all the traditional systems of medicine, the medicinal plants play a major role and constitute their backbone. Several scientific studies conducted throughout the world have revealed and confirmed the dramatic medicinal properties of plants containing various phytochemicals like flavanoids, carotenoides, alkaloids etc.

Recognition and development of the medicinal and economic benefits of traditional medicinal plants is on the increase in both developing and industrialized countries, although it varies greatly from region to region (WHO-Traditional Medicine (TRM), 1998).

In the last few decades there has been an exponential growth in the field of herbal medicine which is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. World Health Organization estimates that 80 per cent of the world's population uses medicinal plants in primary health needs (WHO, 2003). Since about 80 per cent of the 7 billion people of the world are in less developed countries, this means that more than 5.6 billion people are likely to use medicinal plants on a frequent basis. Moreover, the modern medicine contains about 25 per cent of drugs derived from plants. Therefore, there is a need to study medicinal plants for their efficacy, safety and quality, and also to search for

potentially valuable medicinal material from which novel curative agents may be created for the benefit of all humankind.

A wide range of medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs, exudates and modified plant organs. While some of the raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many industries (Uniyal *et al*., 2006).

At present more than 20,000 herbal plants are being used for medicinal purposes at the international level. In India, the home for herbal plants, there are about 7000 medicinal plants, which are in use. Among the estimated source of 3.6 lakhs plant species spread over the earth, 40 per cent are available in our country. Recent survey indicated that about 7195 plant species are being used by 7800 herbal product industries manufacturing 25000 effective plant based formulations. Over 6.5 lakhs practitioners of Indian systems of medicine in the oral and codified streams use these formulations for preventing, promotive and curable application.

At the international level the herbal medicine trade has been estimated to be about 36-43 million dollars. India is one of the major exporters of crude drugs and one among the major biodiversity centers in the world. India has been producing herbal plants worth Rs. 2300 crores. Out of this, the quantity available for export amounts to Rs. 500 crores which represent 0.04 per cent of the world trade (Mohideen *et al.,* 2011).

Phyllanthus amarus, a distinguished botanical has been used worldwide since many years because of its rich medicinal importance. *P.amarus* is an erect annual

herb having large number of phytochemicals that are attributed to its leaves, stems and roots. A wide array of studies conducted revealed the anti inflammatory, antidiabetic, antimicrobial, antihyperlipidemic, antioxidant, antispasmodial, chemoprotective, antihypercalciuric, antiviral and diuretic properties associated with *P. amarus* (Thyagarajan *et al*., 1988; Patel *et al*., 2011).

P.amarus has been used since ages by the folk because of its rich ethanomedicinal importance. A number of phytochemicals associated with the herb renders it a broad spectrum medicinal valued herb. Therefore the chemical standardization of the raw material of plant and the formulations containing *P.amarus* is under immense invention and thus more work is required to ascertain *P. amarus* as a valuable herb (Kiran *et al* ., 2011).

In this background an attempt has been made to improve the postharvest handling of *Phyllanthus amarus* Schum & Thonn.) with an aim of making the material available for an extended period of 3-4 months other than its normal available period. Therefore the entire study is divided under the following objectives

1) To develop a suitable drying technique for 'Kizharnelli'

2) To find out the suitable packaging material to store the dried samples and extractives with minimum quality deterioration.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

From past to present, medicinal plants are considerably useful and helpful to cure the human ailments and diseases. Different parts of medicinal herbs are used to prepare different therapeutic medicine in many countries for several centuries. The bioactive substances originated from these plants possess the potential health benefits towards human body. India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. In the last few decades there has been an exponential growth in the field of herbal medicine which is getting popularized in developing and developed countries owing to its natural origin and lesser side effects.

'Kizharnelli'(*Phyllanthus amarus* Schum and Thonn), an important medicinal plant of the tropics, belongs to the family Euphorbiaceae and has long history in traditional system of medicine in every tropical country. *Phyllanthus amarus* Schum. & Thonn., which is widely spread throughout the tropical and subtropical countries of the world including India, is most commonly used in the Indian Ayurvedic system of medicine in problems of stomach, genitourinary system, liver, kidney and spleen (Zavar, 2011)

Phyllanthus amarus has long history of usage by the folk because of its rich medicinal values. It has been reported to possess potent antiinflammatory, antihepatotoxic, analgesic, hypotensive, antispasmodic, antibacterial, antiviral, diuretic, antimutagenic and hypoglycemic properties (Thyagarajan *et al*., 1988).

The postharvest process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active ingredients in the product sold (Silva and Casali, 2000).

Timely and right processing of medicinal plant produce, after it has been harvested is imperative to preserve the quality and enhance the shelf life of the produce. The produce should be properly dried before it is packed for shipping or storage. (NMPB, 2009).

Recent surveys indicate that poor post harvest handling and lack of knowledge on suitable packaging systems for herbs as major factors contributing to wastage, poor quality and limited market opportunities. In this background an attempt has been made to develop a suitable drying and storage method for whole plant and extractives of 'Kizharnelli'.

A review of research works done on different aspects of 'Kizharnelli' was undertaken and is presented here under the titles.

2.1 'Kizharnelli'- ethanopharmacological relevance

2.2 Drying of herbs

2.3. Storage of herbs

2.4. Extraction and phytochemistry

2.1 'KIZHARNELLI'- ETHANOPHARMACOLOGICAL RELEVANCE

Phyllanthus amarus Schum. & Thonn. (Euphorbiaceae) is an annual herb, growing as a weed throughout India, commonly known as Jamgli amli, Jaramla, or Bhuiamla. Traditionally it is useful in treatment of dropsy, jaundice, diarrhoea, dysentery, and intermittent fever, diseases of the urinogenital system, scabies, ulcers, and wounds.

Phyllanthus amarus is well known for the biologically active compounds it possesses, which include different classes of organic compounds of medicinal

importance including alkaloids, flavanoids, steroids, terpenoids, lignans, lipids and coumarins. Phyllanthin and hypophyllanthin are the important lignans isolated from *P.amarus* (Row *et al.,* 1967).

P. amarus is a member of the Euphorbiaceae family (Spurge family), which groups over 6500 species in 300 genera. Euphorbiaceae is a large family of upright or prostrate herbs or shrubs, often with milky acrid juice (Lewis, 1977).

P. amarus is always sold as fresh and dry plant materials in the herb markets. Decoctions are used in herbal baths and after labor, cramps, asthma, uterus complaints, and to treat stomachache (May, 1982).

According to Heyde (1990) *P.amarus* extracts are used as blood purifiers, for light malaria fevers and anaemia.

According to Unanader *et al.* (1991*)* the aerial parts of the herb *Phyllanthus amarus* Shum & Thonn. have been widely used in folk medicine in India and other tropical countries for the treatment of various diseases and disorders such as jaundice, diarrhoea, constipation, kidney ailments, ulcers, ringworm, malaria, genitourinary infections, hemorrhoids and gonorrhea.

Traditionally *P.amarus* is useful in treatment of dropsy, jaundice, diarrhoea, dysentery, intermittent fever, diseases of the urinogenital system, scabies,ulcers and wounds. In a number of countries, the aerial part of *P.amarus* is highly valued in traditional medicine for its healing properties. This plant is traditionally used around the world in the treatment of liver ailments and kidney stones. The Spanish name ‘chanca piedra’ means “stone breaker or shatter stone”. In South America, ‘chanca piedra’ has been used to eliminate gall bladder and kidney stones, and to treat gall bladder infections. (Foo and Wong, 1992).

The use of *P.amarus* is gaining momentum because of its novel antiviral activity against hepatitis B virus and for several other biological activities such as kidney and gall bladder stones, for cold, flu, tuberculosis and other viral infections; liver diseases, and disorders including hepatitis, jaundice and liver cancer (Unander *et al.,* 1993)

Phyllanthus amarus is a common pantropical weed that grows well in moist, shady and sunny places (Cabieses, 1993).

Jayarama *et al.* (1997) reported that *P.amarus* acts against liver cell toxicity and improves the immune system of patients and also found effective against hepatitis A.

Infusion of leaves, stem and root of *P. amarus* are used in Brazilian folk medicine for treating kidney problems, intestinal infection and liver problems (Calixito *et al.,* 1998).

Different plant parts of *P.amarus* are ethnobotanically used in various diseases and disorders e.g. leaves as expectorant and diaphoretic, fruits as carminative, laxative, astringent, diuretic, diaphoretic and tonic to the liver (Kirtikar and Basu, 2001).

Khatoon *et al.* (2006) conducted pharmacognistic studies on *P. amarus* and reported that the plant contains 6.23 per cent total ash, 10 per cent alcohol extract, 22.25 per cent water soluble extract, 0.2 per cent phyllanthin, 0.3 per cent hypophyllanthin. 0.7 per cent phenols, 1.9 per cent sugar and 0.7 per cent tannins.

Kumaran and Karunakaran (2007) reported that methanol extracts of powdered air dried *P. amarus* showed high antioxidant activities.

According to Igwe *et al.* (2007) *P. amarus* was first identified in central and southern India in 18th century but is now found in many countries including Philippine, Cuba, Nigeria among others. It is commonly called 'carry me seed', 'stone breaker', wind breaker' or 'gulf leaf flower'.

Among a few potent hepatoprotective phytomolecules reported in the scientific literature, against various types of liver damages, phyllanthin and hypophyllanthin have largely attracted the scientific community (Negi *et al*., 2008).

The extract of *P.amarus* along with a small amount of turmeric is taken orally for 3-5 days to cure jaundice (Prasad *et al*., 2008).

Annamalai and Lakshmi (2009) reported that the interest in *P.amarus* has increased in recent years based on the efficacy of the herb against Hepatitis B virus and all parts of this wonder plant is medicinally important.

In the market many drugs which contain *P.amarus* have been released under name like 'Ayurviva' against liver disorders, hepatoprotective, loss of appetite, general debility and convalescence and 'Lovanthin' against Hepatitis-B (Kumar *et al*., 2010).

P. amarus has been described in Ayurveda by the Sanskrit name – Bhoomyaamalakee. It was described to have the properties of Rasa, Guna, Veerya and Vipaaka. The Ayurvedic literature has shown its uses as Kaasahara (antitussive), Shwaasahara (antispasmodic, antidyspnoic), Kaphapittahara (which relieves the Kapha Pitta Dosha), Pipaasaaghna (which relieves Polydipsia), Raktapittahara (hemorrhage disease), Paanduhara (antianaemic), Kaamalaahara (which cures jaundice), Kushthaghna (indicated in leprosy), Daahaghna (refrigerant, relieves burning sensation), Kshatakshayaghna (indicated in Trauma) and Mootrarogahara (which cures urinary disorders) (Patel *et al*., 2011).

In the Ayurvedic system of medicine *P.amarus* is considered as an acrid alexipharmic and it is used in thirst, bronchitis, asthma, leprosy, anaemia, urinary discharge, anuria, hiccups and as a diuretic. In the Unani system of medicine, the herb is used as a stomachic and is also good for sores and chronic dysentery. The fresh root is believed to be an excellent remedy for jaundice. The infusion of the roots and leaves is a good tonic and diuretic when taken as repeated doses (Mohideen *et al*., 2011).

2.1.2 Antimicrobial properties of herbs

Phyllanthus amarus can help to control infection caused by *Staphylococcus aureus* which is a major pathogen of human infections varying from food poisoning or minor skin infections (Adegoke and Komolafe, 2008).

Khanna and Kannabiran (2008) evaluated antimicrobial activity of saponin fraction from the roots of *Hemidesmus indicus* and found that pure saponin extract exhibit remarkable antimicrobial activity against *Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, Aspergillus flavus, Aspergillus niger* and *Aspergillus fumigus.*

P. amarus was examined against ocular infections causing bacteria *P. aeruginosa, Micrococcus lylae, Bacillus licheniformis, Staphylococcus hominis, S. aureus, Staphylococcus haemolyticus, Micrococcus luteus, Bacillus lentus, Bacillus firmus* and *Pseudomonas stutzeri* using agarwell diffusion method. Results revealed that *P. amarus* exhibited remarkable bioactivity against *M. lylae, S. haemolyticus, B. lentus, B. firmus, P. stutzeri, P. aeruginosa* and *S. aureus* (Koday *et al*., 2009).

The antibacterial activities of the methanol, ethanol, acetone and chloroform extracts of *Glycyrrhiza glabra* plant roots were tested against five bacterial species- *Bacillus coagulans, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and found that ethanol and methanol

extracts shows inhibition against *B. coagulans, P. aeruginosa* and *S. typhi* but no inhibition effect against *P. aeruginosa* and *S. aureus* (Nisha *et al.,* 2010).

Akinjogunla *et al.* (2010) assessed the antibacterial activity of extracts of the root and leaf of *Phyllanthus amarus* against extend spectrum lactamase (ESBL) producing *Escherichia coli* and the results obtained showed that ethanolic extracts of *P. amarus* exhibits inhibitory activities against ESBL- *E. coli* at varying degrees of concentration.

Alexander *et al.* (2010) screened *Alpinia galangal* for potential antimicrobial activity and found that the extract was found most effective against two bacterial species - *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* followed by *Staphylococcus aureus, E. coli*, and *Vibrio species.*

A study conducted by Adgoke *et al.* (2010) revealed that the ethanolic extracts of *P.amarus* shows antimicrobial potentiality against *Staphylococcus aureus, Pseudomonas spp. Klebsiella spp and Eschersia coli.*

Ajitha Rani *et al.* (2011) reported that ethanolic and methanolic extracts of *Desmodium triflorum* have activity against *E. coli* and *Streptococcus aureus.*

Methanol extract of S*olanum nigrum* is mildly potent as antibacterial agent against *E. coli, B. subtilis* and *S. aureus* (Zubair *et al*., 2011).

Okolo *et al.* (2012) reported that the results of the antimicrobial screening on the crude extract of *P. amarus* showed activities against *Pseudomonas auregenosa, Escherichia coli, Staphylococus aureus and Candida albican* at minimum inhibitory concentration as low as 100 ppm.

2.2 DRYING OF HERBS

Water is a significant component of biological materials. The physical and chemical properties of herbs are determined by their moisture content. The first step in many postharvest operations is the removal of water through drying.

Drying is the oldest preservation technique of herbs and spices. In developing countries, traditional sun drying method is commonly used for drying herbs and spices. Although it is the cheapest method, the dry products are of poor quality due to contamination by insects, birds and dust.

The major objective in drying agricultural products is the reduction of the moisture content to a level which allows safe storage over an extended period. Drying also brings about substantial reduction in weight and volume and minimize packaging, storage and transportation costs (Okos *et al.,* 1992).

The leaves of aromatic and medicinal plants are often dried before extraction to reduce moisture content. During the process, many compounds which are dragged to the leaf surface by the evaporating water are lost (Moyler, 1994).

Since it results in reduction of the weight and volume of the plant, drying process may also contribute to a regular supply and facilitate the marketing of plants with positive consequences for transport and storage (Calixto, 2000).

According to Lorenzi and Matos (2002), drying of medicinal species is a preparation process, carried out to meet the needs of the pharmaceutical industry, which does not have the adequate conditions to use fresh plants on the scale required by industry.

Medicinal plants can be dried in a number of ways: in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms or buildings; by direct sunlight, if appropriate; in drying ovens/rooms and solar dryers; by indirect fire; baking; lyophilization; microwave; or infrared devices. When possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. (WHO, 2003).

Chemical changes are the most important in the post harvest of medicinal plants and can be influenced by drying. Drying can promote changes in product appearance and smell and thus modifies final quality of the product (Fennel *et al*, 2004).

During drying, heat-sensitive active ingredients in the plant materials are lost. Proper control of the drying process is important in minimizing the loss of the medicinal compounds of the plant parts (Tanko *et al*., 2005).

Drying decreases water activity which ultimately retards the microbial growth, helps to conserve the desirable qualities and reduces the storage volume. However enzymatic and or nonenzymatic processes that may occur during drying of the fresh plant tissues may lead to significant changes in the composition of phytochemical (Capeka *et al.,* 2005).

According to Schmidt *et al*. (2005) inadequate drying leads to microbial contamination and changes in the phytochemical composition.

Drying is the most common and fundamental method for preservation of medicinal plants because it allows for the quick conservation of the medicinal qualities of the plant material in an uncomplicated manner (Muller and Heindal, 2006).

Some herbs or crops are perishable in their fresh state and may deteriorate within a few days after harvest. One way to preserve these plant products is to dry them in order to conserve their desirable qualities, reduce storage volume and to extend their shelf life (Lim and Murtijaya., 2007)

Drying is one of the most critical and fundamental unit operations in the post-harvest processing of medicinal plants. The quality of drug and consequently the earnings are significantly influenced by the drying regime. To prepare high-quality products, considerable attention should be devoted to the drying process (Mahapatra and Nguyen, 2007).

According to Oztekin and Martinov (2007) drying of medicinal plants must meet the following requirements; 1) moisture content has to be brought down to an equilibrium level, 2) there should be minimum quality reduction in terms of active ingredients, colour, flavour and aroma and 3) microbial content must be below the prescribed limits.

Most of the herbs and spices are marketed as dried, because, due to high water content in the fresh state they undergo severe deterioration after microbial growth and biochemical changes (Verma and Chauhan, 2011).

2.2.1 Natural Drying

Sun drying is the cheapest method of drying which has been practised since ancient times. Here drying process is slow and the product will develop dark colour and may often contain deposits 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° C (Gupta and Pareek, 1993).

In the case of natural drying in open air, medicinal plant materials are spread out in thin layers on drying frames and stirred or turned frequently. In order to achieve adequate air circulation, the drying frames should be located at a sufficient height above ground. Efforts should be made to achieve uniform drying and so avoid mould formation (WHO, 2003).

Pragathi and Dahiya (2003) found that indirect solar drying method was comparatively better than direct solar drying in terms of nutritional value of dried aonla fruit.

For indoor drying, the drying time and drying temperature should be determined on the basis of the plant part concerned, and any volatile natural constituents such as essential oils. For example cinnamon bark is typically sundried when the quality of the bark is high (Cai *et al.,* 2004).

Solar driers were used successfully in drying of spices and medicinal plants. Those dryers may reduce the drying time by 65 per cent compared to natural drying (Mohamed *et al*., 2005).

The optimal method for drying medicinal plants is to spread the unwashed, dust free, plants on racks in a well- ventilated room away from sun light and excessive heat. Turning or agitation should occur daily, and the plants should not undergo further processing until the drying is completed (Ahmed *et al.,* 2007*).*

2.2.2 Artificial drying

To overcome the problems of drying viz., unhygienic surroundings and long duration of drying, the mechanical drier appeared in to the drying scenario. In the air circulating type of electric dehydrators, temperature inside the chamber can be precisely controlled to obtain desired quality of product without spoilage and dependence on weather in a short period of time.

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

Roots of *Rauvolfia serpentina* could be dried in a mechanical drier to reduce the moisture content to about 8 percent or less for safe storage (Gauniyal *et al*., 1988).

2.2.3. Effect of drying

A literature search was undertaken on effects of different drying methods on the quality of medicinal plants. The results showed that drying methods had a significant effect on chemical composition of medicinal plants.

Drying is an important operation that normally occurs in the early post harvest processing of medicinal plant materials involving the removal of moisture from the plant tissues, therefore decreasing the weight and preserving the plant material from further deterioration that may occur due to the presence of moisture.

In *Mellisa officinalis* L., flowering tops are dried in shade in order to preserve its natural colour (Gulati and Tajuddin, 1979).

After initial sun drying for 2 to 3 days, *Hyoscyamus* is dried in shade for 6-7 days with constant racking with sticks. The fresh herb loss 80-86 per cent of its weight on drying. The characteristic odour disappears on drying but the bitter taste then become more pronounced (Anup Kumar *et al*., 1984).

Drying in shade or artificial drying at 30-40°C proved to be the most appropriate treatments (Silva and Constantinescus, 1986).

Salabi *et al.* (1988) reported that samples of mint (*Mentha arvensis L.*) dried in oven at 60° C reduced the essential oil content by 89.5-91 per cent, whereas air

drying at 27-30° C had no effect on the essential oil content which remained at 1.71-2.76 per cent.

Leela and Angadi (1992) recommended shade drying of menthe herbage for 3 days at 30° C for obtaining maximum yield of good quality essential oil.

Baritaux *et al.* (1992) compared the chemical composition of essential oil of basil (*Oscimum basilicum*) submitted to drying with air heated to 45°C, with those obtained from fresh plant (control). The composition of essential oil of air dried basil showed a chromatographic standard very different from that obtained in control. The content of metilchavicol and euginol decreased during drying, however the levels of trans-bergamotene, linalool and 1,8 cineole significantly increased.

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

Costa *et al*. (1998) found better visual quality in leaves of Guaco (*Mikania glomerata* Sprengel) when dried in an oven with forced air at 37ºC than in a chamber with desiccant at ambient temperature. Drying at ambient temperature, the leaves were dark spots, which may indicate the need of short time to dry this species.

Meisheri (2001) suggested that the best eco-friendly method for dehydration of medicinal herbs that can keep its chemical composition, colour, flavour and taste intact at very economically viable scale is the dehydration of herbs at room temperature.

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.

Research work done by Akola and Udaipur centers of All India Co-ordinated Research Project on Medicinal and Aromatic Plants revealed that in the case of safed musli (*Chlorophytum brovillianum*), oven drying was better in comparison to sun drying in terms of loss of saponin content (Anon., 2002).

