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INVESTIGATIONS ON NUTRACEUTICAL, QUALITATIVE PHYTOCHEMICAL ANALYSIS OF EXTRACTS FROM GIANT POTATO

A PROJECT REPORT

Submitted by

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BONAFIDE CERTIFICATE

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The Report of the project work submitted by the above students in partial fulfillment for the award of Bachelor of Technology degree in Biotechnology of Anna University was evaluated and confirmed to be the report of work done by the above students. It was submitted for evaluation and viva-voce held on 27.04.2009.

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ABSTRACT

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Roots and tubers will play an increasingly important role in meeting the food requirements, feed uses and income needs of the world's food system as we enter the new millennium. Potatoes are members of the Solanaceae family, while giant potatoes belong to the Convolvulaceae Family. A study on the proximate analysis, the trace elements and the caloric values of the *Ipomoea mauritiana Jacq* species were performed and it was found to contain high amount of iron of 38.03 parts per million besides various components. The caloric value for carbohydrates was 304.96 which was found to be comparatively higher, the fat and protein caloric values was calculated to be 5.22 and 21.08 respectively. The total caloric content per 100g of dry weight was 331.26. The qualitative phytochemical analysis was performed for fractionated hexane, chloroform, methanol and water extracts and various tests were performed for the detection of many compounds some of which were alkaloids, flavonoids, saponins, oils, fats, fibres, proteins and carbohydrates. Thin Layer Chromatography was performed on silica gel plates for the various extracts of hexane, chloroform and methanol and then the standardization and separation of unknown compounds were performed. The Relative Front values were calculated for each compound of the hexane extract separated based on the distance travelled by the compound and the solvent front.

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INTRODUCTION

1. INTRODUCTION

Due to increasing market demands on protein ingredients, novel proteins have been purified from various sources (El Nasri & Tinay 2007; Lamsal, *et al.*, 2007; Løkra, *et al.*, 2007). However, for a novel protein to be useful for food processing application, it should possess desirable functional and nutritional qualities. The functional properties of proteins, such as solubility and emulsifying activities, are in turn highly dependent on many factors, such as pH and the type and amount of salts present. For instance, the presence of NaCl improved the emulsifying properties of cowpea, fenugreek and sesame proteins (El Nasri & Tinay, 2007; Inyang & Iduh, 1996; Ragab *et al.*, 2004). On the other hand, addition of $CaCl_2$ prior to emulsification increased the average droplet diameter and reduced creaming stability of the emulsion. (Ye & Singh, 2000).

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Goldfrank, *et al.*, 1982). The convolvulceae comprise nearly tropical species. A wide variety of low-molecular N-containing secondary metabolites has been found in this family. Plant chemicals are often classified as primary and secondary metabolites (Goldsoetin, 1981). Primary metabolites are widely distributed in nature, occurring in one form or the other in all organisms. In higher plants, such compounds are often concentrated in seeds and vegetative storage organs and are required for physiological development because of their role in basic cell metabolism (Harbone, 1972).

Secondary metabolites are biosynthetically derived from primary metabolites but are limited in distribution in the plant kingdom. Secondary compounds have no apparent function but often have an important ecological role in the plant. Bell (1980) has suggested that secondary products from plants ward off potential predators attract pollinators and serve as chemical defenses against many of the microorganisms. These metabolites are to be synthesized and stored in specialized cells at distinct

developmental stages. As a result, secondary metabolites are used commercially as biologically active compounds (Luckner, 1972; Mann, 1978; Robinson, 1983). Secondary products have not been produced for their physiological functions as in the case of primary compounds like amino acids but, they serve as a chemical interface between the producing plant and its environment. Most plant derived biologically active secondary metabolites have been found in medicinal applications as drugs or as model compounds for drug synthesis. The search for other secondary products in plant still continues (Ramawat, 2000).