The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials. For example, shade drying is preferred to maintain or minimize loss of colour of leaves and flowers; and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances. The drying conditions should be recorded. (WHO, 2003).

Trials conducted by Dambrauskine *et al.* (2003) on the effect of drying methods on raw material quality of aromatic plants revealed that natural drying took 3-7 days for drying , where as mechanical drying using convection dryer took one day and drying by active ventilation took 2-4 days for drying. The quality of raw materials changed the least when aromatic plants were dried naturally. Convection drying slightly reduced the amount of essential oils in the oreganum and contact reduced the amount of essential oils in sage.

Omidbaigi *et al.* (2004) reported that the oil content of shade dried Roman Chamomil flower was found to be larger (1.9 % w/w) than that of sundried (0.4%) and oven dried at 40°C (0.9%).

Mehta *et al.* (2005) did comparative evaluation of different drying methods like sun drying, solar drying, shade drying and tray drying at 60°C for drying liquorice (*Glycyrrhiza glabra*) and reported that tray drying recorded the shortest

time of 36 hours followed by solar drying (52) hours, sun drying (64 hours) and shade drying (76 hours). Glycyrrhizin content of the product dried under shade was 9.81 per cent, tray drier, 8.76 per cent, solar drier, 8.36 per cent and on sun drying, 8 per cent.

Costa *et al.* (2005) studied two drying methods, viz. oven with forced ventilation at 40°C, and room temperature with dehumidifiers in lemon grass and concluded that the most abundant component in the essential oil was citral, and had the highest concentrations in the leaves dried in the dehumidifiers.

Mendes *et al.* (2006) investigated the effect of natural and artificial drying on the composition of the essential oil of *Cymbopogon nardus* and concluded that, especially for this plant, the drying operation did not influence the composition of the volatile compounds.

Muller and Heindal (2006) reported that maximum allowable temperatures depend mainly on the chemical composition of the active ingredients of the medicinal plant species under consideration.

David *et al*. (2006) evaluated the influence of the drying air temperature on *Ocimum selloi* Benth essential oil composition. They observed that the main components of essential oil were elimicin (69.8%), trans-caryophyllene (6.0%), germacrene D (3.7%) and bicyclogermacrene (3.5%), and found that increasing temperature above 40°C reduced the levels of these components.

Sefidkon *et al.* (2006) evaluated the influence of three drying methods viz. sun drying, shade drying and oven drying at 45°C on yield and chemical composition of the essential oil of *Satureja hortensis.* It could be concluded that drying of aerial parts of *S. hortensis* in the oven at 45°C is most suitable and is recommended for fast drying, and high oil yield as well as for a high percentage of carvacrol.

Colour and essential oil content change during the storage and the changes are linked to the temperature level and duration of the drying process. For an equal duration of drying, product properties change more for higher temperatures and at a constant temperature longer drying time causes more changes (Arabhosseini *et al.,* 2007).

Different drying treatments led to significant reduction ($p < 0.05$) in antioxidant properties of *P. amarus* methanolic extracts, with microwave drying causing the highest decrease in total phenolic compound and antioxidant activity exhibited by the reduction in both radical scavenging activity and FRAP(Ferric reducing antioxidant power). On the other hand, boiling water extracts appeared to exhibit significantly stronger antioxidant potentials ($p < 0.05$) even in dried plant materials due to greater solubility of compounds, breakdown of cellular constituents as well as hydrolysis of tannins (Lim and Murtijaya, 2007).

Yuan Zhang and Zhezhi Wang (2007) reported that in *Glechoma longituba* different drying methods caused some variation of the relative proportions of the components and the higher amount of germacrene D (19.0%) was obtained by shade drying.

The alkaloid content of *Tinospora cordifolia* viz., tinosporine was significantly influenced by different methods of drying. The highest alkaloid content (0.045%) was observed in mechanically dried 2.5 cm stem bits of *Tinospora cordifolia* at 40°C as compared to sun dried stem bits of equal size with 0.033% tinosporine content (Padmapriya *et al*., 2009).

Generally high temperatures influence essential oil quantity and quality in aromatic and medicinal plants not only during drying; reduction in active ingredients continues during storage period as well (Martinazzo *et al*., 2009).

Jalal *et al.* (2009) studied the effect of three drying methods, viz. sun drying, shade drying and oven drying at 45°C on the essential oil percentage of *Rosmarinus officinalis* and reported that the samples dried in shade had the higher essential oil percentage compared to samples that dried in sun and oven.

A study conducted by Balakumbhan *et al.* (2010) on influence of drying methods on drying rate and quality of *Gloriosa superba* seeds proved that alkaloid content was much higher in the sample dried under ambient room temperature shade, though the shade drying took longer time to achieve the desired moisture content.

Medicinal quality of Neem (*Azadirachta indica*) leaves dried under shade, oven dried at 45°C and at 70°C varied in final moisture content, colour, crispness and also phenolic contents. Additional processing like grinding will also influence the composition and extraction of active ingredients. The phenolic content were higher in powder obtained from shade dried leaves compared to the oven dried leaves at 45°C or at 70°C. The extracts from finer particle sizes (< 250 μm) had 26.2 per cent and 10 per cent higher phenolics compared to the larger-sized particles (> 250 μm) from oven-dried leaves at 70°C and shade-dried leaves respectively (Sejali and Anuar, 2010).

Young *et al.* (2010) reported that the quantity of essential oil is high if extracted from fresh herbs followed by freezed herbs, shade dried herbs and hot wind dried herbs.

In *Sechium edule* (chayote) dehydration at 55° C and 65° C yielded better quality product as assessed from the time of drying, colour changes, reconstitution and sensory acceptability (Sharma and Varma, 2010).

Phoungchandang and Saentaweesuk (2011) reported that drying reduces the moisture content and hence inhibits microbial growth and certain biochemical changes, but leads to loss of aroma in ginger.

2.3 STORAGE OF HERBS

Medicinal plant materials must be stored under specified conditions in order to avoid contamination and deterioration. Avoid formation of moulds, which may produce aflatoxins. Materials that need to be stored at temperatures other than room temperature should be stored at low temperatures to avoid decomposition of phyto constituents or deterioration of quality. Low humidity may be maintained using a desiccant in the container if necessary. Medicinal plant materials requiring protection from light should be kept in a light resistant container or the container may be placed inside a suitable light-resistant (opaque) covering.

The changes of cardiac glycoside complex in leaves of *Digitalis purpurea* during storage at different air humidities have been studied and reports showed that at higher RH rapid decomposition of glycosides occurs (Rada,1963., Kucera, 1971).

Lee (1995) reported that cathinone, the principle active component of shoots of *Catha edulis* is readily converted into cathine upon drying and storage of the cut plant materials.

Storage of essential oils of *Mellissa officinalis* extracted from dried aerial parts in glass bottles for one year at 27°C± 4°C influenced the proportional content of some constituents. Storage markedly increased the concentration of geranial and geraniol from 18.08 to 54.73 per cent and decreased the concentrations of beta caryophyllene, caryophylleneoxide and citronellal (Shalaby *et al.,* 1995).

During storage at 24°C for 64 weeks, the levels of alkamides fell by over 80% in *Echinaceae purpurea*. Alkamides levels also dropped significantly during storage at -18°C (Perry *et al*., 2000).

Krenn *et al* (1998) reported that long terms storage of sundew herb (*Drosera* spp.) resulted in a considerable loss of naphthoquinones (plumbagin as well as 7

methyl juglone). The decrease after 2.5 years was about 60 per cent on an average. A similar decrease in the naphthaquinone content was observed in commercial samples within one year.

Nambiar (2002) reported that type of storage influenced the quality of medicinal herbs. According to him, dried herb stored best in the whole form and most buyers choose this form of storage in air tight containers in a dry dark place as heat robs of their flavour whilst dampness causes ground herbs to cake or deteriorate.

Misra (2009) reported that medicinal and aromatic plants are mostly perishable and utmost care should be taken during the post harvest processing for improving the quality and efficacy of active ingredients.

2. 3.1. Effect of different forms of materials on storage

During storage, whole intact dried thallus of *Cladonia* spp., showed considerable stability of biological activity during storage. Ethanolic extracts also showed cytotoxic activity (Osniecimska *et al*., 1985).

Varlamov *et al.* (1999) reported that for Jerusalem artichoke used in food, pharmaceutical and chemical industries, it is best to store dried produce in the form of dried cut tubers, rather than as powder which tends to cake. Drying, comminution, and extraction with water or organic solvents are some of the techniques for processing medicinal plants.

Shakanjavi concentrates packed in plain glass bottles, amber glass bottles, PET bottles, laminate bricks and polythene pouches stored at 38°C and 80 per cent RH showed a decrease in ascorbic acid and sensory scores due to storage. The preservative used was potassium metabisulphite (KMS) (Sogi *et al*., 2000).

Storage of fresh plant materials of *Echinacea purpurea* at 20°C and 60 per cent RH for 30 days showed no significant loss of alkalymides and cichoric acid. Whereas storage of dried, crushed plant material showed degradation of alkylamides at 20 and 30°C, especially when held in light, but no loss occurred when stored at 50°C in the dark. Cichoric acid was found to be stable at 5, 20 and 30°C provided that the moisture content remained low or enzymic activity was eliminated by blanching (Wills and Stuart, 2000).

Storage of Azadirachtin A of varying strengths of 4.06 per cent to 33 per cent under ambient conditions (12-33.5° C and 41-78 per cent RH) for a period of 19 months revealed a degradation of 59.52 per cent to 81.27 per cent suggesting a need of due care and caution in storing these concentrates (Jitendra Kumar and Parmar, 2001).

2.3.2. Effect of packaging materials on storage

Based on the systemic studies conducted on the ERH, sorption isotherms, packaging in different types of containers and their storage at room temperature for months, Pruthi and Saxena (1984) suggested packaging and storage of dried *Prunica granatum*. L in friction top tins gives good storage life.

Pakkonen *et al*. (1990) conducted a study on drying, packaging, and storage effect on quality of basil, majoram, and wild majoram and reported that odour and taste of freeze dried basil and freeze dried and air dried majorams were sensitive to storage conditions. Intensity of odour and taste of dried herbs could be maintained for 2 years at 23°C in air tight packaging.

Gauniyal *et al.* (1988) suggested that dried roots of *Rauvolfia serpentina* (dried to moisture content 8 per cent or less) broken to small pieces of 15 to 20 cm should be packed in air tight containers for storage in a cool dry place.

Major source of medicinal plants are hilly areas and coastal zones from where medicinal plants are collected rather than cultivated. These are transported without proper packing. Mostly jute and polythene bags are used for packaging during transportation. Enormous amounts of plants and their parts are either spoiled or contaminated by microorganisms especially by fungi due to improper packing (Khan *et al*., 2006).

Thakur *et al.* (2012) reported that glass jars and aluminium laminated pouches are the best materials for storage of dried chilgoza nuts when compared to polythene.

2.3.4 Microbial contamination in storage

According to Silverman and Goldblith (1965) improper packaging and storage conditions may assist microbial survival.

Malmsten *et al.* (1991) reported that after one year of storage microbial counts of herbs were higher in dried Dill stored in vacuum packages than those stored in packages containing oxygen.

Roy and Chourasia (1989) reported that on examining the various medicinal like *Cyperus rotundus, Gmelina arborea, Hygrophila spinosa, Meusa ferrea and Solanum nigrum* the common microflora under storage were *Aspergillus, Alternaria, Curvularia, Fusarium* and *Rhizopus.*

Exceeding the thresholds for microbial count is the most frequent reason for rejection of medicinal plant material from growers by the pharmaceutical industry (Baier and Bomme, 1996).

Medicinal plants may be associated with a broad variety of microbial contaminants, which are represented by bacteria, fungi, and viruses. This microbiological background depends upon several environmental factors and exerts

an important effect on the overall quality of herbal products and preparations (Kniefe *et al*., 2002).

Practices used in harvesting, handling, storage, production and distribution make medicinal plants subject to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins (Mandeel, 2005).

Khan *et al.* (2006) reported that usually plant parts of medicinal plants are stored under unhygienic conditions which expose them to wide variety biological and chemical contaminants. Contamination of medicinal herbs with mycelium and spores of *Alternaria sp. Fusarium oxysporum, Penicillium oxysporum, Botrytis cinerea* and *Aspergillus niger* were reported.

Gupta *et al.* (2008) observed that contamination of medicinal herbs with aflatoxins after harvesting can be minimized by controlling the water activity and storage temperature, as *Aspergillus flavus* did not grow in any of the samples of medicinal herbs with water activity above 0.81 and when stored below 10±2°C.

2.4 EXTRACTION AND PHYTOCHEMISTRY

2.4.1. Extraction of Herbs

Plants are a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another. Plants have been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the

plants given in Ayurveda. Extraction of the bioactive plant constituents has always been a challenging task for the researchers.

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and potential health hazard of the extractants (Eloff, 1998).

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician (Remington, 2005).

Dry extracts of natural products have many advantages compared to the liquids extracts, because they are more stable and easy to handle. However, natural dry extracts generally show deficient physical mechanical properties, bad compressing behavior and they are very sensitive to moisture that impedes the direct compression (De Sauza *et al*., 2006).

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Extraction

methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Ncube *et al*., 2008).

The purpose of standardized extraction procedures for medicinal plant parts is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans (Handa *et al*., 2008)

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (soxhlet extraction), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents) (Handa *et al*., 2008).

The basic parameters influencing the quality of an extract are plant parts used as starting material, solvent used for extraction and extraction procedure. The effect of extracted plant phytochemicals depends on the nature of the plant material, its origin, degree of processing, moisture content and particle size (Ncube *et al.,* 2008).

Bimakar *et al*. (2010) reported that higher concentrations of bioactive flavanoids were detected in spearmint extract, when extracted with 70% ethanol due to its higher polarity than pure ethanol.

According to Wang (2010) ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material.

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled (Tiwari *et al*., 2011).

2.4.2. Phytochemistry

Today's use of medicinal plants and bioactive phytocompounds worldwide and our scientific knowledge of them comprise the modern field of the phytosciences. The phytosciences have been created from the integration of disciplines that have never been linked before, combining diverse areas of economic, social, and political fields, chemistry, biochemistry, physiology, microbiology, medicine, and agriculture.

Phytochemistry is regarded as the heart of herbal therapy and the phytochemical research plays an important role in the development of herbal medicines. Phytochemicals occur in various parts of plants. Their functions are diverse which include provision of strength to plants, attraction of insects for pollination and feeding, defense against predators, provision of colour, while some are simply waste products.

Important lignans present in *P. amarus* include phyllanthin, hypophyllanthin, niranthin, phyltetralin, nirtetralin,isonirtetralin and hinokinin (Morton, 1991; Sharma *et al*., 1993; Chevellier, 2000; Srivastava *et al*., 2008; Singh *et al.,* 2009).

The highest amount of phyllanthin (0.9%) and hypophyllanthin has been reported in the leaves whereas, in stem these are in minor quantities (Sharma *et al*., 1993).

Handa and Kapoor (2001) reported that the active constituents present in various parts of the plant are lignans, glycosides, flavonoids, alkaloids, ellagitannins and phenylpropanoids and are found in the leaf, stem and root of the plant.

Methanolic extract of leaves and stems of *P. amarus* was found to have potential antioxidant activity as it could inhibit lipid peroxidation, and scavenge hydroxyl and superoxide radicals in vitro. The amount required for 50 per cent inhibition of lipid peroxide formation was 104 µg/mL and the concentrations needed to scavenge hydroxyl and superoxide radicals were 117 and 19 µg/mL respectively (Raphael *et al*., 2002).

The crude extract of phyllanthin was obtained from *P. amarus* using solvents of varied polarity. The presence of pyrrolizidine type of alkaloids was reported in extract of *P. amarus*. These are securinine, dihydrosecurinine, tetrahydrosecurine, securinol-B, phyllanthine, allosecurine, nor-securinine etc. (Kassuya *et al*., 2006)

Assessment of aqueous extract of *P. amarus* showed that it has hepatic cell function enhancement ability which may explain its use traditionally in the treatment of liver problems. Plant also contains high content of carbohydrates, potassium and sodium which is related to the plant's alleged use as tonic and in the treatment of oedema and kidney disorders (Igwe *et al.,* 2007).

The antioxidant activity of methanolic extracts of five species including *P. debilis, P. urinaria, P. virgatus, P. maderaspatensis, P. amarus* from the genus *phyllanthus* was evaluated by various antioxidant assays. All the extracts at the concentration of 50µg/mL showed strong antioxidant activity in all the tested methods. Among the five plants, *P. debilis* has been found to possess the highest

activity and *P. amarus* posses the lowest activity in all tested models (Kumaran and Karunakaran, 2007).

Free radical scavenging activity of 50 per cent ethanolic extract of aerial parts of *P.amarus* extract and phyllanthin was examined using 2, 2-diphenyl-2-picrylhydrazyl (DPHH) assay. The DPHH free-radical scavenging activity was concentration-dependent in both cases and reaches a maximum at concentration of 300 µg /ml extract and 20 µ mol/mL for phyllanthin. No difference in inhibition was noted with further increase in concentration of either of the compounds. Results indicated that phyllanthin exhibited very high antioxidative property as compared to *P. amarus* extract which is clearly evident by its low IC50 value of 7.4 mol/mL (Krithika *et al*., 2009).

The aqueous extract of whole plant of *P. amarus* showed significant ($p < 0.05$) potential in scavenging free radicals, and in inhibiting lipid peroxidation. Furthermore, the extract proved to contain a high content of phenolic compounds which were found to have strong and significant ($p < 0.05$) positive correlations to free-radical scavenging potential, lipid peroxidation inhibition capacity and cyto-protective efficiency against Cr (VI)-induced oxidative cellular damage (Guha *et al*., 2010).

Khan *et al.* (2010) reported that concentration of phyllanthin is higher in leaf than fruit, stem, and root.

Mahalakshmi *et al.* (2010) reported that phyllanthin content of different genotypes of *P. amarus* varies significantly from 0.68-0.8 per cent while that of hypophyllanthin varies from 0.22 - 0.3 per cent.

Phytochemical screening of the leaf, root and stem of *Phyllanthus amarus* showed the presence of alkaloids, tannins, flavonoids, saponin, glycosides but showed absence of steroids (Akin-Osanaiye *et al*., 2011).

2.4.2.1 *Analytical techniques*

HPLC is a dynamic adsorption process and is a separation technique conducted in the liquid phase in which a sample is separated into its constituent components by disturbing between the mobile phase and stationery phase. The technique utilises a liquid mobile phase and the stationary phase may be a liquid or solid phase (Synder and Kirkland, 1979).

A variety of methods are available for analyzing pharmaceutical compounds. High Performance Liquid Chromatography (HPLC) is one of the best methods for analyzing a variety of natural and synthetic compounds. It is because it offers high performance over ambient pressure (Ahuja *et al*., 2005).

Phyllanthin was extracted from the plant *P. amarus* by soxhlet and supercritical-fluid extraction (SFE) and isolated by column chromatography. A HPTLC method was established and validated for analysis. The method was used for quantitative analysis and macro and micro fingerprinting analysis of phyllanthin. This study revealed that SFE enabled more efficient isolation of phyllanthin than Soxhlet extraction (Hamrapurkar *et al.*, 2010; Annamalai and Laxmi, 2009).

HPLC can be employed for the routine analysis of natural and synthetic compounds in pharmaceutical formulations and in bulk drug preparations as well as for the quality assurance of related extract and market samples (Arya *et al*., 2011).

A simple, specific and precise RP-HPLC method has been developed and validated for the estimation of phyllanthin, present in *P. amarus*. Furthermore, the developed method was also used to successfully quantify the phyllanthin in plant extract. The mobile phase optimized for RP-HPLC was methanol–water 66:34 (% v/v) which was very simple and cost effective. The detection was carried out using variable wavelength UV–vis detector set at 229 nm. Linearity for the developed

method was found over the concentration range 1–50 µg/mL with a correlation coefficient of 0.999 (Alvari *et al*., 2011).

A HPLC analysis method was developed and validated to obtain an easily performable and inexpensive method for the standardization of crude extract of *P. amarus* and ellagic acid. Ethanolic extract of whole plant of *P. amarus* was dissolved in Dimethyl Sulfoxide, ultrasonicated for 15 min, and diluted with 50% methanol. Analysis was performed using water and methanol containing 0.06 per cent TFA and the peaks were detected at 254 nm. Ellagic acid showed a linear relationship in the range of 1.74–20.91 µg/mL and a single-point calibration was allowed. The method was shown to be precise with respect to time (RSD of 1.84%, 3 days, n = 6) and concentration (RSD of 2.54%, three levels, n = 6). The overall mean content of ellagic acid was 2.06 per cent. A recovery experiment was performed and it showed an accuracy of 100.4 per cent (Dhooghe *et al*., 2011).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation on "Drying and Storage studies in 'Kizharnelli'(*Phyllanthus amarus* Schum and Thonn.) was carried out at the Department of Processing Technology and Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur and Regional Analytical Laboratory, Aromatic and Medicinal Plants Research Station, Odakkali, Ernakulam during 2011-2012.

In this study an attempt has been made to develop a suitable drying and storage technique for both whole plant and extractives of a well known medicinal plant, 'Kizharnelli' (*Phyllanthus amarus* Schum and Thonn.)

3.1 SITE AND CLIMATE

The area is located at 10°32 N latitude, 70°10' E longitude and 22.25 m above mean sea level. The area has a tropical monsoon climate.

3.2 EXPERIMENTAL DETAILS

The whole programme was carried out as two experiments, as detailed below:

3.3 DRYING AND STORAGE STUDIES OF THE WHOLE PLANT

3.3.1 Procurement of 'Kizharnelli'

'Kizharnelli' plants were collected from fields through the medicinal plant supplier to Oushadhi,Thrissur (A Kerala Government Organisation).

Harvested plants were cleaned well in tap water and taken to the laboratory for further post harvest studies. Plant materials were pooled together and samples were drawn from the pooled materials by quartering method to get composite samples.

Plate 1. 'Kizharnelli' (*Phyllanthus amarus*) single plant

Plate 2. 'Kizharnelli' ((*Phyllanthus amarus*) in field

3.3.2 Drying

3.3.2.1 *Treatments*

T_1: Whole plant sundried

T_2: Whole plant shade dried

T_3: Whole plant dried in a mechanical drier (MD) at 40°C to 50°C

T_4: Whole plant chopped in to pieces (WC) and sun dried

T_5: WC and shade dried

T_6: WC and dried in a MD at 40-50°C

3.3.2.2 *Drying methods*

3.3.2.2.1 *Sun drying*

Weighed samples were dried under sun with three replications till a constant weight was obtained. Temperature during drying ranged between 23°C and 33°C.

3.3.2.2.2 *Shade drying*

Samples were dried in open condition within a room till they attained constant weight for consecutive days and the temperature within the room ranged between 21°C and 31°C.

3.3.2.2.3. *Mechanical drying*

Mechanical drying of the samples was done using a cabinet drier at 30° to 40° C. A cabinet drier with inner dimensions as 0.9 x 1 x 0.61 m with 2.5 KW heating capacity was used for drying.

Whole plant

Whole plant chopped into pieces

Plate 3. Forms of plant materials used for drying

3.3.2.3 ***Layout***

The experiment was laid out in a Completely Randomized Design (CRD) with three replications of 200 gms each of both whole plant and chopped plant materials.

3.3.2.4 ***Observations***

Observations on both physical and chemical changes after drying were taken as detailed below.

3.3.2.4.1 ***Physical observations***

3.3.2.4.1.1 ***Recovery percentage***

Recovery of the dried samples were calculated on initial weight basis as suggested by Srivastava and Tandon (1968) and expressed as percentage.

$$\boldsymbol{Recovery\ percentage} = \frac{\boldsymbol{Final\ weight}}{\boldsymbol{Initial\ weight}} \times \mathbf{100}$$

3.3.2.4.1.2 ***Residual moisture***

Moisture content was estimated by drying 10 g of the samples in hot air oven at 70 ± 2°C till the samples attained constant weight. The moisture content was expressed in percentage (Ranganna, 1986).

3.3.2.4.1.3 ***Colour changes***

Change in colour due to different treatments was assessed visually and recorded.

Sun drying

Shade drying

Mechanical drying

Plate 4. Methods of drying of *P. amarus*

3.3.2.4.1.4 ***Drying rate***

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

3.3.2.4.2 ***Chemical analysis***

Samples were analysed for phyllanthin and hypophyllanthin content. Quantitative estimation of phyllanthin and hypophyllanthin was carried out by High Performance Liquid Chromatography (HPLC).

250 mg of air dried sample was extracted in 50 ml chloroform in an extractor for one hour. It was taken out and filtered in a vacuum filter. Filtrate was vacuum dried and made up to 2.5 ml using HPLC grade methanol in a small tube and injected onto the column of HPLC machine.

HPLC analyses were performed in a Dionex ultiMate®3000 column compartment HPLC system. HPLC grade solvents were used and analyses were performed on Purospher RP 18e column. The mobile phase was a solution of tetrahydrofuran : water : acetonitrile (1:6:3) at a flow rate of 1 ml/min; the detection wavelength was 210 nm, which was close to the absorption maxima for both compounds. The injection volume for standards and samples was 5 µL, and phyllanthin and hypophyllanthin were eluted at retention times of 25.13 and 27.40 minutes respectively.

3.3.3 Storage of dried samples

The experiment was carried out with an objective of finding out a suitable method for storage of dried whole plant and plant powder of *Phyllanthus amarus,* so

Plate 5. HPLC system

as to reduce the quality deterioration, reduce the storage space and also to improve its convenience of use.

3.3.3.1 ***Preparation of samples for storage***

Dried whole plant samples were stored as such and chopped materials were powdered in a grinder. These two forms of materials were filled in three different packaging materials and kept for storage.

3.3.3.2 ***Treatments***

Whole plant of *P.amarus* dried through different methods were stored under different treatment combinations

T_1 : Sun dried whole plant in 300 gauge polythene bags

T_2 : Sun dried whole plant in metal containers

T_3 : Sun dried whole plant in aluminium laminated pouches

T_4 : Shade dried whole plant in 300 gauge polythene bags

T_5 : Shade dried whole plant in metal containers

T_6 : Shade dried whole plant in aluminium laminated pouches

T_7 : Mechanically dried whole plant in 300 gauge polythene bags

T_8 : Mechanically dried whole plant in metal containers

T_9 : Mechanically dried whole plant in aluminium laminated pouches

Powdered samples of *P. amarus* were stored in different combinations as below

Metal containers

Polythene bag

Aluminium laminated pouch

Plate 6. Types of packaging materials for dried *P. amarus*

T_1 : Sun dried plant powder in 300 gauge polythene bags

T_2 : Sun dried plant powder in metal containers

T_3 : Sun dried plant powder in aluminium laminated pouches

T_4 : Shade dried plant powder in 300 gauge polythene bags

T_5 : Shade dried plant powder in metal containers

T_6 : Shade dried plant powder in aluminium laminated pouches

T_7 : Mechanically dried plant powder in 300 gauge polythene bags

T_8 : Mechanically dried plant powder in 300 gauge polythene bags

T_9 : Mechanically dried plant powder in aluminium laminated pouches

3.3.3.3 ***Layout***

The experiment was laid out in Completely Randomised Design (CRD) with 100 g each.

3.3.3.4 ***Observations***

Observations for physical, chemical parameters and microbial load in the stored samples were taken at bimonthly intervals.

3.3.3.4.1 ***Physical observations***

Physical observations like residual moisture and colour changes of different samples under different packages were determined at bimonthly intervals as stated in 3.3.2.4.1.2 and 3.3.2.4.1.2 respectively.

3.3.3.4.1.1 ***Moisture sorption behavior***

Moisture sorption studies were conducted by exposing 'Kizharnelli' powder to different relative humidities ranging from 5 to 65 per cent created using sulphuric acid of various normalities placed in the bottom of the desiccators held at constant temperature (30± 2) as described by Ranganna (1995).

Table. 3.1. Relative humidities corresponding to different normalities of sulphuric acid

Relative humidity	***Normality of sulphuric acid solution***
5	22
15	18
25	15.8
35	13.9
45	12.3
65	9.2
85	5.2
95	2.3

The critical and danger points were evaluated according to the equilibrium method (Wink, 1946). Equilibrium moisture curves (sorption isotherms) were plotted to find the equilibrium moisture content.

3.3.3.4.2 ***Chemical analyses***

For storage study composite samples were taken at random from the top, middle and bottom of the container or packages as suggested by World Health Organization norms (WHO, 2002) with respect to each treatment for the quantitative

estimation of phyllanthin and hypophyllanthin content. Estimations were carried out by High Performance Liquid Chromatography (HPLC) method as stated in 3.3.2.4.2

3.3.4 Quantitative estimation of total microbial population

The quantitative assay of micro flora was carried out by serial- dilution and plate count technique (Johnson and curl, 1972). The sample [10g (powdered samples sieved through 1 mm sieve) or 10 ml liquid sample] was added to 90 ml sterile distilled water in 250 ml conical flasks and shaken for 30 minutes in an orbital shaker gives 10^{-2} dilution. One milliliter of 10^{-2} sample dilution was transferred to another test tube containing 9 ml sterile distilled water to get 10^{-3} dilution. Later 10^{-4}, 10^{-5}, 10^{-6} dilutions were prepared from this by serial dilutions.

3.3.4.1 *Estimation of fungal population*

One ml of 10^{-3} dilution was pipetted into a sterile petridish using a micropipette. About 20 ml of the melted and cooled Potato Dextrose Agar (PDA) media was poured to the Petridish and it was swirled. After solidification, kept for incubation at room temperature.

Three Petridishes were kept as replicate for each sample. The Petriplates were incubated at room temperature for 4-5 days. The fungal colonies developed at the end of three days were counted and expressed as CFU/g of the sample.

3.3.4.2 *Estimation of bacterial population*

Bacterial population was estimated using 10^{-6} dilution on Nutrient Agar medium. The method that used for the estimation of fungal population was followed for estimation of bacterial population. The dishes were incubated for 48 hours at room temperature. The bacterial colonies developed were counted and expressed as CFU/g of the sample.

3.3.4.3 *Estimation of actinomycetes population*

The population was estimated using 10^{-5} dilution of the sample. The media used was Kenknight's agar medium and the same method was followed as in the estimation of fungal population. The dishes were incubated at room temperature for a week and the actinomycetes colonies were counted and expressed as CFU/g of the suspension.

Culture media composition for fungi, bacteria and actinomycetes are given in Appendix II

3.4 DRYING AND STORAGE STUDIES OF THE EXTRACTIVES

3.4.1 Preparation of raw materials for extraction

'Kizharnelli' plants were air dried under ambient conditions. The dried plants were ground using a grinder to obtain powder.

3.4.2 Treatments

T_1: Extraction using ethyl alcohol

T_2: Vapour heat treatment for 30-40 minutes followed by mechanical pressing

3.4.3 Solvent extraction

Solvent extraction was carried out in a batch process using analytical grade solvents. The extraction was performed in a soxhlet apparatus composed of a 500 ml round bottom flask, 100 ml extractor and a condenser. The extractor was loaded with 5 g sample in a filter paper packet. The solvent used was ethyl alcohol (95%) which was taken in round bottom flask. The whole apparatus was heated to a temperature of 100° C in a water bath. When the liquid reaches the overflow level a siphon aspirates

the solution of extractor and unloads it back to the flask. The operation was repeated until the complete extraction was achieved.

3.4.3 Extraction through vapour heat treatment

The plant materials of known weight were taken in a kettle and were steamed for 30-40 minutes. Then juice was expressed from the heated material. The extract was filtered using nylon net.

3.4.4 Storage of extractives

Storage conditions have a considerable effect on quality of the herbs. So an attempt has been made to find out a suitable method for storage of extractives of 'kizharnelli'.

3.4.4.1 *Treatments*

T_1: Extractives stored as such in glass containers

T_2: Extractives stored in glass containers with 600 ppm sodium benzoate as preservative

T_3: Extractives after vacuum concentration to thick consistency and stored in glass containers

T_4: Vacuum concentrated extractives stored in glass containers with 600 ppm sodium benzoate as preservative

T_5: Extractives after vacuum oven drying to powder form and stored in glass containers

T_6: Extractives after vacuum oven drying to powder form and stored in aluminium foil laminated pouches

3.4.4.2 *Layout*

The experiment was laid out in Completely Randomized Design (CRD) with three replications of 10 g each for each treatment for both extractives.

3.4.4.3 *Observations*

3.4.4.3.1 *Physical observations*

Physical observations like residual moisture and colour changes of different samples under different packages were determined at bimonthly intervals as stated in 3.3.2.4.1.2 and 3.3.2.4.1.2 respectively.

3.4.4.4 *Chemical observations*

Estimation of phyllanthin and hypophyllanthin were carried out by High Performance Liquid Chromatography as stated in 3.3.2.4.4

3.4.4.4 *Quantitative estimation of microbial population*

The quantitative assay of microflora was carried out by serial dilution plate technique as stated in 3.3.4

3.5. TABULATION AND STATISTICAL ANALYSIS

Observations under each experiment were tabulated and analysed statistically in a Completely Randomised Design (CRD) wherever appropriate as proposed by Panse and Sukhatme (1976). The treatments were ranked according to Duncans' Multiple Range Test (DMRT) as suggested by Duncan (1955).

RESULTS

4. RESULTS

The results of the studies conducted at the Department of Processing Technology, Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur and Regional Analytical laboratory, AMPRS, Odakkali, Ernakulam during 2011-2012 with the objective of developing suitable drying and storage techniques for whole plant and extractives of Kizharnelli', a well known medicinal plant with hepatoprotective action are presented in this chapter under the following titles.

1. Drying
2. Extraction
3. Storage

4. 1 DRYING

The drying experiment was conducted with an objective of developing an ideal drying method for 'Kizharnelli'. Drying rates of different methods, amount of phyllanthin and hypophyllanthin, and residual moisture content were evaluated. The results are presented in the tables 4.1 to 4.3

4.1.1 Recovery Percentage, Residual Moisture and Colour Change Due to Drying

Both whole plant and chopped materials were dried to a constant weight and recovery percentage, residual moisture, phyllanthin content, hypophyllanthin and colour change due to drying are presented below.

4.1.1.1 *Recovery percentage*

'Kizharnelli' plants were dried as whole plant and also as chopped material. Three methods of drying were adopted viz. sun drying, shade drying and mechanical

Table 4.1 Recovery percentage and residual moisture content of dried *P. amarus*

Treatment	Recovery (%)	Residual moisture (%)
T_1	24.00[a]	5.4[b]
T_2	25.00[a]	5.82[a]
T_3	22.67[a]	4.46[e]
T_4	23.33[a]	5.1[d]
T_5	24.67[a]	5.3[c]
T_6	22.00[a]	4.1[f]

Values with different superscript differ significantly

Table. 4.2. Contents of phyllanthin and hypophyllanthin in dried *P. amarus*

Treatment	Phyllanthin (%)	Hypophyllanthin (%)
T_1	0.0713[d]	0.1246
T_2	0.1202[a]	0.1865[a]
T_3	0.0752[c]	0.1421[c]
T_4	0.0133[f]	0.1213
T_5	0.0870[b]	0.1659[b]
T_6	0.0676[e]	0.1377

(Values with different superscript differ significantly)

T_1: Whole plant sundried
T_2: Whole plant shade dried
T_3: Whole plant dried in a mechanical drier (MD) at 40°C to 50°C
T_4: Whole plant chopped in to pieces (WC) and sun dried
T_5: WC and shade dried
T_6: WC and dried in a MD at 40°C to 50°C

drying. There was not much variations observed in recovery percentage. Highest percentage of dried material was for shade dried whole plant (25%) (T_4) and lowest was for mechanically dried chopped material (22%) (T_5) (Table 4.1). It indicates that there was no effect of chopping on drying recovery.

4.1.1.2 *Residual moisture*

The amount of residual moisture in percentage retained in each samples of both whole plants and chopped materials were analysed. It was found that lowest value was in the case of T_6, when the chopped plant materials were dried mechanically (4.1%)

Chopped materials generally recorded lower values of residual moisture. The maximum value observed was in T_3, whole plant dried under shade (5.82%) followed by sun dried whole plant, T_1 (5.4%) and shade dried chopped material, T_5 (5.3%). (Table 4.1)

4.1.1.3 *Colour change due to drying*

Colour is one of the important attribute of any dried material. Usually the colour nearer to the fresh material is preferred. Visual assessments of the colour of the dried materials were carried out. It was found that shade dried material, both whole plants and chopped materials retained better colour compared to other treatments.

4.1.1.4 *Drying rate*

Rate of drying of *Phyllanthus amarus* were analysed. Rate of drying will depend on several factors like temperature, RH around the drying material, surface area and the very nature of the material. Rate of drying for different drying methods were plotted as drying rate curves and are presented on tables 4.3a and 4.3b.

Table 4.3a Rate of sun drying and shade drying of P.amarus

Duration (days)	Sun drying		Shade drying	
	Percentage of original weight			
	WP	CP	WP	CP
0	100	100	100	100
1	63.67	51.67	68.33	58.33
2	34.33	28.33	46.67	43.33
3	28.67	26.00	30.00	27.67
4	26.67	23.33	28.00	25.50
5	24.50	23.33	27.00	25.00
6	24.00	23.33	26.00	24.67
7	24.00	23.33	25.00	24.67
8	24.00	23.33	25.00	24.67

The values represent average of three replications

Table. 4.3b Rate of mechanical drying of *P.amarus*

Duration (hrs)	Mechanical drying	
	Percentage of original weight	
	WP	CP
0	100	100
4	42.33	39.33
8	27.00	29.00
12	24.00	22.00
16	22.67	22.00
20	22.67	22.00
24	22.67	22.00

The values represent average of three replications

WP: Whole Plant

CP: Chopped Plant material

DRYING CURVES

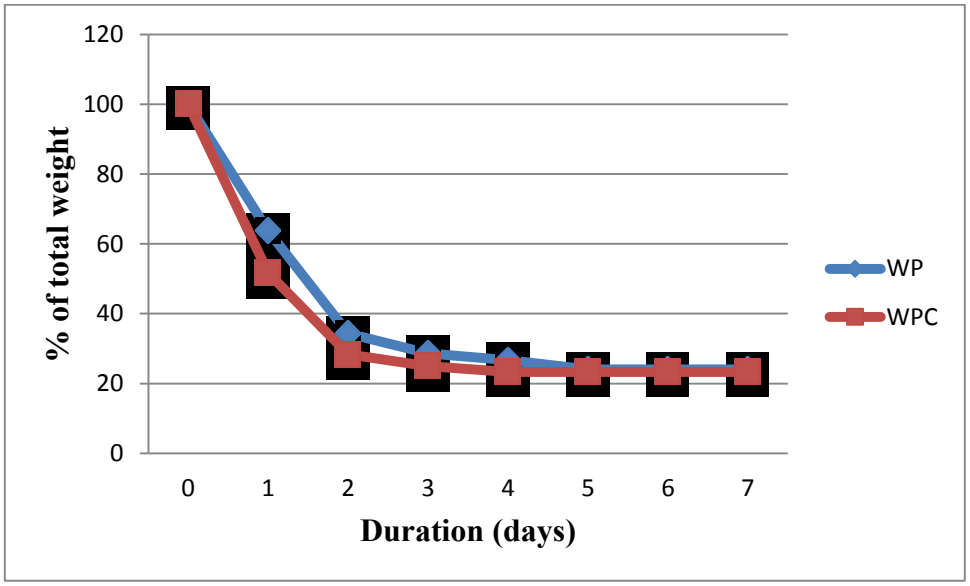


Fig: 1. Drying curve of sun dried *P. amarus*

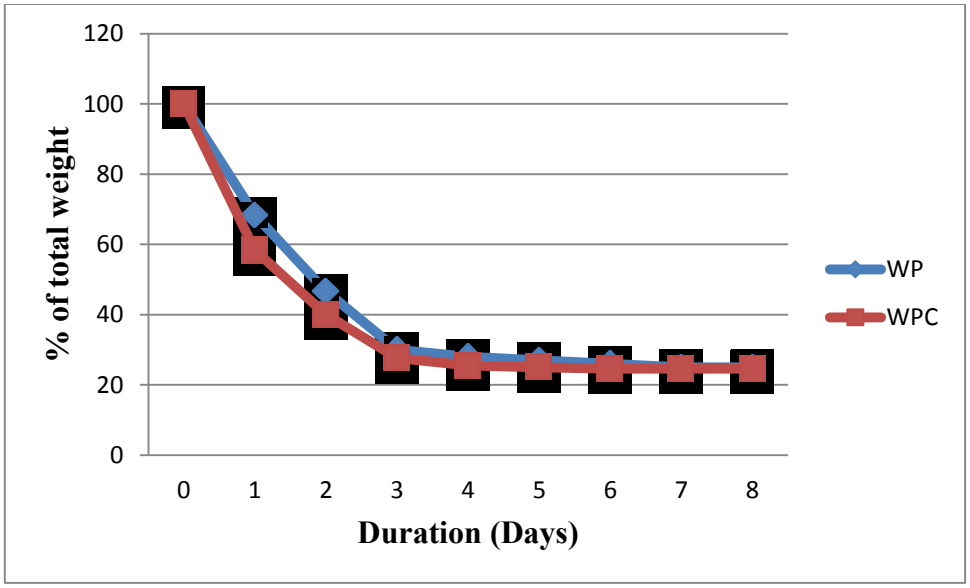


Fig: 2. Drying curve of shade dried *P. amarus*

WP- Whole Plant

WPC-Whole Plant Chopped

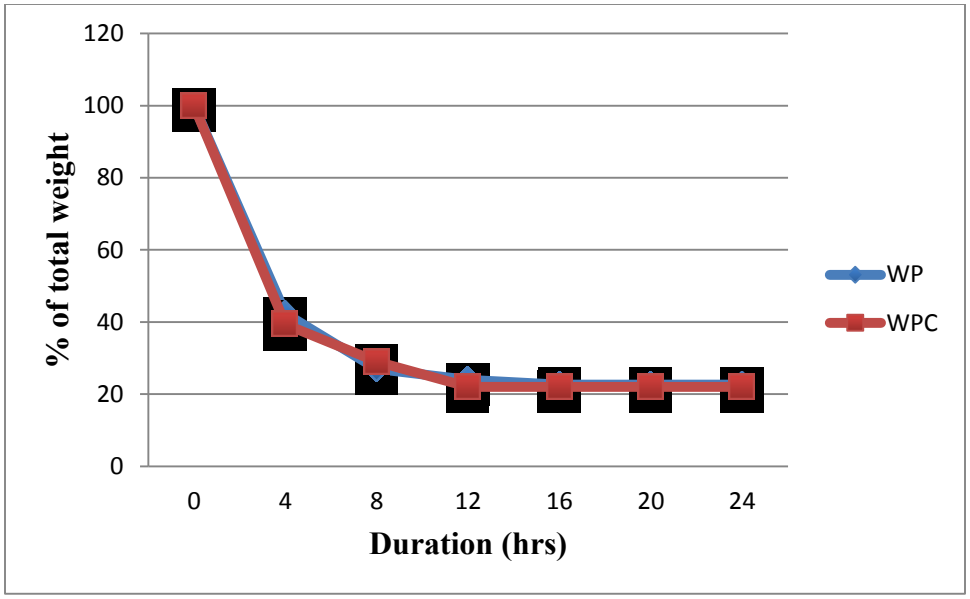


Fig: 3. Drying curve of mechanically dried (at 40-50°C) *P.amarus*

WP- Whole Plant

WPC-Whole Plant Chopped

In the three methods of drying, mechanical drying was faster followed by sun drying and shade drying. On comparing the forms of the materials, chopped materials showed initial faster rate of drying than whole plant. (Fig.1-3)

Mechanical drying of the chopped plant materials (T_6) took only 12 hrs for attaining constant weight which is recorded to be the least time taken where as whole plant took 16 hrs to dry mechanically. Longest time period (7 days) was taken by whole plant when dried under shade (T_2) followed by chopped material when shade dried (T_5) (6days).