Secondary metabolite has been found in the family, Convolvulaceae over the years. Etrolines, pyrrolidines, lipophilic tropanes, hydrophilic tropanes and pyrrolizidine alkaloids as well as cyanogenic glycosides are isolated from this family. Furthermore, different types of amides have been isolated: N-feruloylatyramine, serotonin-hydroxycinnamic acid conjugates of the upobscurine type and linganamides (Britta Tofern, *et al.*, 1999). This family contains a unique group of glycosides which gives hydroxylated fatty acids as aglycone and a mixture of aldohexoses when subjected to acid hydrolysis. On alkaline hydrolysis, they give short chain aliphatic acids and glycosidic acids. The aglycones and sugars most frequently obtained from Convolvulaceous glycosides are: 11-hydroxyhexadecanoic acid, dihydroxy derivatives of tetradecanoic, hexadecanoic and octadecanoic acids and glucose, rhamnose, fucose and 6-deoxy-glucose (Nikolin, *et al.*, 1977). Considering the importance of phytochemical constituents, the present study reports the effective isolation, identification and screening of bioactive compounds from *Ipomoea mauritiana.*

OBJECTIVES

The present study was carried out with the objective to

- Evaluate the nutritional contents of dried powder samples of giant potato
- To screen the qualitative phyto-constituents in the fractionated polar solvent extracts of hexane, chloroform, methanol and water from the powder sample.
- Solvent standardization of thin layer chromatography for hexane extract.

LITERATURE REVIEW

2. LITERATURE REVIEW

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases, these substances serve for plant defense mechanism against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinines and tannins) are responsible for plant pigment, and many compounds are responsible for plant flavor (e.g., the terpenoid, capsaicin from chilli peppers). The family Convolvulaceae consists of 50 genera and 1200 or more species, primarily of tropics and subtropics with ranges extending into north and south temperate regions and particularly abundant in tropical America and tropical Asia. *Ipomoea* the largest genus has about 400 species mostly lians, *Convolvulus* is of more temperate distribution has about 200 species and *Cuscutta* the third largest with 120 species. The family is distinguished by mille sap or latex usually present (Kirithikar-Basu, 2001).

2.1 Plant Description

Table 2.1 Plant description

Order:	Solanales
Family:	Convolvulaceae (Morning- glory family)
Genus:	*Ipomoea*
Species:	*mauritiana Jacq*

Fig. 2.1 *Ipomoea mauritiana Jacq* species

Fig. 2.2 Dried chopped pieces of tuber

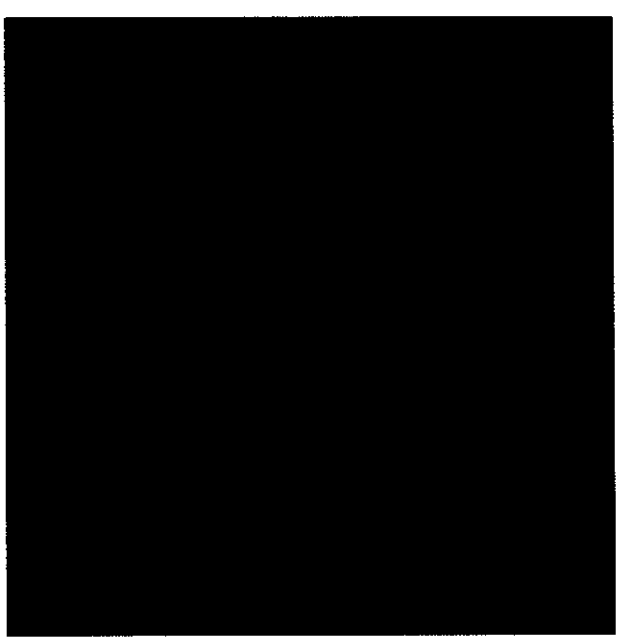
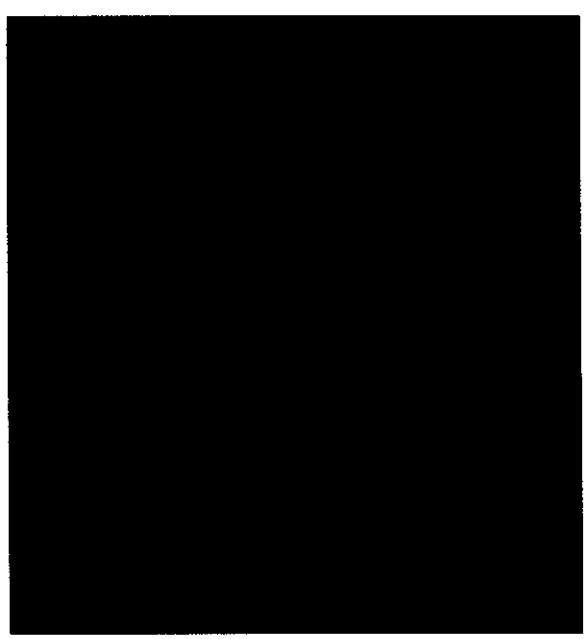