4.1.1.5 *Phyllanthin and Hypophyllanthin Content*

Phyllanthus amarus possess two important lignans namely phyllanthin and hypophyllanthin which renders it hepatoprotective. Both whole plant and chopped materials of *P.amarus* which were dried through different methods were analysed for phyllanthin and hypophyllanthin content and the results are presented in table 4.2

While comparing different drying methods, it was found that drying in shade resulted in highest alkaloid content, followed by mechanical drying. The highest content of phyllanthin was 0.1202%, observed in T_2 (whole plant dried in shade) followed by T_5 (chopped plant dried in shade) with 0.0870% phyllanthin. Least quantity of phyllanthin was in T_4 (0.0133%) when chopped plants were dried under sun.

The amount of hypophyllanthin was also found to be maximum in T_2 (whole plant dried in shade) with 0.1865% followed by T_5 (chopped material dried in shade) with 0.1659 % hypophylanthin. The least percentage of hypophyllanthin (0.1377%) was in T_6 (mechanically chopped plant).

4.2 EXTRACTION

The study of the extractives of *P.amarus* was carried out. The total extraction of the plants were done by two methods viz. vapour heat treatment followed mechanical pressing and solvent extraction using ethyl alcohol.

Recovery percentage, phyllanthin and hypophyllanthin content of both the extractives were analysed and presented in table 4.4

4. 2.1. Recovery percentage and residual moisture content

Vapour heat treatment of fresh plants were done using steam for 30-40 minutes followed by mechanical pressing(T_2) which recorded a recovery of 21% and soxhlet extraction using ethyl alcohol (T_1) gave 18.66 % extract. Residual moisture content of ethanolic extract (T_1) was 6.56 per cent and that of T_2 was 14.4 %.(Table 4.4).

4.2.2 Phyllanthin and hypophyllanthin content

Content of the major alkaloids in both types of extractives were determined using HPLC method. In the case of vapour heat extract (T_2) there was no phyllanthin and the content of hypophyllanthin was negligible (0.0031%). The ethanol extract (T_1) recorded 0.071% phyllanthin and 0.89% hypophyllanthin. (Table 4.4)

4.3. STORAGE

P. amarus is a rain dependent plant and it is mostly available during rainy season from July to September. So there is a need to develop suitable storage practice in order to make it available through the year. A medicinal herb can be stored as whole plant and as powder and also as extractives after drying.

Table. 4.4 Recovery percentage, phyllanthin and hypophyllanthin content of extractives

Treatments	Recovery (%)	Phyllanthin (%)	Hypophyllanthin (%)	Residual moisture (%)
T_2	18.66	0.071	0.89	6.56
T_2	21.00	0	0.003	14.40

The values represent average of three replications

Storage conditions and packaging materials have great importance during storage and adequate storage prolongs the product usefulness. Improper packaging may lead to high moisture ingress, degradation of chemical constituents and growth of microbial populations which in turn catalyze the spoilage and quality deterioration.

4.3.1 Storage of dried plant material

P. amarus dried through different methods were stored in two different forms viz. whole plant as such and as powder after grinding. The packaging materials used were 300 gauge polythene bags, rigid metal containers and aluminium foil laminated pouches. All the samples after packing were stored under room conditions for a period of six months.

Residual moisture, colour changes, Moisture sorption behavior, contents of phyllanthin and hypophyllanthin, and microbial populations of stored samples were taken at bimonthly intervals.

4.3.1.1 *Moisture sorption behaviour*

Moisture isotherm curve is the most useful representation of moisture relations and generally gives all information necessary for determining the degree of protection required for the product and the extent of barrier property required for the packaging material.

Moisture sorption behaviour of the plant powders was studied and the results are presented in table 4.5 to 4.7 and figures 5 to 7.

Sun dried P. amarus powder

Moisture sorption behaviour of sun dried powders at 30° C on exposure to different relative humidities is given in table 4. 4. The product remained free flowing up to 45 per cent RH beyond which caking was observed. Critical point (C), the stage at which the product become just lumpy was found to be at 15 per cent equilibrium

moisture content (65% RH) and danger point, five percent lower ERH than critical point was found to be 8 per cent equilibrium moisture (45% RH). (Fig.5)

Table. 4. 5. Equilibrium relative humidity (ERH) data for sun dried *P. amarus* powder at 30°C

Equilibrium Relative Humidity (ERH %)	Equilibrium Moisture Content (EMC%)	Physical observation
5	0.9	Product free flowing, colour not affected
15	2	Product free flowing, colour not affected
25	3	Product free flowing, colour not affected
35	4.9	Product free flowing, colour not affected
45	8	Slightly caking ,colour change
65	15	Fully caked, colour become pale
80	17	Fully caked, colour lose

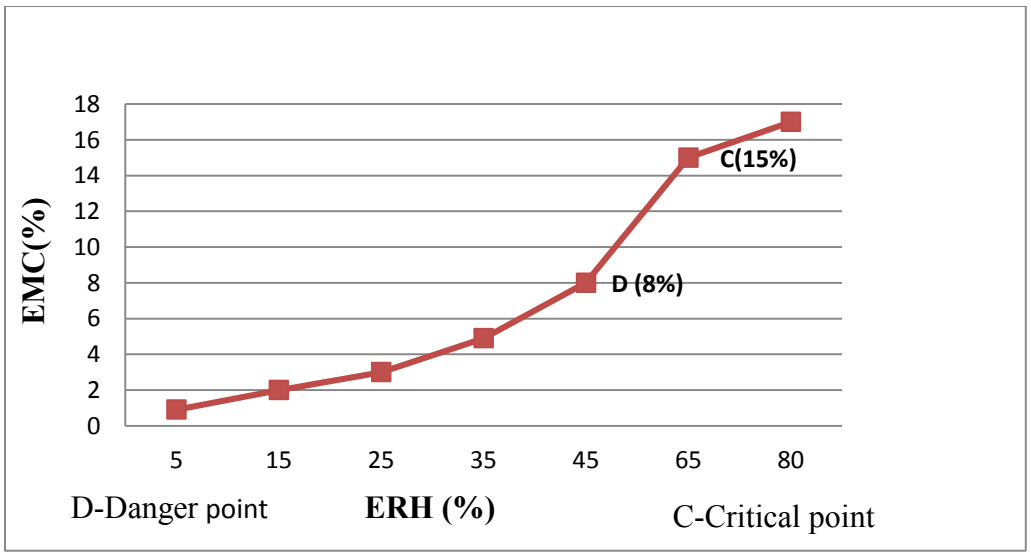


Fig. 6. Moisture absorption isotherm of sundried *P. amarus* powder

Shade dried P. amarus powder

Moisture sorption behaviour of shade dried powders at 30° C on exposure to different relative humidities is given in table 4.5. The product remained free flowing up to 45 % RH beyond which caking is observed. Critical point(C), the stage at which the product become just lumpy was found to be at 13 per cent equilibrium moisture content (65% RH) and danger point, five per cent lower ERH than critical point was found to be 8.1 per cent equilibrium moisture(45% RH) (Fig.6).

Table. 4.6. Equilibrium relative humidity (ERH) data for shade dried *P. amarus* powder at 30°C

Equilibrium Relative Humidity (ERH %)	Equillibrium Moisture Content (EMC %)	Physical observation
5	1	Product free flowing, colour not affected
15	2.4	Product free flowing, colour not affected
25	3.5	Product free flowing, colour not affected
35	4.9	Product free flowing, colour not affected
45	8.1	Slightly caking ,colour change
65	13	Caking , colour become pale
80	15	Fully caked, colour lose

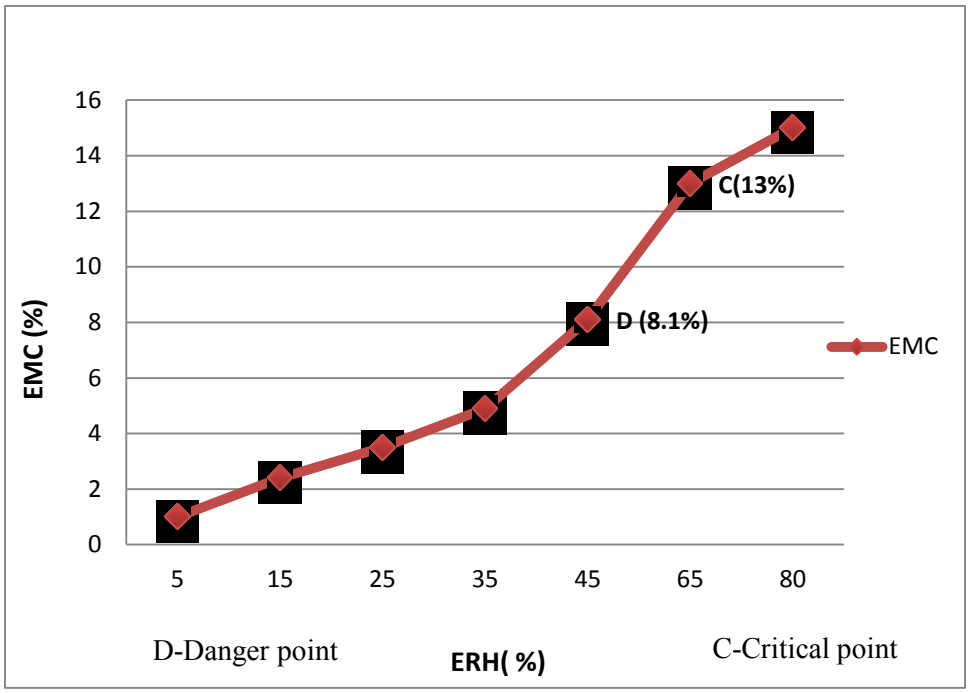


Fig. 7. Moisture absorption isotherm of shade dried *P. amarus* powder

Mechanically dried P. amarus powder

Moisture sorption behaviour of mechanically dried powders at 30° C on exposure to different relative humidities are given in table 4.6. The product remained free flowing at up to 45 % RH beyond which slight caking was observed. Critical point (C), the stage at which the product become just lumpy was found to be at 12.6 per cent equilibrium moisture content (65% RH) and danger point was five per cent lower ERH than critical point was found to be 7.6 per cent equilibrium moisture (45% RH). (Fig.7).

Table. 4.7. Equilibrium relative humidity (ERH) data for mechanically dried *P. amarus* powder at 30°C

Equilibrium Relative Humidity (ERH %)	Equillibrium Moisture Content (EMC%)	Physical observation
5	0.3	Product free flowing, colour not affected
15	1.1	Product free flowing, colour not affected
25	2.4	Product free flowing, colour not affected
35	5.4	Product free flowing, colour not affected
45	7.6	Slightly caking ,colour change
65	12.6	Caking , colour become pale
80	13.4	Fully caked, colour lose

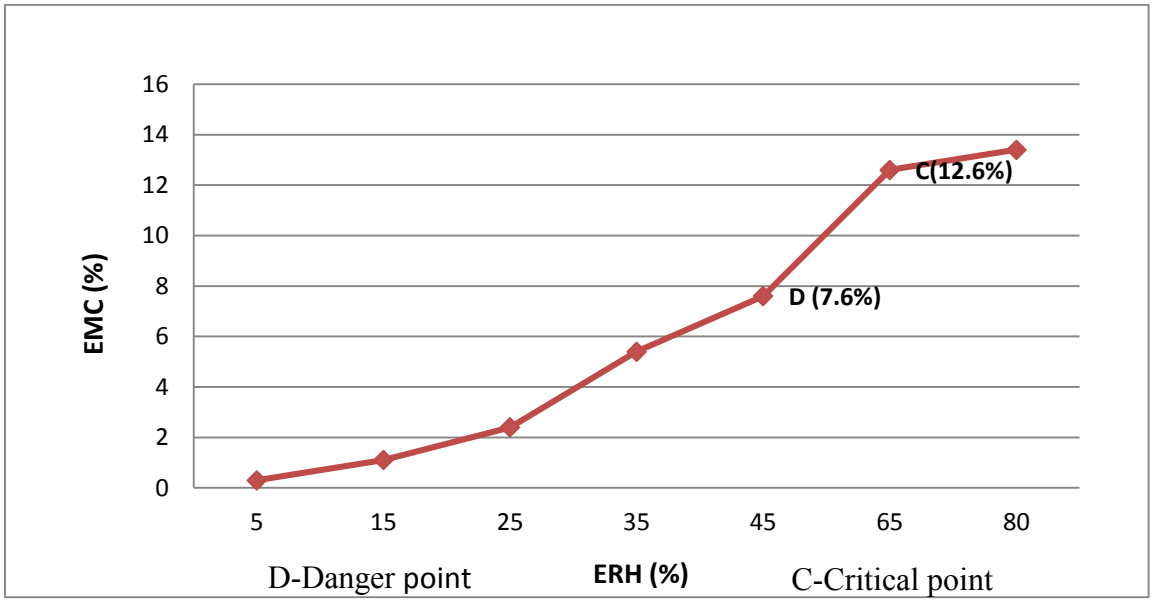


Fig. 8. Moisture absorption isotherm of mechanically dried *P amarus* powder

4.3.1.2 *Residual moisture*

Moisture is the foremost factor that influences the storage quality of any dried product. Higher amounts of moisture attract the microbes and thus enhance the quality deterioration of the product.

An increase in the percentage of residual moisture was observed with the advancement of storage. (Tables. 4.8 &4.9)

Plant powders stored in different packaging materials showed a higher percentage of absorption of moisture during storage than whole plant. Among the packaging materials aluminium foil laminated pouches showed least ingress of moisture and metal containers absorbed maximum amount of moisture within 6 months of storage.

Sun dried whole plant stored in aluminium foil laminated pouches (T_3) showed least ingress 0.41% followed by shade dried whole plant stored in aluminium foil laminated pouches. Maximum moisture absorption was recorded in T_8 (mechanically dried whole plant stored in metal containers) with 2.46% increase in residual moisture percentage. (Table 4.8)

In the case of powdered samples T_3 (sun dried powder stored in aluminium laminated pouches) and shade dried powder stored in aluminium laminated pouches(T_6) showed the least ingress (0.77 % & 0.80% respectively) of moisture compared to other treatments. Mechanically dried plant powder stored in metal containers T_8) absorbed maximum moisture (2.66%) followed by T_5, shade dried powder stored in metal containers (2.53%) after six months of storage. (Table 4.9)

4.3.1.3 Colour change

Loss of colour of materials during storage will affect its quality. Colour changes of the stored samples *of P. amarus* were assessed visually. It was found that green colour was retained in all the shade dried and mechanically dried samples. But the colour was changed to pale green in sun dried samples.

Table 4.8 .Residual moisture percentage of stored whole plant samples of *P. amarus*

Treatment	Residual moisture (%)			
	0 MAS	**2 MAS**	**4 MAS**	**6 MAS**
T_1	5.40^{b}	6.50^{cd}	7.01^{c}	7.11^{cd}
T_2	5.40^{b}	7.01^{b}	7.30^{b}	7.50^{b}
T_3	5.40^{b}	5.64^{g}	5.70^{f}	5.81^{f}
T_4	5.82^{a}	6.71^{c}	6.94^{c}	7.25^{c}
T_5	5.82^{a}	7.30^{a}	7.74^{a}	7.92^{a}
T_6	5.82^{a}	6.10^{ef}	6.26^{e}	6.40^{e}
T_7	4.46^{c}	5.76^{fg}	6.12^{e}	6.32^{e}
T_8	4.46^{c}	6.33^{de}	6.67^{d}	6.92^{d}
T_9	4.46^{c}	4.72^{h}	5.01^{g}	5.28^{g}

Values with different superscript differ significantly

Values represent average of three replications

T_1 : Sun dried whole plant in 300 gauge polythene bags
T_2 : Sun dried whole plant in metal containers
T_3 : Sun dried whole plant in aluminium laminated pouches
T_4 : Shade dried whole plant in 300 gauge polythene bags
T_5 : Shade dried whole plant in metal containers
T_6 : Shade dried whole plant in aluminium laminated pouches
T_7 : Mechanically dried whole plant in 300 gauge polythene bags
T_8 : Mechanically dried whole plant in metal containers
T_9 : Mechanically dried whole plant in aluminium laminated pouches

Table 4.9. Residual moisture content of stored powdered samples of *P.amarus*

Treatment	Residual moisture (%)			
	0 MAS	**2 MAS**	**4 MAS**	**6 MAS**
T_1	5.10^{b}	6.52^{b}	6.97^{b}	7.23^{b}
T_2	5.10^{b}	6.8^{a}	7.00^{b}	7.40^{b}
T_3	5.10^{b}	5.70^{c}	6.05^{d}	6.25^{d}
T_4	5.3^{a}	6.61^{ab}	7.06^{b}	7.30^{b}
T_5	5.3^{a}	6.85^{a}	7.51^{a}	7.83^{a}
T_6	5.3^{a}	5.72^{c}	5.90^{d}	6.10^{de}
T_7	4.1^{c}	5.40^{d}	5.61^{e}	5.81^{e}
T_8	4.1^{c}	5.93^{c}	6.47^{c}	6.76^{c}
T_9	4.1^{c}	4.41^{e}	4.61^{f}	4.87^{f}

Values with different superscript differ significantly

Values represent average of three replications

T_1 : Sun dried plant powder in 300 gauge polythene bags
T_2 : Sun dried plant powder in metal containers
T_3 : Sun dried plant powder in aluminium laminated pouches
T_4 : Shade dried plant powder in 300 gauge polythene bags
T_5 : Shade dried plant powder in metal containers
T_6 : Shade dried plant powder in aluminium laminated pouches
T_7 : Mechanically dried plant powder in 300 gauge polythene bags
T_8 : Mechanically dried plant powder in 300 gauge polythene bags
T_9 : Mechanically dried plant powder in aluminium laminated pouches

4.3.1.4 Percentage of phyllanthin and hypophyllanthin

The packaging material which retains phyllanthin and hypophyllanthin with minimum loss is regarded to be the best packaging material for storage.

It was found that phyllanthin content of sun dried *P.amarus* reduced highly in all the treatments irrespective of the form of the plant material and packaging materials in which they were stored. Among the packaging materials, aluminium laminated pouches retained comparatively better percentage of phyllanthin even after 6 months of storage.

Hypophyllanthin reduced abruptly after two months of storage in all the treatments. Sun dried samples showed maximum loss compared to other drying methods.

4.3.1.4.1 *Change in content of phyllanthin and hypophyllanthin during the storage of dried whole plant of P.amarus*

Initial phyllanthin status of dried whole plants were 0.071%, 0.120% and 0.075% in sun dried, shade dried and mechanically dried samples respectively. Whole plant stored in aluminium laminated pouches showed minimum percentage of reduction after six months of storage. Mechanically dried whole plant stored in aluminium laminated pouches (T_9) showed less degradation of phyllanthin content (16%) followed by whole plant dried under mechanically stored in 300 gauge polythene bags. (T_6) which retained about 81% phyllanthin. (Table 4.10)

Decrease in hypophyllanthin content with the progress of storage was quite drastic. Hypophyllanthin content in whole plant samples before storage were 0.13%, 0.19% & 0.14% in sun dried, shade dried and mechanically dried samples respectively. At the end of storage period the maximum degradation (89%) was recorded in sun dried whole plant stored in metal containers (T_2) and the final content phyllanthin was 0.0145%. Least loss of hypophyllanthin (72.11%) was found in

Table 4. 10. Effect of storage treatments on phyllanthin content of dried whole plant samples of *P. amarus*

Treatments	Phyllanthin content (%)			
	0 MAS	2 MAS	4 MAS	6 MAS
T_1	0.071	0.046 (35.21%)	0.035 (50.70%)	0.025 (64.79%)
T_2	0.071	0.037 (47.89%)	0.026 (63.39%)	0.015 (78.87%)
T_3	0.071	0.048 (32.40%)	0.04 (43.67%)	0.033 (53.52%)
T_4	0.12	0.112 (6.67%)	0.102 (15%)	0.092 (23.33%)
T_5	0.12	0.101 (15.83%)	0.092 (23.33%)	0.079 (34.17%)
T_6	0.12	0.115 (4.17%)	0.106 (11.67%)	0.095 (20.83%)
T_7	0.075	0.070 (6.7%)	0.066 (12%)	0.061 (18.6%)
T_8	0.075	0.063 (16%)	0.058 (22.67%)	0.053 (29.33%)
T_9	0.075	0.069 (8%)	0.064 (14.67%)	0.063 (16%)

(Values in brackets shows the percentage loss)

CD = 0.0008
CV = 0.76%

T_1 : Sun dried whole plant in 300 gauge polythene bags
T_2 : Sun dried whole plant in metal containers
T_3 : Sun dried whole plant in aluminium laminated pouches
T_4 : Shade dried whole plant in 300 gauge polythene bags
T_5 : Shade dried whole plant in metal containers
T_6 : Shade dried whole plant in aluminium laminated pouches
T_7 : Mechanically dried whole plant in 300 gauge polythene bags
T_8 : Mechanically dried whole plant in metal containersT_9 : Mechanically dried whole plant in aluminium laminated pouches

Table 4. 11. Effect storage treatments on hypophyllanthin content of whole plant samples of *P.amarus*

Treatments	Hypophyllanthin content (%)			
	0 MAS	2 MAS	4 MAS	6 MAS
T_1	0.13	0.061 (53%)	0.026 (80%)	0.015 (88.46%)
T_2	0.13	0.058 (55%)	0.02 (84.61%)	0.014 (89%)
T_3	0.13	0.077 (40.77%)	0.035 (73%)	0.02 (84.62%)
T_4	0.19	0.132 (30.53%)	0.086 (54.74%)	0.043 (77.37%)
T_5	0.19	0.122 (35.79%)	0.065 (65.79%)	0.021 (85.78%)
T_6	0.19	0.142 (38.57%)	0.094 (50.53%)	0.053 (72.11%)
T_7	0.14	0.086 (38.57%)	0.038 (72.86%)	0.031 (77.86%)
T_8	0.14	0.083 (40.71%)	0.03 (78.57%)	0.026 (81.42%)
T_9	0.14	0.088 (37.14%)	0.049 (65%)	0.035 (75%)

(*Values in brackets shows the percentage loss)*

CD =0.0008

CV (%)= 0.9

T_1 : Sun dried whole plant in 300 gauge polythene bags
T_2: Sun dried whole plant in metal containers
T_3: Sun dried whole plant in aluminium laminated pouches
T_4 : Shade dried whole plant in 300 gauge polythene bags

T_5 :Shade dried whole plant in metal containers
T_6 : Shade dried whole plant in aluminium laminated pouches
T_7 : Mechanically dried whole plant in 300 gauge polythene bags
T_8 : Mechanically dried whole plant in metal containers
T_9 : Mechanically dried whole plant in aluminium laminated pouches

shade dried whole plant stored in aluminium laminated pouches (T_6) and the percentage of hypophyllanthin at the end of storage period was 0.053%.(Table 4.11)

4.3.1.4.1 ***Change in content of phyllanthin and hypophyllanthin during the storage of plant powder of P.amarus***

Phyllantin content of the powdered samples of *P.amarus* dried under sun, shade and mechanical drying were 0.013%, 0.087% and 0.068% respectively. However the content was reduced with the advancement of storage period. Mechanically dried powder stored in aluminium laminated pouches (T_9) showed least degradation (16.02%) with a final content of 0.057 % phyllanthin. Powdered material dried under shade and packed in aluminium laminated pouches (T_6) recorded highest amount of phyllanthin (0.070%) with a reduction of only 19.54% after six months of storage. Phyllanthin content in powdered material dried under sun and stored in metal containers (T_2) was found to be minimum (0.003%) with a reduction of 76.9 percent from the initial content. (Table 4.12)

The amounts of alkaloid hypophyllanthin in powdered samples at the time of storage were 0.12%, 0.17% and 0.13% in sun, shade, and mechanically dried samples respectively. After six months of storage drastic reduction in the content was observed. Powdered samples dried under sun lost almost 90% of hypophyllanthin irrespective of the packaging material. Shade dried samples stored in aluminium laminated pouches (T_6) retained comparatively better percentage of hypophyllanthin (0.039%) with 77% loss. (Table 4.13)

4. 3. 1. 5. **Enumeration of microbial populations**

Growth of microbial population is a major problem during storage in all dried materials. When it comes to medicinal plants it become more dangerous since it will affect the quality of drug prepared from it. So, proper prevention against the growth of microbes should be taken before storage.

Table 4.12. Effect storage treatments on phyllanthin content of powdered samples of *P.amarus*

Treatment	Phyllanthin content (%)			
	0 MAS	**2 MAS**	**4 MAS**	**6 MAS**
T_1	0.013	0.011 (15.38%)	0.007 (46.15%)	0.005 (61.53%)
T_2	0.013	0.008 (38.46%)	0.005 (61.53%)	0.003 (76.9%)
T_3	0.013	0.012 (7.7%)	0.009 (30.76%)	0.007 (46.15%)
T_4	0.087	0.074 (14.94%)	0.069 (20.69%)	0.064 (26.44%)
T_5	0.087	0.07 (19.54%)	0.066 (24.14%)	0.055 (36.78%)
T_6	0.087	0.077 (11.49%)	0.071 (18.39%)	0.07 (19.54%)
T_7	0.068	0.062 (9.11%)	0.058 (15.14%)	0.055 (19.70%)
T_8	0.068	0.061 (10.88%)	0.055 (18.97%)	0.048 (30%)
T_9	0.068	0.065 (4.85%)	0.063 (10%)	0.057 (16.02%)

(*Values in brackets shows the percentage loss)*

CD =0.0008

CV (%)= 1.25

T_1 : Sun dried plant powder in 300 gauge polythene bags
T_2 : Sun dried plant powder in metal containers
T_3 : Sun dried plant powder in aluminium laminated pouches
T_4 : Shade dried plant powder in 300 gauge polythene bags
T_5 : Shade dried plant powder in metal containers
T_6 : Shade dried plant powder in aluminium laminated pouches
T_7 : Mechanically dried plant powder in 300 gauge polythene bags
T_8 : Mechanically dried plant powder in 300 gauge polythene bags
T_9 : Mechanically dried plant powder in aluminium laminated pouches

Table 4.13. Effect of storage treatments on hypophyllanthin content of powdered samples of *P.amarus*

Treatment	Hypohyllanthin content (%)			
	0 MAS	**2 MAS**	**4 MAS**	**6 MAS**
T_1	0.12	0.057 (52.5%)	0.024 (80%)	0.012 (90%)
T_2	0.12	0.051 (57.5%)	0.018 (85%)	0.011 (90.8%)
T_3	0.12	0.067 (44.16%)	0.028 (76.67%)	0.013 (89%)
T_4	0.17	0.108 (36.47%)	0.066 (61.17%)	0.035 (79.41%)
T_5	0.17	0.1 (41.17%)	0.048 (71.76%)	0.024 (85.83%)
T_6	0.17	0.117 (31.18%)	0.086 (49.41%)	**0.039 (77%)**
T_7	0.13	0.081 (37.7%)	0.032 (75.38%)	0.024 (81.5%)
T_8	0.13	0.075 (42.30%)	0.027 (79.23%)	0.018 (86.15%)
T_9	0.13	0.086 (33.85%)	0.046 (0.462%)	0.026 (80%)

(*Values in brackets shows the percentage loss)*

CD =0.00056

CV (%) = 0.65%

T_1 : Sun dried plant powder in 300 gauge polythene bags

T_2 : Sun dried plant powder in metal containers

T_3 : Sun dried plant powder in aluminium laminated pouches

T_4 : Shade dried plant powder in 300 gauge polythene bags

T_5 : Shade dried plant powder in metal containers

T_6 : Shade dried plant powder in aluminium laminated pouches

T_7 : Mechanically dried plant powder in 300 gauge polythene bags

T_8 : Mechanically dried plant powder in 300 gauge polythene bags

T_9 : Mechanically dried plant powder in aluminium laminated pouches

Bacteria, fungi and actinomycetes are the major groups of microorganisms found in stored samples of medicinal plants.

Assessments of microbial population during different phases of storage of *P. amarus* were done. Initially, there were only fungi and bacteria. Actinomycetes were found to be absent. With the advancement of storage time presence of actinomycetes were also found, however they were less in number.

There was increase in microbial population with increase in storage period. Aluminium foil laminated pouches showed least count of microbes compared to other packaging materials.

4.3.1.5.1 ***Microbial growth in whole plant samples***

Initial bacterial count of whole plant samples were 0.16x 10^6 CFU /g, 1.33x 10^6 CFU /g and 1.67x10^6 CFU /g in sun dried whole plant, shade dried whole plant and mechanically dried plant respectively. Before storage, fungal populations were 2.33 x10^3 CFU /g in sun dried whole plant, 4.33 x10^3 CFU /g in shade dried whole plant and 1.67 x10^3 CFU /g in mechanically dried whole plant. Actinomycetes were absent in all the samples.

After 3 months of storage, maximum bacterial population (6 x10^6 CFU /g) was recorded in T_7 (mechanically dried whole plant stored in 300 gauge polythene bag), followed by shade dried whole plant stored in metal containers (T_5). Sample T_9 (mechanically dried whole plant in aluminium laminated pouches) recorded least count (1.67x10^6 CFU /g) after 3 months of storage.

The maximum fungal population (27.3x10^3 CFU /g)was found in T_2 (whole plant packed in metal containers) and least (5.67x10^3 CFU /g) was in T_7 (mechanically dried whole plant in aluminium laminated pouches).

Actinomycetes count was very less and recorded maximum (1.66 $x10^5$ CFU /g, 1.33x $x10^5$ CFU /g) in mechanically dried whole plant stored in metal containers (T_8) and 300 gauge polythene bags (T_7).

At the end of six months of storage, bacterial population increased in all the samples but did not differ significantly among the samples. However shade dried whole plant packed in 300 gauge polythene bags (T_7) and that in metal containers (T_8) recorded maximum bacterial count ($20.67x10^6$ CFU /g and $19.67x10^6$ CFU /g respectively).

Total fungal count was also increased considerably and least count ($13.33x10^6$ CFU /g) was noticed in whole plant dried mechanically and stored in aluminium foil laminated pouches (T_9). Maximum fungal population ($40x10^3$ CFU /g) was noticed in shade dried whole plant stored in metal containers (T_5) and that in 300 gauge polythene bags (38.66 $x10^3$ CFU /g) at the end of six months.

Actinomycetes count was comparatively less and was least (1.33 $x10^5$ CFU /g) in T_9 (whole plant dried mechanically and stored in aluminium laminated pouches). Mechanically dried samples stored in metal containers (W_8) and whole plant sun dried and kept in metal containers (T_2) recorded maximum count (5.33x $x10^5$ CFU /g). (Table 4. 14)

4.3.1.5.2 ***Microbial growth in powdered samples***

Powdered samples recorded $0.67x10^6$ CFU/ g of bacterial population initially.

The initial population increased gradually and a maximum population of $8x10^6$ CFU/ g were found in T_8 (mechanically dried plant powder stored in metal containers) and least population (4 $x10^6$ CFU/ g was found in T_6 (shade dried plant powder stored in aluminium laminated pouches) after 3 months of storage. At the end of the storage period, it was found that sun dried and shade dried powders stored

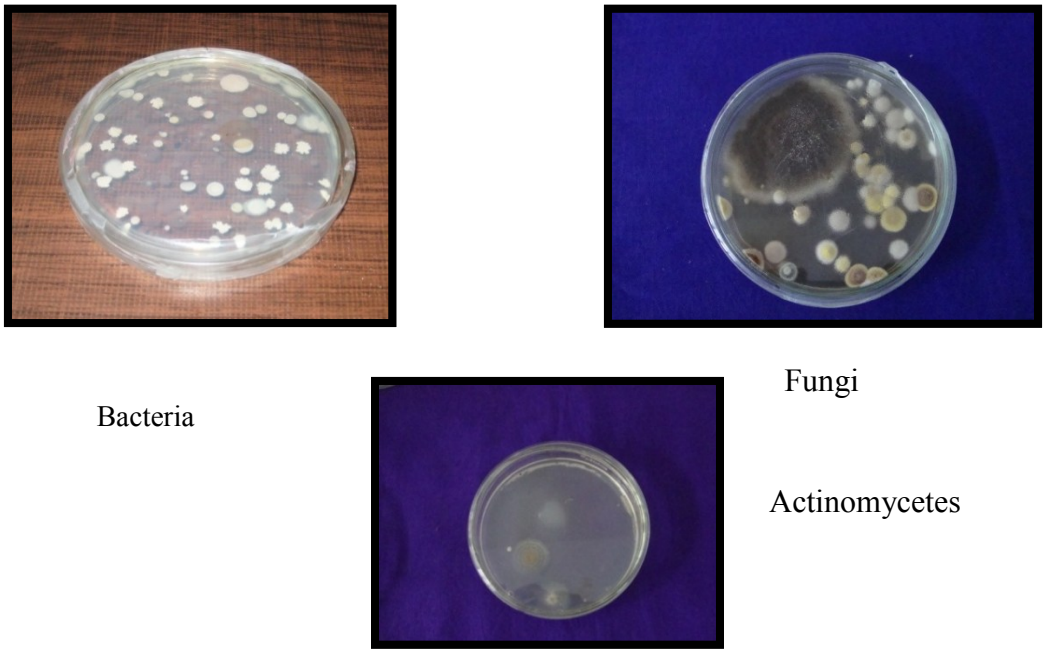


Plate 7. Microbial growth observed in whole plant samples of *P. amarus* at the end of 6 months of storage

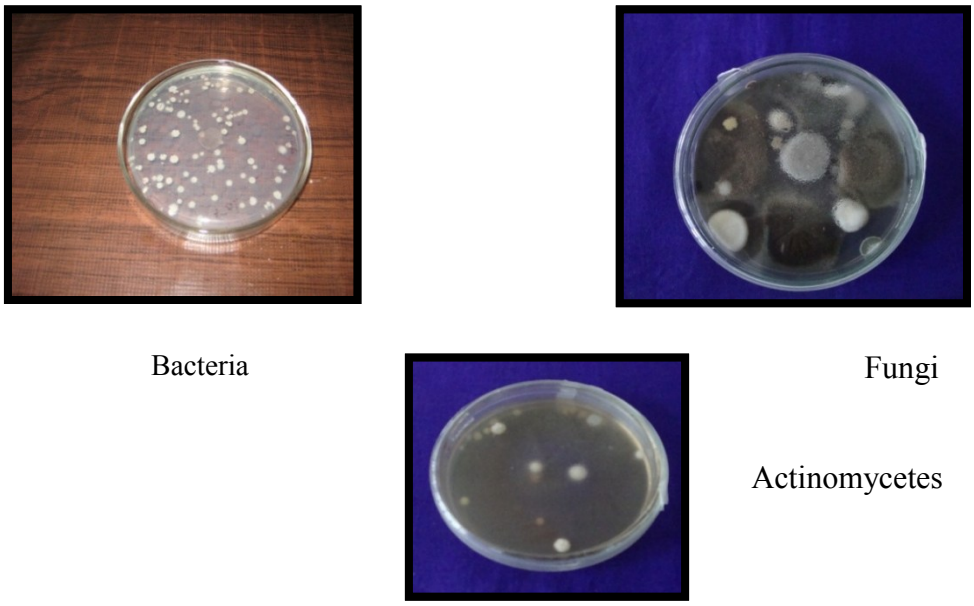


Plate 8. Microbial growth observed in powdered samples of *P. amarus* at the end of 6 months of storage

in metal containers(T_5) had the maximum bacterial population ($27.67x10^6$ CFU/ g followed by that stored in 300 gauge polythene bags.(T_4).

The fungal populations in powdered samples before storage were $1.67x\ 10^3$ CFU /g, $4x10^3$ CFU /g and $0.66x10^3$ CFU /g in sun dried, shade dried and mechanically dried samples respectively. With the advancement of storage period,the count increased and at the end 3 months T_5 (shade dried whole plant stored in metal containers)recorded maximum population ($19.66\ x10^3$ CFU /g) and the least count of fungal growth was in T_9 ($1.33x10^3$ CFU /g).

After six months, sun dried powder stored in aluminium foil laminated pouches showed least count ($11.33x10^3$ CFU /g) followed by mechanically dried powder stored in aluminium laminated pouches (T_9) with a population of $12.66x10^3$ CFU /g.

Actinomycetes populations were absent before storage and after six months only a slight increase in population was noticed. Sun dried powder stored in metal containers (T_2) showed a maximum population of $6.67x10^5$ CFU /g at the end of storage. The least population was recorded in powder stored in aluminium laminated pouches after mechanical drying (T_9) (Table 4.15).

4.3.2 Storage of extractives

Two types of extractives of *P. amarus* were taken *viz*. ethanolic extracts and extract obtained from vapour heat treatment followed by mechanical pressing. Due to the complete deterioration of phyllanthin content and negligible quantity of hypophyllanthin the extracts obtained through mechanical pressing were discarded. Ethanolic extracts were concentrated through different methods and were kept for storage for six months in glass containers and also in aluminium laminated pouches.

Table. 4.14. Effect of storage treatments on microbial population of whole plant samples of *P. amarus*

Treatments	Total microbial count in CFU/g								
	Bacteria (x 10^6)			Fungi (x 10^3)			Actinomycetes (x10^5)		
	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS
T_1	1.66^a	2.67^a	11.33^a	2.33^a	19.30^{ab}	28.67^{ab}	0	0.67^b	1.67^b
T_2	1.66^a	2.00^a	15.00^a	2.33^a	27.33^a	33.00^{ab}	0	0.33^b	1.33^b
T_3	1.66^a	2.00^a	10.00^a	2.33^a	15.6^{abc}	20.00^{bc}	0	0.66^b	2.00^b
T_4	1.33^a	2.67^a	20.70^a	4.33^a	25.33^a	38.60^a	0	0.67^{ab}	1.70^b
T_5	1.33^a	5.00^a	18.33^a	4.33^a	20.66^{ab}	40.00^a	0	0.33^b	2.00^{ab}
T_6	1.33^a	2.00^a	8.67^a	4.33^a	15.67^{abc}	20.00^b	0	0.66^{ab}	1.33^b
T_7	1.67^a	6.00^a	15.33^a	1.67^a	10.67^{bc}	23.67^{bc}	0	1.60^a	3.33^{ab}
T_8	1.67^a	2.00^a	19.60^a	1.67^a	7.33^{bc}	28.33^{ab}	0	1.30^a	5.33^a
T_9	1.67^a	1.67^a	12.00^a	1.67^a	5.67^c	13.33^c	0	0.33^a	4.33^{ab}

T_1 : Sun dried whole plant in 300 gauge polythene bags

T_2 : Sun dried whole plant in metal containers

T_3 : Sun dried whole plant in aluminium laminated pouches

T_4 : Shade dried whole plant in 300 gauge polythene bags

T_5 : Shade dried whole plant in metal containers

T_6 : Shade dried whole plant in aluminium laminated pouches

T_7 : Mechanically dried whole plant in 300 gauge polythene bags

T_8 : Mechanically dried whole plant in metal containers

T_9 : Mechanically dried whole plant in aluminium laminated pouches

Table 4.15. Effect of storage treatments on microbial population of powdered samples of *P. amarus*

Treatments	Total microbial count in CFU/g								
	Bacteria (x 10^6)			Fungi (x 10^3)			Actinomycetes (x10^5)		
	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS
T_1	0.67	5.66^{ab}	9.67^{a}	1.67^{a}	12.33^{bc}	14.00^{a}	0	1.33^{a}	2.00^{a}
T_2	0.67	4.33^{b}	14.00^{a}	1.67^{a}	13.33^{abc}	27.00^{a}	0	2.66^{a}	6.67^{a}
T_3	0.67	6.00^{ab}	9.00^{a}	1.67^{a}	9.67^{bcd}	11.33^{b}	0	1.00^{a}	1.60^{a}
T_4	0.67	6.00^{ab}	26.66^{a}	4.00^{a}	15.70^{a}	24.66^{a}	0	1.33^{a}	4.67^{a}
T_5	0.67	6.00^{ab}	27.67^{a}	4.00^{a}	19.66^{a}	27.33^{a}	0	1.66^{a}	5.33^{a}
T_6	0.67	4.00^{b}	14.33^{a}	4.00^{a}	16.6^{ab}	20.67^{a}	0	1.00^{a}	1.33^{a}
T_7	0.67	6.30^{ab}	20.00^{a}	0.67^{a}	4.30^{de}	18.33^{a}	0	1.00^{a}	2.00^{a}
T_8	0.67	8.00^{a}	19.67^{a}	0.67^{a}	7.00^{cde}	15.00^{a}	0	2.66^{a}	3.00^{a}
T_9	0.67	5.30^{ab}	14.33^{a}	0.67^{a}	1.33^{e}	12.66^{ab}	0	1.00^{a}	1.00^{a}

Values with different superscript differ significantly

T_1 : Sun dried plant powder in 300 gauge polythene bags

T_2 : Sun dried plant powder in metal containers

T_3 : Sun dried plant powder in aluminium laminated pouches

T_4 : Shade dried plant powder in 300 gauge polythene bags

T_5 : Shade dried plant powder in metal containers

T_6 : Shade dried plant powder in aluminium laminated pouches

T_7 : Mechanically dried plant powder in 300 gauge polythene bags

T_8 : Mechanically dried plant powder in 300 gauge polythene bags

T_9 : Mechanically dried plant powder in aluminium laminated pouches

Table 4.16 Residual moisture percentage of stored samples of ethanolic extractives of *P.amarus*

Treatments	Residual moisture (%)			
	0 MAS	2 MAS	4 MAS	6 MAS
T_1	6.56^a	6.67^a	6.70^a	6.75^a
T_2	6.56^a	6.58^a	6.65^b	6.70^a
T_3	4.84^b	4.93^b	5.75^c	5.80^b
T_4	4.84^b	4.90^b	5.85^c	5.95^b
T_5	3c	3.15^c	3.20^d	3.26^c
T_6	3c	3.10^c	3.12^d	3.15^c

Values with different superscript differ significantly

Values represent means of three replications

T_1: Extractives stored as such in glass containers
T_2: Extractives with 600 ppm sodium benzoate and stored in glass containers
T_3 : Vacuum concentrated extractives stored in glass containers
T_4: Vacuum concentrated extractives with 600 ppm sodium benzoate stored in glass containers.
T_5 : Extractives after vacuum oven drying to powder and stored in glass containers
T_6 : Extractives after vacuum oven drying to powder and stored in aluminium laminated pouches

Residual moisture, colour change, change in phyllanthin content and microbial population of the extractives were assessed at bimonthly intervals and are presented in tables 4.15 to 4.18

4.3.2.1 ***Percentage of residual moisture***

Residual moisture content of the extractives were 6.56% (T_1 & T_2), 4.84 % (T_3 & T_4) and 3% (T_5 & T_6) at the time of storage. After six months all the samples showed negligible increase in moisture content varying from (0.14% to 1.11%). However extractives stored as such in glass containers with 600 ppm sodium benzoate (T_2) showed least ingress of moisture ((0.14%) (Table 4.16).