Fig. 2.3 Grounded coarse between fine tuber powder



2.2 Vernacular Names

Tamil : Palmuthamki, Palmuthamkai

Hindi : Kazun, Pan-kazun

English : Giant Potato

The giant potato *(Ipomoea mauritiana)* is a type of morning glory plant. Like the sweet potato, it belongs to the *Ipomoea* genus. It grows as a vine. Its origins are uncertain, but it has been recorded in West Africa including in Gambia and riparian forests of Benin, as well as Australia's Northern Territory. It is naturalised in many parts of the world including Taiwan and Hawaii (where it is called Likam). *Ipomoea mauritiana* is a twining shrub, Branchlets hirsute. Leaves triangular-cordiform, 2-4 x 2-3 cm, chartaceous, glabrous, base cordate, apex gradually acute, apiculate, basal lobes triangular, acute or obtuse; petiole 1 to 2 cm. Flowers shortly pedicellate, in subumbellate cymes; peduncle up to 10 cm. Calyx-lobes 5, subequal, obovate, 6 x 4 mm, truncate, scarious, obtuse-apiculate, hardly enlarged in fruit. Corolla cream, with a purple throat, 4 cm across, salver-form tube 3.5 cm long. Stamens 5 up to 2 cm; anthers 2.5 mm. Ovary conical, 1.5 mm; style 2.5 cm; stigma capitate, (sub) globose. Capsule globose, 0.8 cm across; seeds white-velvetty.

2.3 Phenols and phenolic acids

Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds which are in highest oxidation state. The common herbs Tarragon and Thyme both contain caffeic acid, which is effective against viruses (Wild, 1994), bacteria (Weinmann, 1997) and fungi (Duke, 1985).

Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two –OH groups, and pyrogallol has three. The sites and number of hydroxyl group on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity.

Some authors have found that more highly oxidized phenols are more inhibitory (Scalbert, 1991). The mechanisms thought to be responsible for phenolic toxicity to microorganisms include, enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987).

Phenolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often cited as antimicrobial as well. Eugenol is a well-characterized representative found in clove oil. Eugenol is considered as both bacteriostatic and fungistatic (Thomson, 1978).

2.4 Importance of Quinones

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds, being colored, are responsible for the browning reaction in cut or injured fruits and vegetables and are the intermediates in the melanin synthesis pathway in human skin. Their presence in Henna gives its dyeing properties (Fessenden and Fessenden, 1982).

The switch between diphenol (or hydroquinone) and diketone (or quinone) occurs easily through oxidation and reduction reactions. The individual redox potential of the particular quinone-hydroquinone pair is very important in many biological systems; witness the role of ubiquinone (coenzyme Q) in mammalian electron transport systems. Vitamin K is a complex naphthoquinone. Its antihemorrhagic activity may be related to its ease of oxidation in body tissues (Harris, 1963). Hydroxylated amino acids may be made into quinones in the presence of suitable enzymes, such as a polyphenoloxidase.

2.5 Bioactive flavonoids and flavonols

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol. Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection, they have been found in *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes (Tanaka and Iinuma, 1996). Catechins, the most reduced form of the C3 unit in flavonoid compounds, deserve special mention. These flavonoids have been extensively researched due to their occurrence in Oolong green teas. It was noticed some time ago that teas exerted antimicrobial activity (Toda, *et al.*, 1989) and that they contain a mixture of catechin compounds. These compounds inhibited *Vibrio cholerae*, *Streptococcus mutans* (Batista, 1994), *Shigella*, and other bacteria and microorganisms in *in vitro* (Sakanaka, 1989). The catechins inactivated cholera toxin in *Vibrio* and inhibited isolated bacterial glucosyltransferases in *S. mutans*, possibly due to complexing activities described for quinones above. This latter activity was borne out in *in vivo* tests of conventional rats. When the rats were fed a diet containing 0.1% tea catechins, fissure caries (caused by *S. mutans*) was reduced by 40% (Ooshima, 1993).

Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids such as swertifrancheside, glycyrrhizin (from licorice), and chrysin against HIV (Critchfield, 1996; Kaul, *et al.*, 1985) provide a summary of the activities and modes of action of quercetin, naringin, hesperetin, and catechin in *in vitro* cell culture monolayers. While naringin was not inhibitory to herpes simplex virus type 1 (HSV-1), poliovirus type 1, parainfluenza virus type 3 and RSV, the other three flavonoids were effective in various ways. Hesperetin reduced intracellular replication of all four viruses; catechin inhibited infectivity but not intracellular replication of RSV and HSV-1; and quercetin was universally effective in reducing infectivity. The authors propose that small

structural differences in the compounds are critical to their activity and pointed out another advantage of many plant derivatives: their low toxic potential.

Delineation of the possible mechanism of action of flavones and flavonoids is hampered by conflicting findings. Flavonoids lacking hydroxyl groups on their b-rings are more active against microorganisms than are those with the 2-OH groups (Chabot *et al.*, 1992); this finding supports the idea that their microbial target is the membrane. Lipophilic compounds would be more disruptive of this structure. However, several authors have also found the opposite effect; i.e., the more hydroxylation, the greater the antimicrobial activity. This latter finding reflects the similar result for simple phenolics. It is safe to say that there is no clear predictability for the degree of hydroxylation and toxicity to microorganisms.

2.6 Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum* (Fessenden *et al.*, 1982).

Solamargine, a glycoalkaloid from the berries of *Solanum khasianum*, and other alkaloids may be useful against HIV infection (McMahon *et al.*, 1995; Sethi, 1979) as well as intestinal infections associated with AIDS (McDevitt, *et al.*, 1996). Bieber *et al.* (1986) have reported that numerous species of the genus *Ipomoea* are widely used in folk medicine all over the world. Antimicrobial glycosides from *I.* species were reported (Valette and Liber, 1938; Carlson *et al.*, 1948). Chemical investigations indicate indole alkaloids and resin glycosides as the most common constituents in the Convolvulaceae (Wagner, 1993).

Bieber *et al.* (1986) have reported the screening program of higher plants from Brazil, exceptionally high number of *Ipomoea* species showed

antimicrobial activity. Especially the methanolic extract of leaves of *I. bahiensis* caused strong inhibition of growth of various respresentative microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Neurospora crassa* and *Streptococcus faecalis*.

Glycosides present as monomers in the plant were described only in the case of *I. muricata* (Khanna and Gupta, 1967), *I. digitata* (Matin *et al.*, 1969), *I. purpurea* (Kikolin *et al.*, 1978), and *I. leari* (Sarin *et al.*, 1973). Compared with these known glycosides, 1a, b and 2a, b are more complex, because they contain not only a hydroxy fatty acid and three different sugars but also short chain acids 3 and 4. The unique structural features of Convolvulaceae glycosides and their multiple pharmacological and antimicrobial properties were investigated. (Bieber *et al.*, 1986). Nair *et al.*, have reported the two varieties of *Ipomoea sepiaria* and number of chemical difference between them. The typical variety of *I. sepiaria* contain methoxy quercetin and 3,4-dimethoxy quercetin, proanthocyanins and p-hydroxybenzoic acid as against 3'-OMe quercetin, quinones, syringic acid, p-coumaric acid, melilotic acid and saponins of variety stipulacea.

2.7 Medicinal properties of Coumarins

Coumarins are phenolic substances made of fused benzene and a-pyrone rings (O'Kennedy and Thornes 1997). They are responsible for the characteristic odor of hay. As of 1996, at least 1,300 had been identified (Hoult and Paya, 1996). Their fame has come mainly from their antithrombotic (Thastrup, *et al.*, 1985), anti-inflammatory (Piller, 1975), and vasodilatory (Namba, 1988) activities. Warfarin is an well-known coumarin which is used both as an oral anticoagulant and, interestingly, as a rodenticide (Keating and O'Kennedy, 1997). It may also have antiviral effects (Berkada, 1978). Coumarins are known to be highly toxic in rodents (Thastrup, 1985) and therefore are treated with caution by the medical community. However, recent studies have shown a "pronounced species-dependent metabolism" (Weinmann, 1997), so that many *in vivo* animal studies cannot be extrapolated to humans. It appears that

toxic coumarin derivatives may be safely excreted in the urine in humans (Weinmann, 1997).