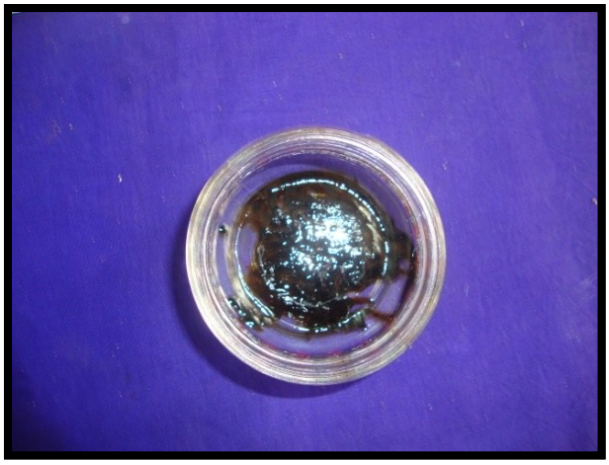
4.3.2.2 .*Colour changes*

Dark green coloured extract did not show considerable change in colour up to the end of storage period in all the samples.

4.3.2.3 ***Phyllanthin and hypophyllanthin content***

Phyllanthin content of the extractive as such was 0.069% initially and that of hypophyllanthin was 0.89%. Extractives were stored for six months as extractives as such, as vacuum concentrated form and also as powder form. Under different storage conditions the contents of these alkaloids were reduced. After six months of storage the highest amounts of phyllanthin (0.059% and 0.057%) were retained by T_6 (Dried and powdered extractives stored in aluminium laminated pouches) and T_4 (vacuum concentrated extractives stored in glass containers with 600 ppm benzoate) respectively. Extractives stored as such in glass containers (T_1) lost 52% of phyllanthin and recorded a final content of 0.033% after 6 months of storage. (Table. 4.17)

Hypophyllanthin reduced drastically after 4 months of storage in all the samples. Powdered extractives stored (T_5 &T_6) showed slightly lower reduction



Ethanolic extract

Vapour heat extract

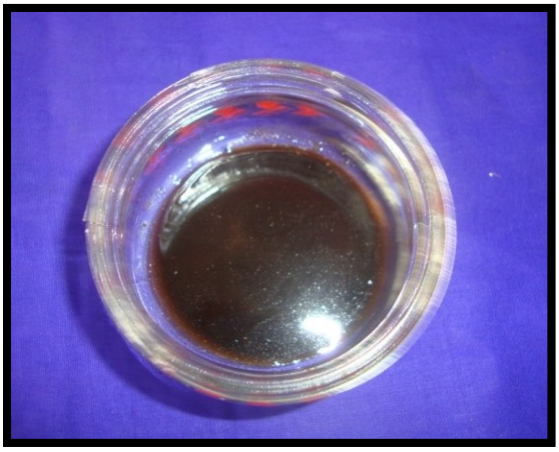


Plate 9. Extractives of *P.amarus*

Table 4.17. Effect of storage treatments on phyllanthin content ethanolic extractives of *P.amarus*

Treatments	Phyllanthin content (%)			
	0 MAS	**2 MAS**	**4 MAS**	**6 MAS**
T_1	0.069	0.063^{d}	0.041^{e}	0.033^{f} (52.17%)
T_2	0.069	0.057^{f}	0.054^{d}	0.049^{e} (28.98%)
T_3	0.071	0.061^{e}	0.057^{c}	0.054^{d} (23.94%)
T_4	0.071	0.064^{c}	0.060^{b}	0.057^{b} (19.71%)
T_5	0.072	0.066^{a}	0.0570^{c}	0.055^{c} (23.61%)
T_6	0.072	0.065^{b}	0.063^{a}	0.059^{a} (18.05%)

(*Values with different superscript differ significantly*)

T_1: Extractives stored as such in glass containers

T_2: Extractives with 600 ppm sodium benzoate and stored in glass containers

T_3: Vacuum concentrated extractives stored in glass containers

T_4: Vacuum concentrated extractives with 600 ppm sodium benzoate stored in glass containers.

T_5 : Extractives after vacuum oven drying to powder and stored in glass containers

T_6 : Extractives after vacuum oven drying to powder and stored in aluminium laminated pouches

Table. 4.18. Effect of storage treatments on hypophyllanthin content of ethanolic extractives of *P.amarus*

Treatments	Hypophyllanthin content (%)			
	0 MAS	**2 MAS**	**4 MAS**	**6 MAS**
T_1	0.85	0.73^{a}	0.32^{f}	0.11^{e} (87%)
T_2	0.85	0.76^{a}	0.33^{e}	0.12^{d} (85.88%)
T_3	0.86	0.71^{a}	0.36^{d}	0.15^{C} (82.65%)
T_4	0.86	0.71^{a}	0.46^{c}	0.17^{b} (80.23%)
T_5	0.89	0.78^{a}	0.49^{a}	0.18^{a} (79.77%)
T_6	0.89	0.79^{a}	0.47^{b}	0.18^{a} (79.77%)

(Values with different superscript differ significantly)

T_1: Extractives stored as such in glass containers

T_2: Extractives with 600 ppm sodium benzoate and stored in glass containers

T_3 : Vacuum concentrated extractives stored in glass containers

T_4:Vacuum concentrated extractives with 600 ppm sodium benzoate stored in glass containers.

T_5 : Extractives after vacuum oven drying to powder and stored in glass containers

T_6 : Extractives after vacuum oven drying to powder and stored in aluminium laminated pouches

(79.77%) compared to other treatments with a final content of 0.18% hypophyllanthin. Extractives stored as such (T_1) lost 87% of hypophyllanthin and retained a hypophyllanthin content of 0.11% at the end of storage period. (Table 4.18)

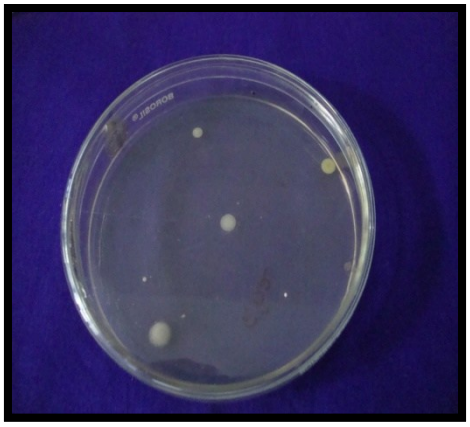
4.3.2.4 Growth of microbial populations

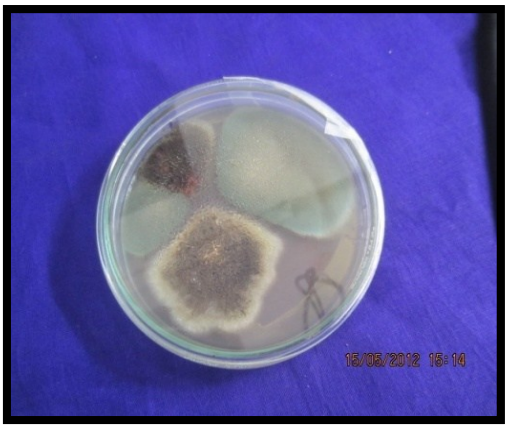
Enumeration of the microbial population was done to assess the quality of the extract. Ethanolic extract showed comparatively lesser count of microorganisms during storage. Initial bacterial count was zero and only a slight increase was noticed with the advancement of storage. Extract as such stored in glass containers (T_1) recorded $3x10^6$ CFU/ml of bacteria which was the maximum. Extractives in the form of powder stored in aluminium laminated pouches (T_6) showed least count of bacteria ($1x10^6$ CFU/ml).

Fungal populations in the samples were $1x10^3$ CFU/ml initially and it also slightly increased after six months. Extractives stored as such (T_1) showed maximum count (4.67×10^3 CFU/ml) at the end of six months. Extractives in the form of powder stored in glass containers (T_1) and aluminium laminated pouches (T_6) showed least count of 2×10^3 CFU/ml of fungal population. Actinomycetes were found to be absent throughout the storage period in all the samples. (Table 4. 19)

Glass bottles **Aluminium laminated pouch**

Plate 10. Packaging materials for the storage of ethanolic extractives of *P.amarus*





Bacteria **Fungi**

Plate 11. Microbial growth in stored ethanolic extractives at the end of 6 months of storage

Treatments	Total microbial count in CFU/ml suspension								
	Bacteria (x 10^6)			Fungi (x 10^3)			Actinomycetes (x10^5)		
	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS
T_1	0	1.6a	3.00^a	1^a	2.33^a	4.7^a	0	0	0
T_2	0	0.67a	2.33^{ab}	1^a	1.7^a	2.67^{ab}	0	0	0
T_3	0	1.33a	2^{ab}	1^a	3.33^a	4.00^a	0	0	0
T_4	0	1.00a	1.6^{ab}	1^a	1.67^a	2.67^{ab}	0	0	0
T_5	0	1.00a	1.60^{ab}	1^a	1.00^a	2^b	0	0	0
T_6	0	0.33a	1^b	1^a	1.00^a	2^b	0	0	0

Table. 4.19. Effect of storage treatments on microbial population of ethanolic extractives of *P. amarus*

Values with different superscripts differ significantly

T_1: Extractives stored as such in glass containers

T_2: Extractives with 600 ppm sodium benzoate and stored in glass containers

T_3 : Vacuum concentrated extractives stored in glass containers

T_4: Vacuum concentrated extractives with 600 ppm sodium benzoate stored in glass containers.

T_5 : Dried and powdered extractives stored in glass containers

T_6 : Dried and powdered extractives stored in aluminium laminated pouches

DISCUSSION

5. DISCUSSION

Herbal drugs constitute a major share of all the officially recognized systems of medicines for the treatment of wide range of diseases. The history of medicinal plants is associated with the development of civilizations. In all the regions of the world, history of people shows that these plants have always occupied an important place in medicine , food etc.

'Kizharnelli' (*Phyllanthus amarus* Schum & Thonn) is an important anti-jaundice plant which contain several alkaloids. *P. amarus* has a big history of usage by the folk because of its rich medicinal values and has been reported to possess potent antihepatotoxic, anti-inflammatory, analgesic, hypotensive, anti bacterial and hypoglycaemic properties. A large number of phytochemicals have been found in the plants of which phyllanthin and hypophyllanthin are the two important alkaloids which are responsible for the hepatoprotctive effect of the plant.

P.amarus is a season bound plant, it is available only from June to September of an year. Since the demand of drug is there irrespective of seasons, there is a need for ensuring availability of raw materials throughout the year.

Thus an attempt has been made to develop drying and storage practices for *P.amarus* which ascertains the form in which the plant material should be dried or extracted as well as an ideal packaging material to store the dried product or extractive without much loss in quality of the active ingredient for a longer period of time. The results of these experiments are discussed in this chapter.

5.1 DRYING

Some herbs or crops are perishable in their fresh state and may deteriorate within a few days after harvest. One way to preserve the plant products is to dry them

in order to conserve their desirable qualities, reduce storage volume and extend shelf life. Moisture plays a crucial role in the keeping quality of any dried material. Usually medicinal plants are harvested at a moisture percentage of 70-80% on wet basis. During drying, moisture content reduces to a lower level and for proper storage moisture content should be less than 12%. Drying method that provides rapid reduction in the moisture content without affecting the quality of the active ingredients of the plants must be needed for medicinal plants. Therefore an attempt has been taken to dry the fresh plants of *P.amarus* by adopting natural drying methods like sun drying and shade drying and also mechanical drying using a cabinet dryer with an aim to reduce the drying time and increase efficiency.

While drying, whole plant as such and chopped plant materials were tried in combination with three types of drying and results obtained are discussed here.

5.1.1 Recovery percentage

Since removal of water reduces the weight of the material, higher recovery percentage indicates higher moisture content of the sample. Higher recovery percentage of the shade dried samples (both whole plant and chopped material),T_2 & T_5 was due to their higher residual moisture percentage. Low recoveries of the mechanically dried samples were due to efficient and continuous drying and lesser residual moisture content.

Comparing the form of the plant materials, least recovery was noticed in chopped material which is due to the increased surface area of the chopped plant material. The heat transfer and mass transfer during drying will depend basically on the increased surface area. Chopping results in higher surface area and hence maximum escape of moisture leading to low recovery. These results are in accordance with the findings of Sujatha (2002) who reported that chopped materials of Adathoda gave lesser recovery percentage compared to whole plant.

However the margin of variation in recovery percentage of whole plant and chopped material was very less. It could be due to the very delicate nature of the plant which is slender and soft and hence dry easily.

5.1.2 Residual moisture

Residual moisture content is very important in the case of microbial quality of a dried product. Residual moisture content was high in shade dried materials. The lowest value of chopped material dried in a mechanical drier was due to low recovery which was explained above. These findings are similar to that of Gauniyal *et al.* (1988) who found that roots and whole plant samples with thicker texture and lesser area exposed to a drying agent, recorded more moisture in shade and sun drying and lower moisture for mechanical drying. Sujatha (2002) also reported the similar results in the case of drying of Adathoda.

5.1.4 Drying rate

Drying rate is important since it influences the efficiency of a drying method. Drying rate depends on the drying temperature, initial moisture content and texture of the plant material. Drying rates of different plant materials in different drying methods were illustrated by drying curves. Chopped materials showed initial faster rate of drying compared to that of whole plant (Fig. 1-3). Higher temperature and higher surface area will result in higher rate of drying. This is the reason why faster rate of drying was recorded in all the samples dried in mechanical drier at 40°C as well as chopped samples having higher surface area, when compared to whole plant dried under sun or shade. Yuvaraj (2007) reported that *Wedelia chinensis,* when dried mechanically has the highest rate of drying compared to sun and shade drying. Padmapriya *et al.* (2009) also reported that sun drying of *Tinosporia cordifolia* required longer period than mechanical drying and the highest drying rate was observed in mechanical drying of smallest stem bits. Similar results were also

obtained by Mehta *et al.* (2005). They did comparative evaluation of different drying methods like sun drying, solar drying, shade drying and tray drying at 60°C for drying liquorice (*Glycyrrhiza glabra*) and reported that tray drying recorded the shortest time of 36 hours followed by solar drying (52) hours, sun drying (64 hours) and shade drying (76 hours).

5.1.5 Colour change

Colour is one of the important attributes of a dried material. Prolonged exposure of materials to sunlight or exposure to higher temperature will result in change in colour of the product. Hence good green colour retention was found in shade dried materials which is in accordance with the findings of Yuvaraj (2007) in drying of *Wedelia chinensis* and Sejali and Anuar (2011) who found that shade dried neem powder retained good colour than that of sun and mechanically dried.

5.1.6 Phyllanthin and hypophyllanthin percentage.

Herbs and spices are the most sensitive to drying processes which increase deterioration of constituents viz. loss of volatiles, flavours, changes in colour, texture and decrease in nutritional value. Retention of the chemical constituents which impart medicinal value to the plants is the prime concern for maintaining its quality as far as the medicinal plants are concerned.

Phyllanthin and hypophyllanthin are the two important alkaloids of *P.amarus* which makes its medicinally useful. Maximum content of both phyllanthin and hypophyllanthin was recorded in shade dried samples (Fig. 4). Drying under shade will not expose the material to high intensity light and temperature. This may be the reason for its maximum retention of the alkaloids. This result is in accordance with the findings of Mehta *et al.* (2005). They found that glycyrrhizin content of *Glycyrrhiza glabra* dried under shade was 9.81 percent, tray drier 8.76 percent, solar drier 8.36 percent and sun drying 8 percent.

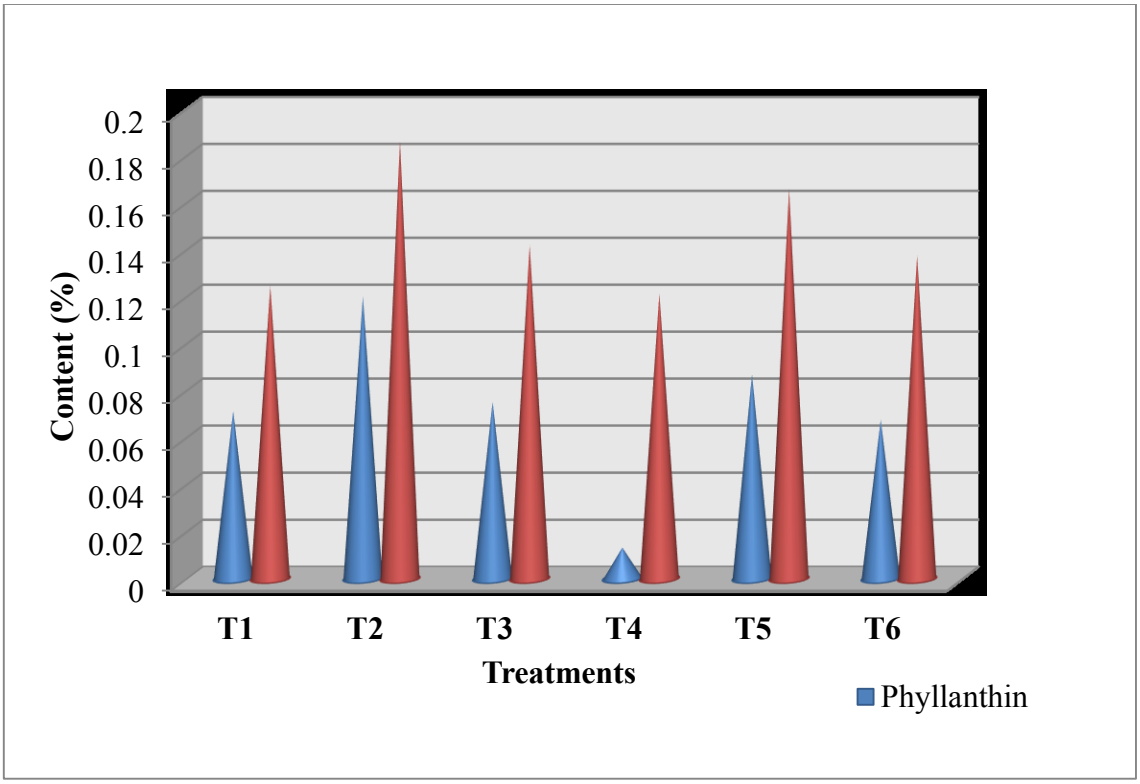


Fig.7. Content of phyllanthin and hypophyllanthin in *P.amarus* dried through different methods

5.2 STORAGE OF WHOLE PLANT AND PLANT POWDER

Medicinal plants are often dried and stored for a long time before use in manufacturing various types of products. Storage of any produce is of utmost importance as inappropriate storage conditions may render the produce unusable, no matter with what care it has been harvested and processed. For safer storage of dried materials the moisture will have to be maintained always below 8-12 per cent. Though there is a continuous demand for the herbal materials and their products in the international market, improper packaging and storage make it difficult to have sustained supply of these materials. Therefore proper packaging and storage are important to maintain the quality.

The storage containers of medicinal plant produce must provide protection from heat, humidity and temperature and at the same time it should not contaminate the produce. Pattenshetty *et al.* (1979) reported that the moisture, time and packing material have definite combined effect on the chemical content of the products and the changes become rapid and significant after 3-4 months of storage. Dried whole plant and plant powders from chopped materials were stored in different packing materials and kept for six months. The quality changes with respect to residual moisture percentage, phytochemistry, and microbiology were assessed at frequent intervals.

5.2.1 Moisture sorption behaviour

Prediction of the moisture exchange between food materials and surroundings is essential as it influences physicochemical properties, drying processes and microbial safety. Moisture content at which vapour pressure of water present in food is equal to that of surroundings is referred to as equilibrium moisture content (EMC).

Moisture sorption isotherms, depicting the relationship of moisture content of a food product with water activity at a constant temperature, are therefore of special

interest in the design of food preservation process such as drying, packaging, storage etc. They are required for the prediction of food stability, shelf life and for estimating drying times (Khalloufi *et al.*, 2000).

In powders obtained from sun dried, shade dried and mechanically dried samples critical point was found to be at equilibrium relative humidity 65 per cent and 15 per cent moisture, 13 per cent moisture and 12.6 per cent moisture respectively (Fig. 5-7). Products having equilibrium relative humidity less than 50 per cent is considered hygroscopic.(Ranganna, 1995). Therefore *P. amarus* powder dried through all the three methods of drying falls under semi hygroscopic group. So special packaging materials are not a necessity in terms of moisture for the storage of *P. amarus* powder. Moderate protection only is needed.

5.2.2 Percentage of residual moisture

Drying results in the alteration of original moisture content of a material. So there will be a tendency for any dried product to absorb moisture and it depends on the barrier proofness of the packaging material and also on the availability of moisture around the product. Therefore a variation in moisture pick up will be observed. Materials stored in aluminuim laminated pouches showed least ingress of moisture than all other packaging materials (Fig. 8 &9). It is due to the high moisture proof barrier property of aluminium laminate. This outcome is well supported by the findings of Pua *et al.* (2008) who found that jackfruit powder packaged in aluminium laminated polythene showed least increase in moisture during storage.

5.2.3 Phyllanthin and hypophyllanthin percentage

Retention of chemical constituents during the storage is an important factor which determines the shelf life of the plant material.

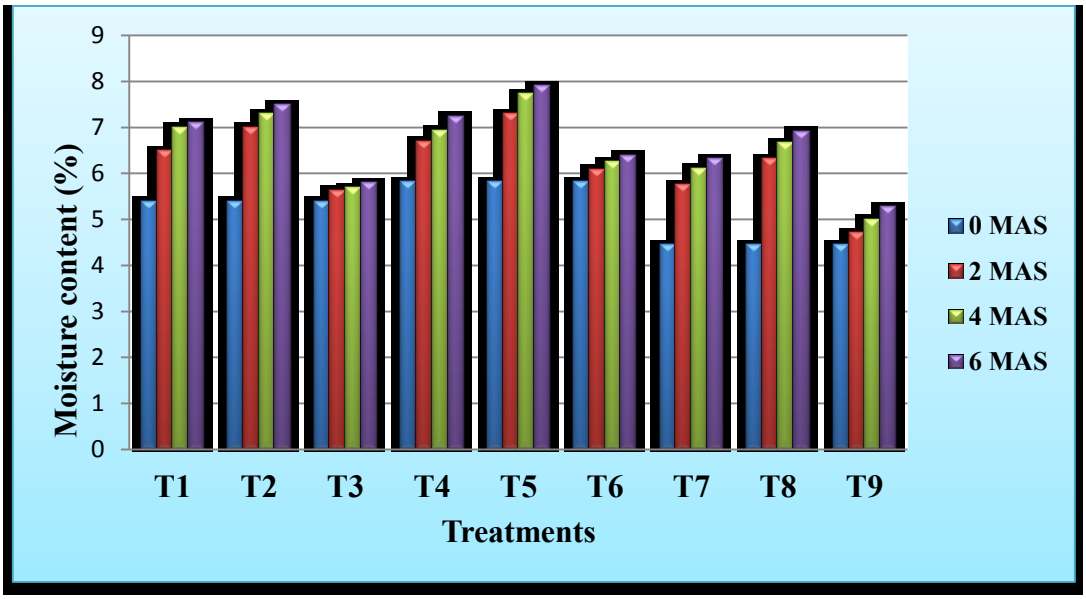


Fig. 8. Percentage moisture ingress during the storage of whole plant samples of *P. amarus*

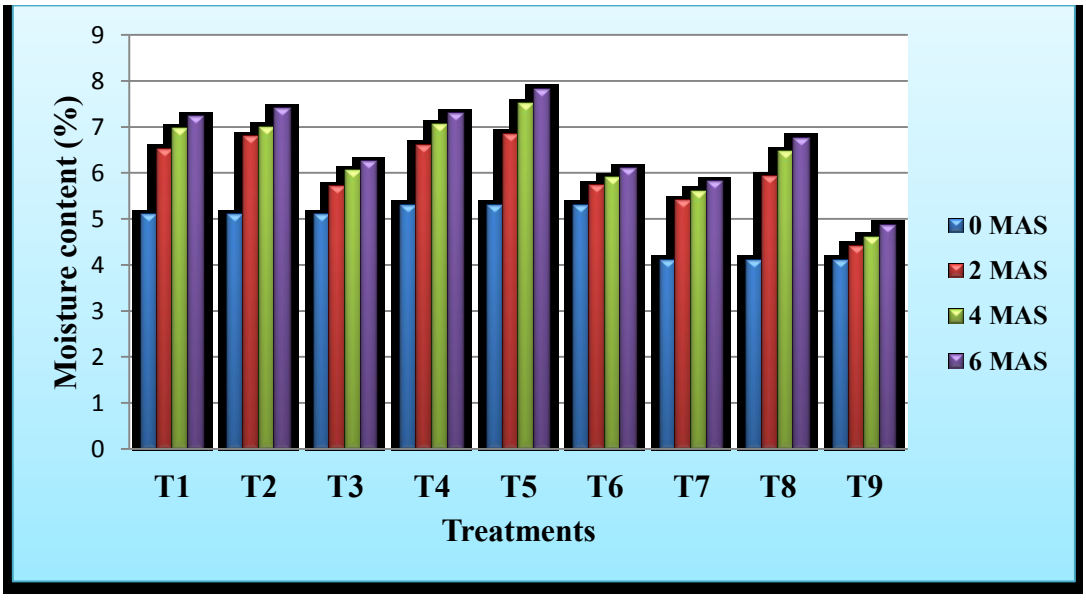


Fig.9. Percentage moisture ingress during the storage of powdered samples of *P. amarus*

Phyllanthin content was reduced highly in sundried samples with the advancement of storage period. Shade dried as well as mechanically dried samples retained highest percentage of alkaloids. This could be due to short time of exposure to drying temperature and also non exposure to direct sunlight. Though the temperature was high exposure time was shorter. Retention of slightly higher percentage of alkaloids in samples stored in aluminium laminated pouches is due to it high barrier proof properties against air and moisture (Fig. 10 -13). Similar results were obtained for Madan *et al.* (2008). They reported that the degradation of saponin content during storage in powder of *Asparagus racemosus* tubers was maximum in sun dried samples and the samples stored in airtight bags after oven drying retained maximum amount of saponin at the end of storage period.

Even though the initial content of hypophyllanthin was high it was found to be degrading drastically irrespective of the drying method and packaging material (Fig.11 & 13). It may be due to the denaturation of hypophyllanthin which needs further studies.

5.2.3 Enumeration of total microorganisms.

Medicinal plants are subject to deterioration by microbial activities during the harvesting, processing, storage and their distribution. Contaminations by pathogenic microbial or natural toxins like mycotoxins have been reported for herbal products and medicinal plants. Attempts have always been made to decontaminate and preserve these medicinal plants so as to get more safe, natural and potent medicines. Medicinal plants are mainly associated with bacteria and fungi. Microbiological background depends on several environmental factors and exerts an important effect on overall quality of herbal products and product preparations (Kniefel *et al.* 2002). Plants used in medicinal preparations have to be assured with quality to ensure the quality of the final product. Results of the study on the quantitative analysis of the stored samples of *P. amarus* are discussed here.

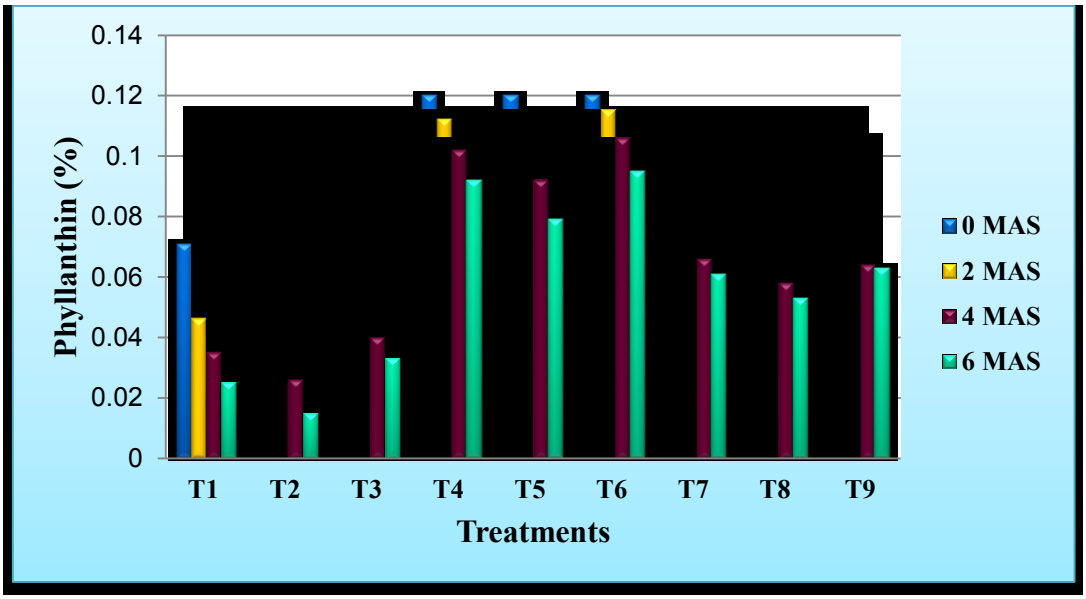


Fig. 10. Effect of storage on phyllanthin content of whole plant samples of *P.amarus*

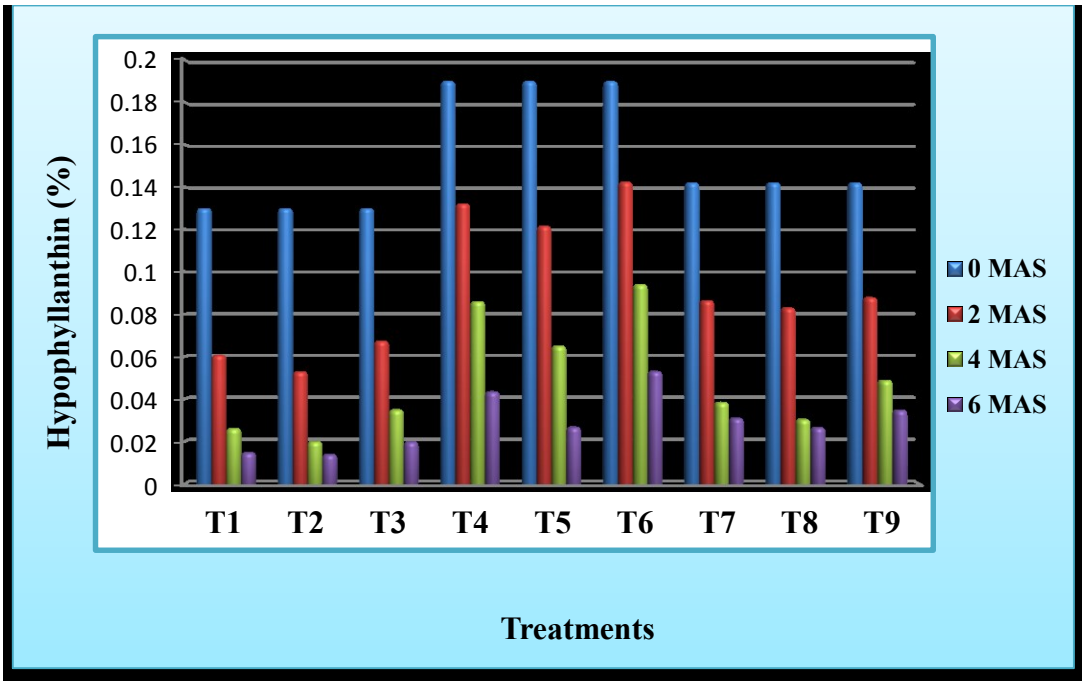


Fig.11. Effect of storage on hypophyllanthin content of whole plant samples of *P.amarus*

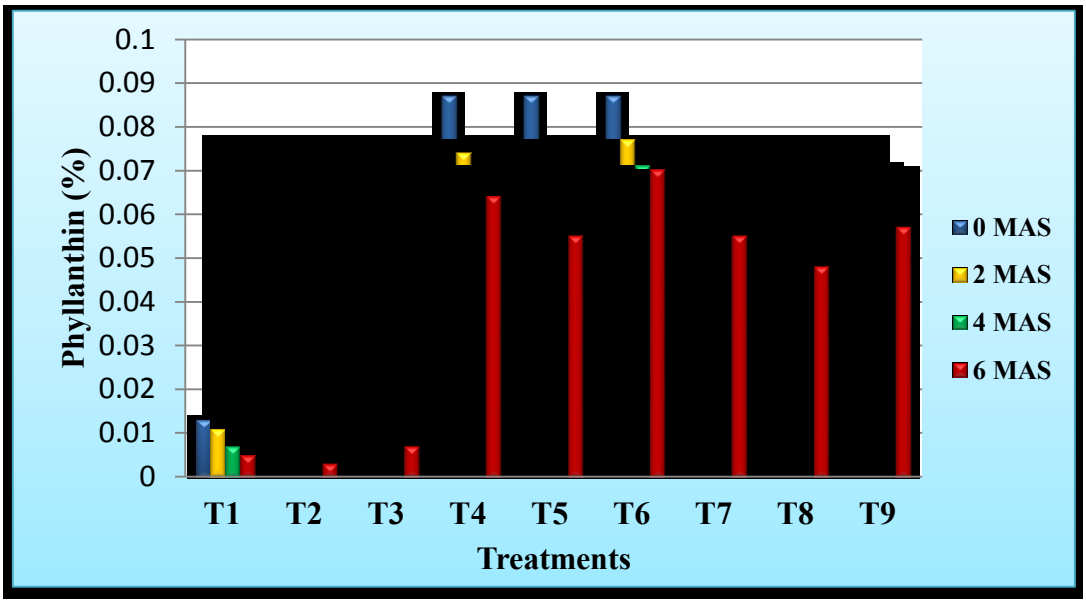


Fig. 12. Effect of storage treatments on phyllanthin content of powdered samples of *P.amarus*

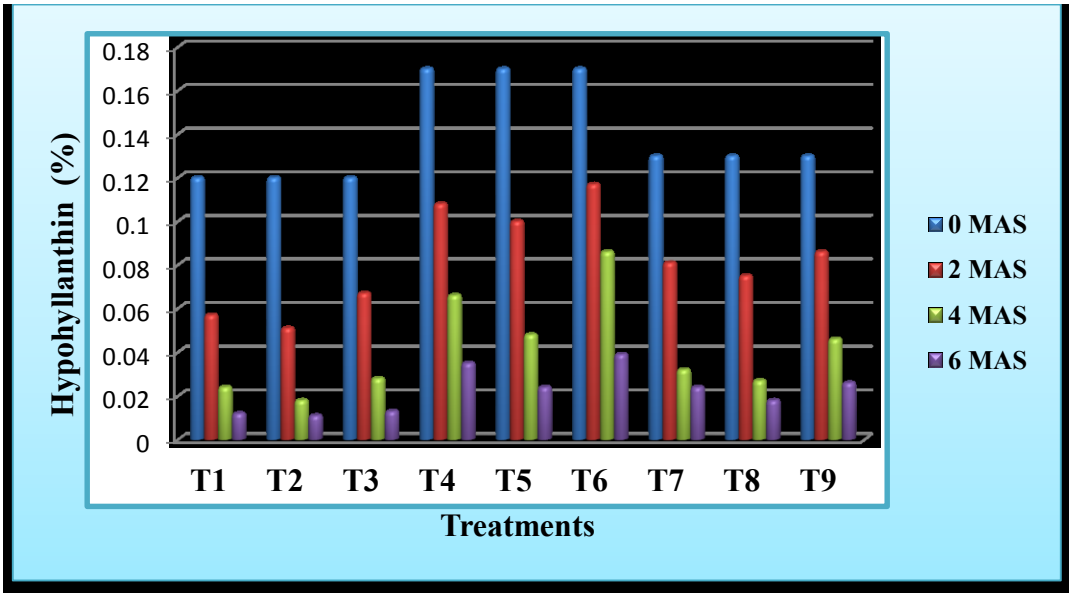


Fig.13. Effect of storage treatments on hypophyllanthin content of powdered samples of *P.amarus*

Initial enumeration of the samples prior to storage itself has shown certain colonies of fungi and bacteria, even after drying. However the load was considerably low. These organisms are either soil borne or air borne. These results are in accordance with the findings of Kniefel *et al.* (2002) who reported that the harvested medicinal plants harbor many microbes and some may be found even after drying.

With the advancement of storage period an increase in microbial population was reported after 3 months of storage (Fig.14 and 15). Low microbial population in aluminium laminated pouches is due to their least ingress of moisture and barrier proofness to air. It is supported by the findings of Negi *et al.* (2012). They reported that aluminium laminated package recorded lesser microbial colonies than polyethylene packages. Increase in residual moisture will increase the water activity. Increased water activity will prompt the growth of microorganisms. Adegoke and Komolafe (2005) reported that *P. amarus* have antimicrobial properties. Since the active constituents impart antimicrobial activity, the degradation of alkaloids might be contributed to the growth of microbes after 3 months of storage.

5.3 STORAGE OF EXTRACTIVES

The phytochemical constituents were extracted by successive solvent extraction using ethyl alcohol. Total extracts of the plants were taken and the extracts of plants have good concentrations of phytochemicals. An attempt has been made to develop a suitable storage technique for the extract. Ethanolic extract of *P.amarus* was stored in different treatment combinations and the results are discussed below.

5. 3. 1 Residual moisture

Residual moisture content of the extractives should be lower than 8 per cent for an extended shelf life. Negligible increase in residual moisture percentage of extractives could be due to its viscous nature and the barrier property of glass

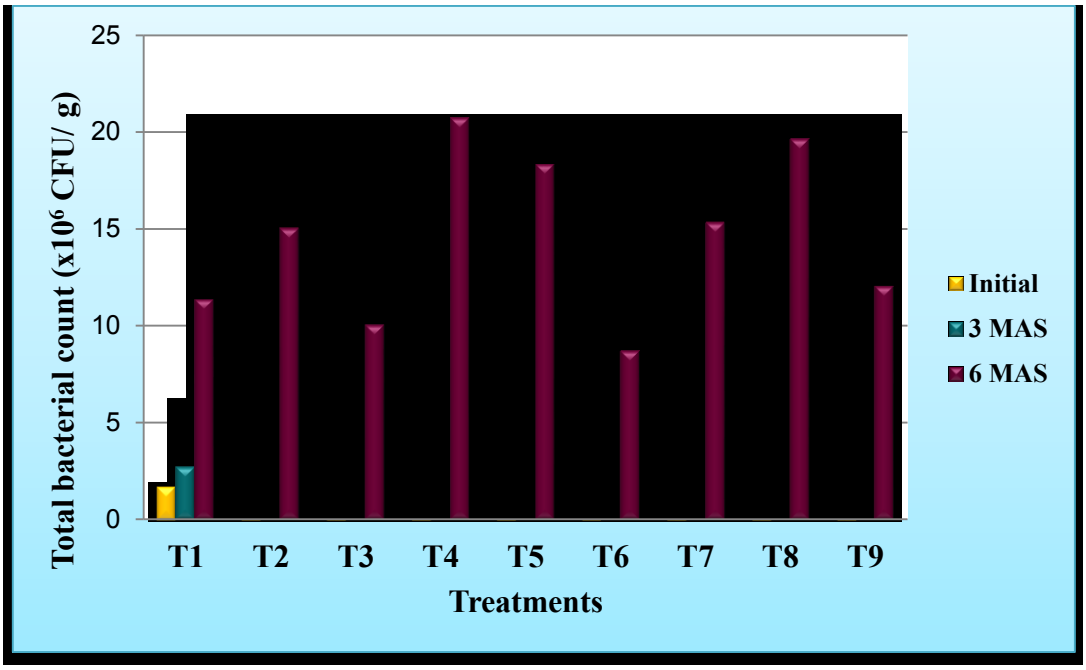


Fig.14. Effect of storage treatments on bacterial count of whole plant samples of *P.amarus*

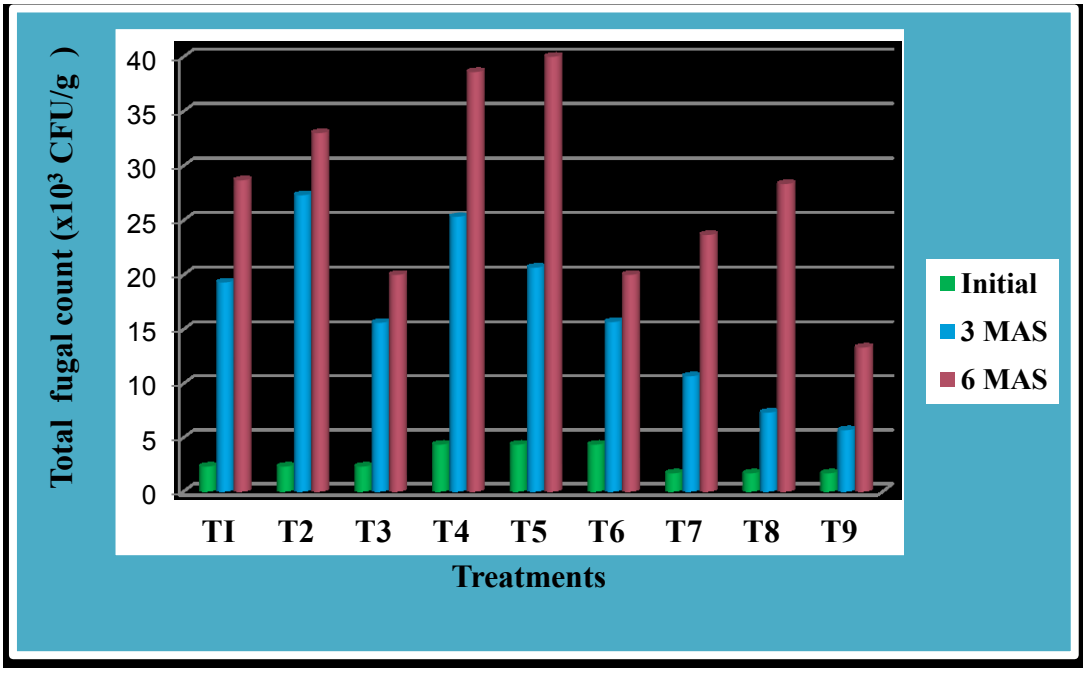


Fig.15. Effect of storage treatments on fungal count of whole plant samples of *P.amarus*

material and aluminium laminated pouch to moisture (Fig.16). It is in accordance with the findings of Sujatha (2002).