Several other coumarins have antimicrobial properties. R. D. Thornes, working at the Boston Lying-In Hospital in 1954, sought an agent to treat vaginal candidiasis in his pregnant patients. Coumarin was found in vitro to inhibit *Candida albicans* in *in vitro* condition. During subsequent *in vivo* tests on rabbits, the coumarin-spiked water supply was inadvertently given to all the animals in the research facility and was discovered to be a potent contraceptive agent when breeding programs started to fail (Thornes, 1997). Its estrogenic effects were described by Soine (1964).

As a group, coumarins have been found to stimulate macrophages (Smith, 1997), which could have an indirect negative effect on infections. More specifically, coumarin has been used to prevent recurrences of cold sores caused by HSV-1 in humans (Berkada, 1978) but was found ineffective against leprosy (Thornes, 1997). Hydroxycinnamic acids, related to coumarins, seem to be inhibitory to gram-positive bacteria (Fernandez, 1996). Also, phytoalexins, which are hydroxylated derivatives of coumarins, are produced in carrots in response to fungal infection and can be presumed to have antifungal activity (Hoult, and Paya, 1996). General antimicrobial activity was documented in woodruff (*Galium odoratum*) extracts (Thomson, 1978). In general, data about specific antibiotic properties of coumarins are scarce, although many reports give reason to believe that some utility may reside in these phytochemicals (Bose, 1958; Hamburger and Hostettmann, 1991; Scheel, 1972).

2.8 Anticancer and antimicrobial glycosides from *Ipomoea* species

Three new tetrasaccharide glycosides, differing from one reported previously in the type of short-chain fatty acids ester-linked to the tetrasaccharide core, have been isolated and identified from an oligosaccharide fraction *Ipomoea stans* by Reynolds *et al.* (1995) Preliminary screening tests showed that the fraction containing these compounds had pronounced cytotoxicity towards three human tumor cell lines as well as specific antibiotic activity against two bacterial strains. Extracts from *Ipomoea stuns* (Convolvulaceae) have been used in Mexican traditional medicine for treating epileptic

seizures (Reynolds *et al.*, 1995). Reynolds *et al.* (1995) have conducted a detailed phytochemical investigation of this species in the hope of isolating and identifying pharmacologically useful compounds. Initially, they have isolated two polysaccharide fractions of different polarity from the roots of *Ipomoea stuns*, with the less polar one was isolated, later.

Numerous species of the genus *Ipomoea* are widely used in folk medicine all over the world especially as powerful cathartics. Pharmacological studies reported antimicrobial, analgesic, spasmogenic, spasmolytic, hypotensive, insecticidal, psychotomimetric and anticancer effects. Chemical investigations indicate indole alkaloids and resin glycosides as the most common constituents in the Convolvulaceae (Bieber *et al.*, 1986).

Bieber *et al.* (1986) reported the screening program of higher plants from north-eastern Brazil and reported that more number of *Ipomoea* species showed antimicrobial activity. Especially methanolic extract of leaves of *I. bahiensist* caused strong inhibition of growth of various microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Neurospora crassa* and *Streptococcus faecalis*.

Chemical investigations of *Ipomoea* glycosides reported in the literature (Smith, *et al.*, 1997; Khanna and Gupta 1967; Kawasaki, *et al.*, 1971) indicate that the glycosidic constituents of *Pharbitis nil* and *I. parasitica*, *I. miricata*, *I. purga*, *I. orizabensis*, *I. operculata* and *I. turpethum* possess mono or dihydroxy $C_{14\text{-}16}$ fatty acids as aglycones with glucose, rhamnose, qunovose and fucose as the sugar components. This communication describes the isolation and structure elucidation of the anti-cancer active glycoside contained in this plant. Ipolearoside, a new glycoside with anticancer activity, has been isolated from *Ipomoea leari* (Sarin, 1973).

Valette and Liber (1938) reported that various plants of the family Convolvulaceae, contain glycosides which have a bactericidal effect. Recently (Smith *et al.*, 1997) it has been noticed that convolvulaceous glycosides bear close resemblance to some of the bacterial metabolites in their general make-up. Some of these bacterial metabolites, i.e. glucoustilagic acid (Lemieux, 1951) possess antibiotic properties. Therefore, it seemed advisable to investigate the seeds of *Ipomoea muricata* (Convolvulaceae) for its active constituents. Misra and Tewari, (1953) reported the

isolation of several constituents from *I. muricata* including muricatin A (4-hydroxy-14-*O*-biglucoside-ethyl stearate) and murication B (14-*O*-biglucoside-γ-stearolactone). Some facts, like the homogeneity of murication A, the interoconversion of murication A and B, the nature of sugars were not elucidated. Muricatin B has a marked physiological activity, (Khanna and Gupta, 1967).

2.9 Nutraceutical properties of *Ipomoea* species

The most serious threat to the survival of humanity is the ever-increasing gap between population growth and food supply. It has been estimated that over 500 million people in the world today are malnourished (F.A.O., 1985). Leaves are reportedly inexpensive, easy to cook and rich in vitamins and minerals (Oke, 1966). The decoction of the entire plant of *Ipomoea* is very efficacious for allaying the dyspnoea of asthmatics (Uphof, 1959). The Lele of *Guinea* reportedly eats the leaves and it is superstitiously considered a good talisman for fecundity (Burkill, 1985) and used for treating anaemic cases by local herbalists in Ghana (Akodam, 1986; Wallace *et al.,* 1998). Only the sweet potato storage roots are traditionally consumed in the United States and the foliage is generally plowed back into the field as an organic fertilizer. In some countries, the fresh or dried foliage is used as an animal feed. The young leaves and petioles are also eaten in several Asian and a few African countries as a leafy or green vegetable. To increase the edible plant portion in ALSS, consumption of greens of sweet potato is promoted.

Ishida *et al.* (2000) compared the nutrient contents of the raw of greens of sweet potato with those of greens from greenhouse bed plants. Protein, ash, fat, total dietary fiber, minerals (Ca, Fe, Na, Mg, and Zn), vitamins (ascorbic acid, carotene, thiamine), oxalic and tannic acids, chymotrypsin and trypsin inhibitors in the greens were analyzed. Some parts of this plant, which are not usually used, were found to be rich in nutritive and functional components. In particular, leaves contain a large amount of protein, showing high amino acid score. Any parts of sweet potatoes was rich in dietary fiber and in particular, leaves were soluble dietary fiber. Mineral content, particularly iron, and vitamin content such as carotene, vitamin B2, vitamin C and vitamin E were high in leaves in comparison with other vegetable parts.

Revaluation of the crops, which are tolerant to environmental changes such as drought, storms and floods, and which can be cultivated in waste land and tropical areas, is necessary to overcome the unbalanced diet and shortage of food production (Ishida *et al.,* 2000). The leaves, stalks and stems of sweet potato are suggested to be valuable as sources of DF. The leaves were characterized to contain the highest amount of soluble dietary fiber (SDF) among each part of sweet potato. The amount of SDF in leaves was 6.83% on dry matter basis in KS and 5.77% in BA (Ishida *et al.,* 2000). *Ipomoea* sp. has been found to be active against some microorganisms, including *Staphylococcus aureus, Escherichia coli,* and *Bacillus megaterium.*

Potatoes are members of the Solanaceae family, which also includes tomatoes, red peppers, and eggplants, while sweet potatoes belong to the Convolvulaceae Family. As we enter the new millennium, roots and tubers will play an increasingly important role in meeting the food requirements, feed uses, and income needs of the world's food system. A good example of the use of crop diversity to combat hunger and malnutrition is the story of sweet potato introduction in Africa, where it was suggested in response to widespread vitamin A deficiency that results in blindness and even death for 250,000–500,000 African children a year. This root is a good source of carbohydrates, an excellent source of vitamin A (in the form of beta-carotene), a very good source of vitamin C and manganese, and a good source of copper, dietary fiber, vitamin B6, potassium and iron (Cardenas, *et al.,*1993).