5.3.2 Phyllanthin and hypophyllanthin content

Phyllanthin and hypophyllanthin are the two lignans which decides the medicinal property of the extract. Loss of phyllanthin during storage is minimum in powdered extracts. It may be due to its less moisture content and less microbial growth. Drastic reduction in hypophyllanthin in all the samples after 4 months may be due to its less storage stability irrespective of packaging material and which needs further studies (Fig. 17 &18).

5.3.2 Microbial quality of extract

Microbial growth in extractives affects its quality. Maximum population was noticed in extractives stored as such (T_1). It is due to its high moisture content. Extractives with sodium benzoate (T_2) showed less microbial growth due to the preservative action of sodium benzoate (Fig. 19 and 20). Least microbial growth in powdered extractives can be correlated with its less moisture content. Moreover it has been reported that ethanolic extract of *P.amarus* possess antimicrobial activity (Oluwafemi and Debri, 2008; Akinjogunla, 2010). Mazumdar *et al.* (2006) reported that the antibacterial activity of *P. amarus* is mainly due to phyllanthin.

After conducting the two experiments it was found that shade drying is the best method to dry *P.amarus* followed by mechanical drying. Since the margin of variation in drying rates of whole plant and chopped material is narrow, it can be concluded that there is no significant effect for chopping of the plant material on drying rate. Sun drying was found to be causing deterioration of phytochemicals which extend even to the storage period. Shade dried and mechanically dried samples proved to be retaining good percentage of phyllanthin and better colour throughout the storage period. Hypophyllanthin was found to get deteriorate abruptly after 3-4

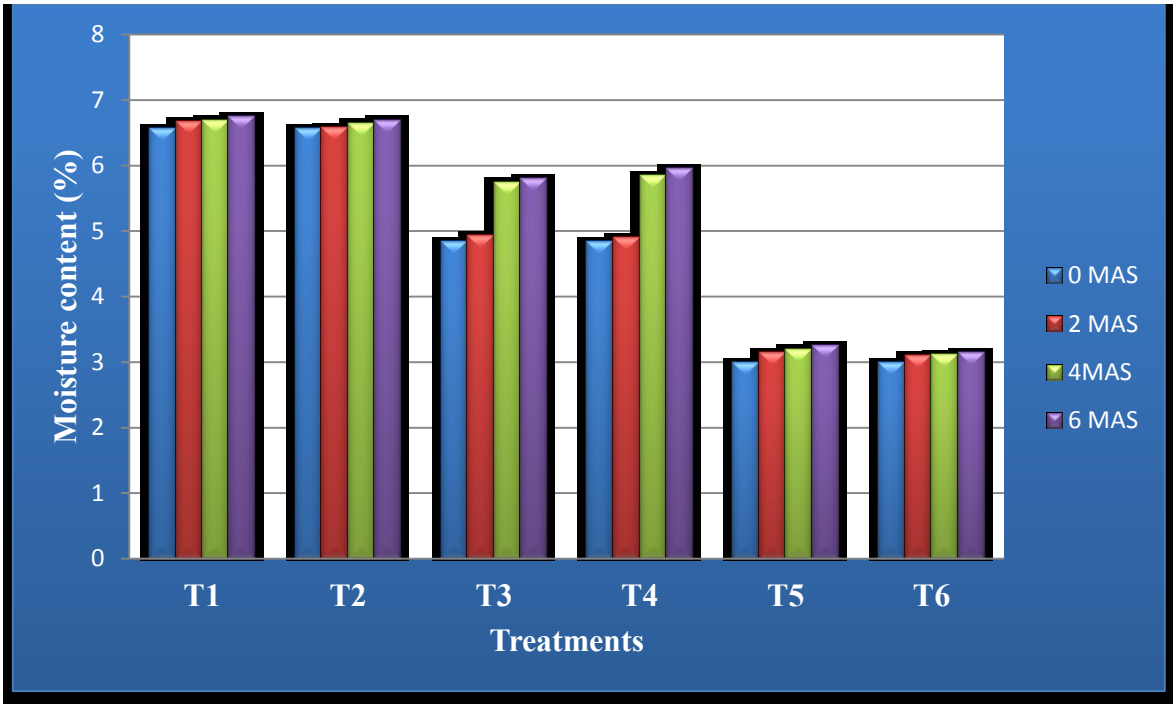


Fig.16. Percentage moisture ingress during the storage of ethanolic extractives of *P. amarus*

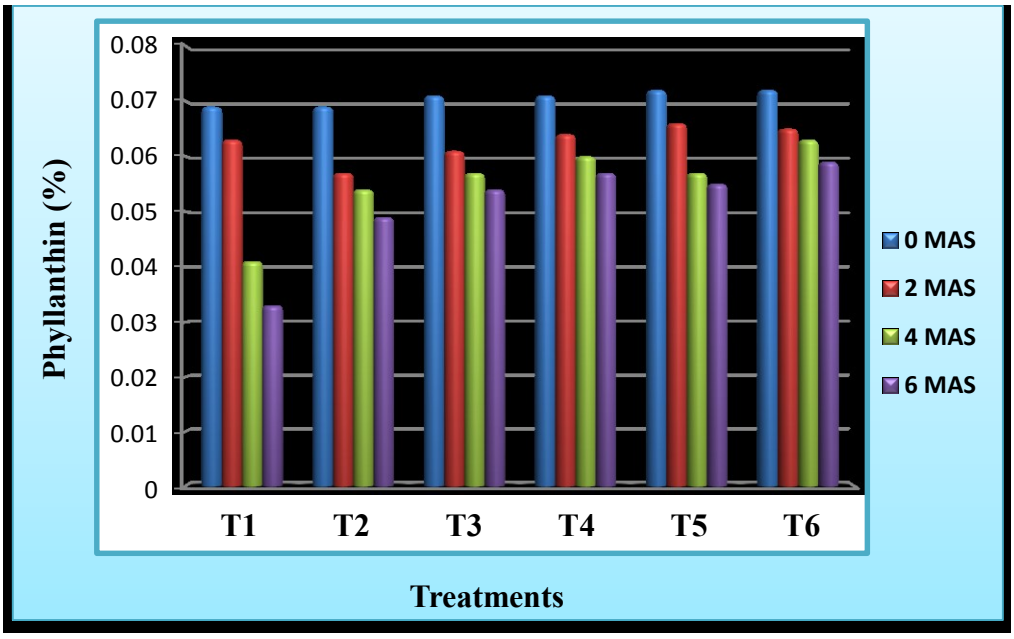


Fig.17. Effect of storage treatments on phyllanthin content of ethanolic extracts of *P.amarus*

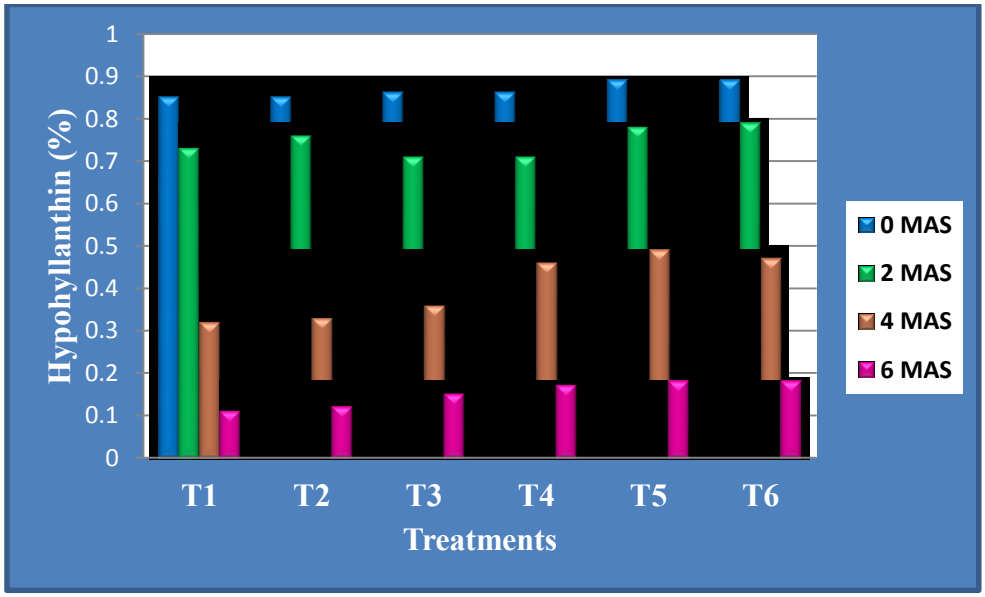


Fig.18. Effect of storage treatments on hypophyllanthin content of ethanolic extracts of *P.amarus*

months of storage irrespective of drying method and packaging materials. Among the packaging materials aluminium laminated pouches proved to be the best in terms of maximum retention of phytochemicals, minimum moisture ingress and least microbial count. Polythene pouches also gave good results. Ethanolic extraction was far better compared to vapour heat extraction. Dried ethanolic extractives retained better percentage of phyllanthin after six months of storage. Though the initial content was higher in extractives, hypophyllanthin percentage decreased faster in all the samples, irrespective of the treatments.

It can be concluded that 'Kizharnelli' (*Phyllanthus amarus*) can be dried under shade as whole plant and can be stored in aluminium laminated pouches without much reduction in physiochemical properties for an extended period of 3-4 months. Dried ethanolic extracts retain good physiochemical and microbial qualities for about 4 months and this method can be adopted by industries as a proper storage technique.

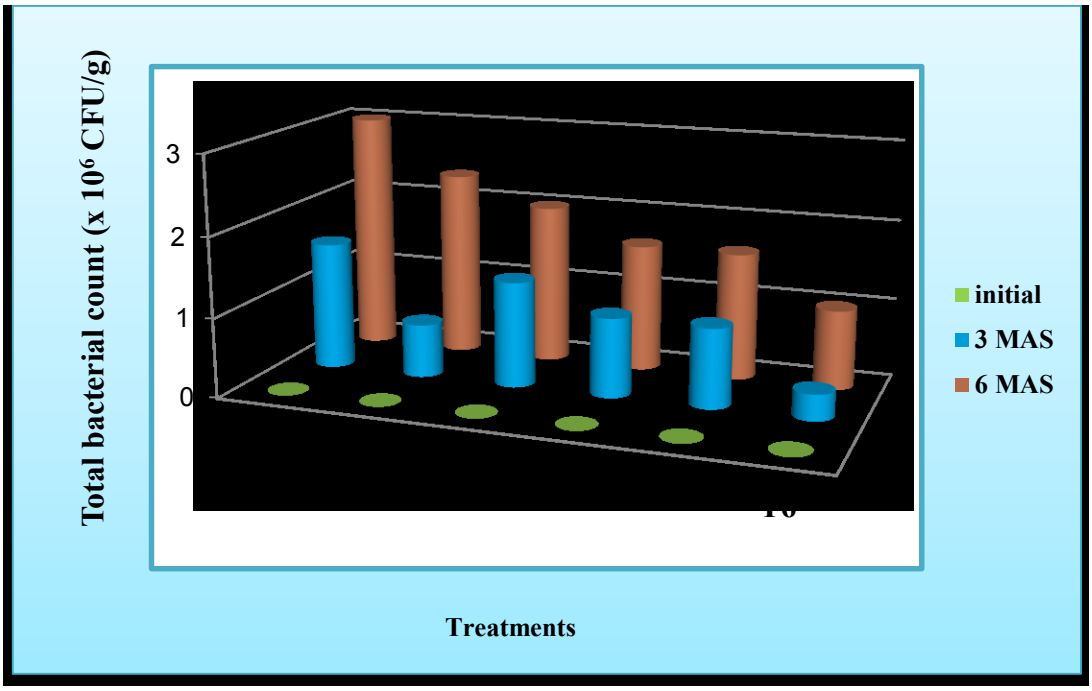


Fig. 19. Effect of storage treatments on total bacterial count of ethanolic extracts of *P.amarus*

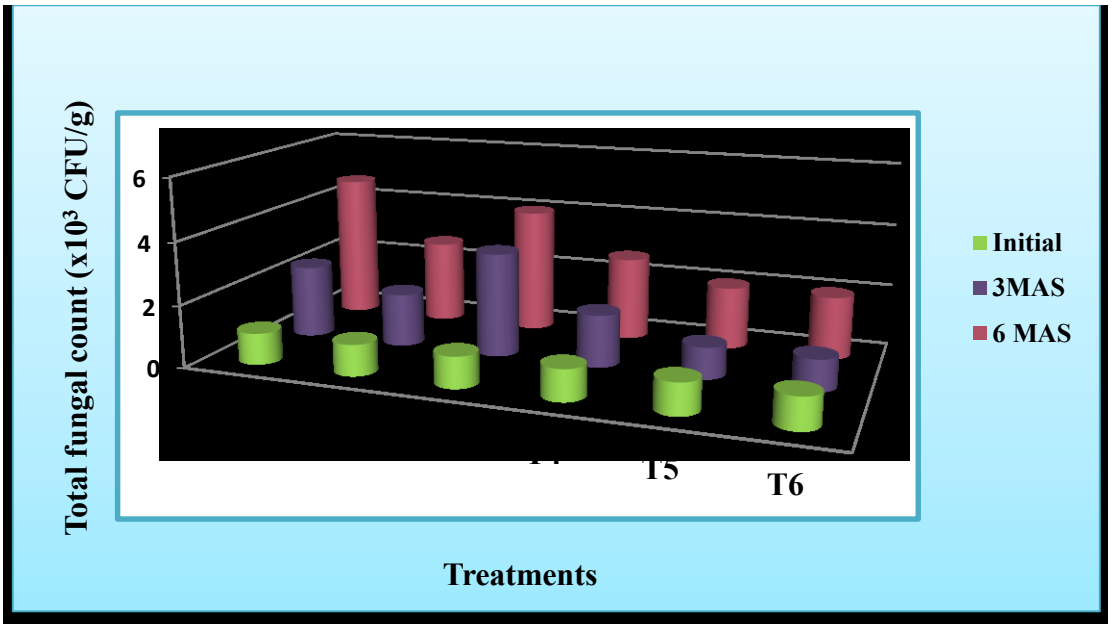


Fig. 20. Effect of storage treatments on fungal count of ethanolic extracts

SUMMARY

6. SUMMARY

The study entitled ‘’ Drying and Storage Studies in ‘Kizharnelli’(*Phyllanthus amarus* Schum &Thonn.) was conducted at Department of Processing Technology, College of Horticulture, Vellanikkara during 2011-2012.

Experiments were done with an objective of developing suitable drying and storage techniques for both whole plant and extractives of ‘Kizharnelli’ without much degradation in quality.

- There was no significant difference exist between the recovery percentage of whole plant and whole plant chopped into pieces. However shade dried whole plant showed the highest recovery of 25% with a residual moisture content of 5.82 percent. Least recovery was showed by mechanically dried chopped plant material (22%) with a residual moisture content of 4.1 percent.

- Mechanical drying in a cabinet drier at 40° to 50° C took least time for drying (12-16 hrs) where as shade drying took the longest time of 6-7 days .Initial rate of drying was found to be higher in chopped plant materials than that of whole plant in all the treatments.

- Shade dried whole plant recorded highest amount of alkaloids, viz. phyllanthin (0.125%) and hypophyllanthin (0.186%) whereas sun dried chopped material recorded least amount of phyllanthin (0.013%) and hypophyllanthin (0.121%).

- Shade drying retained better colour followed by mechanical drying

- Phyllanthin content was found to get reduced gradually with the advancement of storage period in all the treatments.

- Hypophyllanthin content was found to get degraded faster during storage period especially in sundried samples which recorded least content after 4 months of storage.

- Samples stored in aluminium laminated pouches recorded highest retention of phyllanthin & hypophyllanthin over 6 months of storage. However the cost of the packaging material was slightly higher than that of other materials.

- Shade dried samples followed by the samples dried mechanically, retained good percentage of phyllanthin and hypophyllanthin at the end of storage period. Sun dried samples showed faster degradation of these alkaloids with the advancement of storage period.

- Residual moisture content increased with increase in storage period. Samples stored in aluminium laminated pouches showed least ingress of moisture at the end of storage period.

- Microbial population increased during storage and there were no significant difference observed in bacterial population among different treatments. Samples stored in aluminuim laminated pouches and polythene bags showed least fungal count. Actinomycetes population was found to be negligible at the end of 6 months in all the treatments.

- Extraction studies revealed that ethanolic extraction was the best method with 0.07% phyllanthin and 0.89% hypophyllanthin with a recovery of 18.66%. The extract obtained through vapour heat treatment followed by mechanical pressing recorded zero phyllanthin and negligible amount of hypophyllanthin.

- While in storage, residual moisture content of the stored ethanolic extrats showed only slight increase in moisture. Glass bottles as well as aluminium laminated pouches imparted good barrier proofness against moisture. With the advancement of storage and it was found that extract as such stored in glass bottles along with 600 ppm sodium benzoate and dried extract stored in aluminium laminated pouches showed least increase of moisture.

- Studies on the effect of storage on phyllanthin content of ethanolic extracts revealed that there was a gradual reduction in phyllanthin percentage with the advancement of storage period. Dried and powdered extractives stored in aluminium laminated pouches and vacuum concentrated extracts stored in glass bottles showed least reduction in phyllanthin content (18.05% and 19.71% respectively) after 6 months of storage.

- Microbial counts in all the stored samples of ethanolic extracts were negligible.

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APPENDICES

APPENDIX –I

WEATHER PARAMETERS PREVAILED DURING DRYING PERIOD

Weather Parameters	September 2011
Max. Temperature (°C)	30
Min. Temperature (°C)	23.1
Relative Humidity (Morning) %	94.2
Relative Humidity (Noon)%	74.7
Sunshine (hrs)	4.4

APPENDIX-II

NUTRIENT COMPOSITION OF MEDIA

1. Nutrient Agar Media (for Bacteria)

Beef extract	3 g
Peptone	5 g
Sodium chloride	5 g
Agar	18 g
Distilled water	1000 ml
P^H	6.8-7.2

2. Potato Dextrose Agar Media (for Fungus)

Peeled potatoes	250 g
Dextrose	20 g
Agar	18 g
Distilled water	1000 ml
P^H	5.6

3. Kenknight's Agar Media (for Actinomycetes)

Glucose	1 g
$MgSO_4$	0.1 g
KCl	0.1 g
KH_2PO_4	0.1 g
Ammonium Sulphate	0.1 g
Agar	18 g
Distilled water	1000 ml
P^H	7

DRYING AND STORAGE STUDIES IN 'KIZHARNELLI' (*Phyllanthus amarus* Schum.&Thonn.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

In recent years, an increasing interest in medicinal herbs, as natural regrowing raw material could be observed worldwide. After a period of exploding the synthetic chemicals and its ill effects too, the present situation demands a 'green wave' to use nature and natural products.

Phyllanthus amarus Schum.&Thonn., known in Malayalam as 'Kizharnelli' is one of the most important herbs in Indian medicine. Owing to its phyllanthin and hypophyllanthin content, it is widely used as hepatoprotective, anti-inflammatory, antidiabetic, chemoprotective, antioxidant etc. The plant has been traditionally used to promote liver functions and used as a remedy for jaundice. The plants are available mostly during the months of July to September and are short living.

Therefore an attempt has been made to extend the post harvest storage life of *P. amarus* with minimum quality deterioration. Two experiments were conducted during the course of study; with the first experiment to evolve a suitable drying and packaging technique for the plant as such and the second to evolve an extraction technique and the storage studies of the extractives.

Shade drying of whole plant followed with packaging in aluminium laminated pouches was found to be the best drying and storage technique in terms of colour, phyllanthin and hypophyllanthin content for the storage up to 4 months. Hypophyllanthin content was found to be degrading in a faster rate and very less amount was noticed after 4 months of storage. Comparing the drying of whole plant and chopped materials, no advantage was noticed in terms of drying rate. Therefore this additional step of chopping can be avoided. Sun drying, though a common practice of drying; degradation of alkaloids and colour was observed in drying and storage of *Phyllanthus amarus*.

Studies on extraction techniques revealed that the extraction using ethanol was found to be better and regarding to the storage of extractives, dried ethanolic extracts stored in aluminium laminated pouches retained maximum phyllanthin

content after six months of storage. However hypophyllanthin content dropped drastically after 4 months of storage.

The techniques thus developed will help to store ‘Kizharnelli’ for an extended period of 3 to 4 months than its normal available period as stored material.