Sweet potato cultivars contain 0.49% to 2.24% crude protein on a fresh weight basis (Purcell, *et al.,* 1972). With a chemical score of 82, the sweet potato protein (SPP) is of acceptable nutritive value (FAO 1990). Globally, China is the largest sweet potato producer with an annual yield of approx 120 million tons, which accounted for 80% of the worldwide sweet potato production. In China, sweet potatoes are mainly used for the production of starch and other starchy foods; this activity has generated a huge volume of wastewater effluent. Our preliminary study showed that the effluent contains approx 1.5% crude protein and attempts have been made to recover the protein from the wastewater effluent (Cheng *et al.*, 2004; Jaw *et al.*, 2007). Currently there is no information about the functional properties of SPP. In the present study, a SSP was purified from the high-protein type sweet potato variety 55-2 available in

China. The amino acid composition as well as solubility and emulsifying properties of the SPP as influenced by salts and pH were analyzed.

Proximate composition of the sweet potato protein (SPP) was determined. The SSP was composed of 87.0% protein, 0.6% fat, 0.16% crude fiber, 2.19% ash and 1.56% sugar .The moisture, protein, fat and crude fiber contents of the SPP were determined according to the A.O.A.C. (1990).

A diet rich in fruits, vegetables and minimally refined cereals is associated with lower incidence of illnesses such as coronary heart disease, some forms of cancer and neurodegenerative ailments (Dragsted *et al.,* 2006; WHO/FAO, 2003). Plants produce a wide range of redox-active secondary metabolites (i.e. antioxidants) such as ascorbic acid, carotenoids, polyphenols and enzymes with antioxidant activity, which protect the cells from oxidative damage. Free radicals are constantly generated in the body as a result of oxidative metabolism. Certain diseases, smoking, environmental poisons, alcohol and ionizing radiation promote the generation of free radicals. Oxidative stress occurs if the antioxidant defence in the organism is not adequate (Blomhoff *et al.,* 2006). Various studies have demonstrated a close link between oxidative stress and development of different ailments, such as inflammatory conditions, cancer, AIDS, gastric ulcer, hypertension and neurodegenerative ailments (Halvorsen *et al.*, 2002; Hegde *et al.*, 2005; Papaharalambus & Griendling, 2007).

The human body has an antioxidant defence system, and it has been assumed that a diet rich in antioxidants strengthens this system.It has been postulated that a network of antioxidants with different chemical properties may work in a synergistic way, protecting the cells from damage (Blomhoff *et al.*, 2006). Fruits, including berries and nuts, some seeds, vegetables, and some beverages (coffee, tea, red wine and fruit juices) are good sources of antioxidants. Bioavailability and absorption of different kinds of antioxidants in the human body are still poorly understood. While some antioxidants are easily absorbed and their manner of action is fairly well known (e.g.vitamin C), recent research indicates that polyphenols do not work in vivo as antioxidants in the conventional way (Stevenson & Hurst, 2007) but instead, provide substantial protection against oxidative stress by inducing cellular endogenous enzymic protective mechanisms. They appear to be able to regulate antioxidant

enzyme gene transcription and numerous aspects of intercellular signalling cascades involved in the regulation of cell growth, inflammation and many other processes. β-Carotene (provitamin A carotenoids) is available in green leafy and yellow–orange vegetables and fruits. In a study of six cultivars of Indian mangoes and two cultivars of papaya, it was found that the content and bioaccessibility of β- carotene varied among cultivars and that mango gave amounts of β-carotene three times higher than those found in papaya (Veda, *et al.*, 2007). In India, these two fruits are frequently used in milkshakes, which were shown to increase bioaccessibility of b-carotene. Vitamin A insufficiency is one of the main micronutrient deficiencies in developing countries (WHO/ FAO; 2003). Since the various groups of antioxidants function differently, the values of total AA are not directly comparable. Several traditional dietary plants that can decrease mineral and vitamin deficiency are also high in antioxidants (green leafy vegetables, mangoes, papaya, sweet potato, groundnuts, sesame seeds and finger millet).

Starch is the major caloric source in a variety of diets of people worldwide. Thus, starches from various plant species, especially cereals, have received very extensive attention in food research. Starch is also an important ingredient in various food systems as a thickener, gelling agent, and binder. Corn, potato, and cassava are the most common sources of starch for such industries (Tester & Karkalas, 2002). Root and tuber crops are grown worldwide and usually have low commercial value for direct consumption. Even though the information available on such underutilized crops is sparse, it has been proved that the starch of such crops would be a good source for different food industries (Alves *et al.*, 1999; Amani *et al.*, 2005; Brunnschweiler, *et al.*, 2005; Moorthy, *et al.*, 1993).

The total protein, fat and carbohydrate contents of the spent cumin were estimated. Protein was found to be 19% and fat 10%. They were high compared with other cereals, e.g. ragi (7.3% protein, 1.3% fat), wheat flour (11.8% protein, 1.7% fat), milled rice (6.8% protein, 0.5% fat) and jowar (10.4% protein, 1.9% fat) (Gopalan *et al.*, 1989).Spent cumin is a rich source of iron and zinc. These values are also high compared to other cereals, e.g. ragi (3.9 mg iron, 2.3 mg zinc), wheat flour (4.9 mg

iron, 2.7 mg zinc), milled rice (0.7 mg iron, 1.4 mg zinc), jowar (4.1 mg iron, 1.6 mg zinc) per 100 g.Having the above composition, the spent cumin may have a high potential as a basic ingredient for various food formulations.Total dietary fibre in spent cumin is very high insoluble fibre 60% and soluble fibre 5.5%. Insoluble fibre is high compared with other commonly consumed cereals e.g. ragi (9.9% insoluble fibre, 1.6% soluble fibre), wheat flour (9.6% insoluble fibre, 2.9% soluble fibre), rice (3.2% insoluble fibre, 0.9% soluble fibre) and jowar (8.0% insoluble fibre, 1.7% soluble fibre). Riboflavin, niacin and thiamine were estimated in the spent cumin. . Vitamins are organic substances present in small amounts in many foods. They are required for carrying out many vital functions of the body. Spent cumin has significant amounts of all vitamins compare with other cereals, e.g. bajra (0.25 mg riboflavin, 2.3 mg niacin), ragi (0.19 mg riboflavin, 1.1 mg niacin), wheat flour (0.17 mg riboflavin, 5.5 mg niacin), milled rice (0.06 mg riboflavin, 1.9 mg niacin), jowar (0.13 mg riboflavin, 3.1 mg niacin), per 100 g.Some part of the daily requirement of niacin, thiamine and riboflavin can be met with the spent cumin. The antinutritional factors, such as tannin, oxalic acid and phytate, are present in low quantity, thus making it a good candidate as a bionutrient. Phytate, a known potent inhibitor of iron absorption, binds with iron and zinc and forms insoluble complexes, rendering the minerals unavailable. Dephytinization can therefore be one of the tools for improving the bioavailability of iron.

In order to increase the iron bioavailability, the spent cumin was treated with the enzyme phytase in different ratios (1:2000, 1:1000, and 1:50) in order to degrade phyticacid. There was a gradual increase in bioavailability of iron and zinc with increase in enzyme concentration, with an optimum enzyme substrate ratio of 1:1000. There was 1.1-fold increase in bioavailable iron and 1.06-fold increase in bioavailable zinc. After optimizing the phytase concentration, the spent cumin was treated with different concentrations of citric acid (2 mM, 5 mM, and 20 mM),in combination with the phytase, to further enhance mineral absorption. The results showed 1.4–1.5-foldincrease in bioavailable iron and zinc in the presence of a 2–20 mM concentration of citric acid, however, further increase in the concentration of citric acid had no beneficial effect on dialyzability of iron and zinc.

2.10 Novel bioactive phytocompounds against multi drug resistant microbes:

Long before the discovery of the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, humans have used plants to treat common infectious diseases, and some of these traditional medicines are still included as part of the habitual treatment of various maladies. For example, the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*), and tea tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (Wild, 1994; Perrett *et al.*, 1995). That being said, it has generally been the essential oils of these plants rather than their extracts that have had the greatest use in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal, and biliary systems, as well as on the skin. In the case of *Melaleuca alternifolia*, for example, the use of the essential oil (tee tree oil) is a common therapeutic tool to treat acne and other infectious troubles of the skin.

Antimicrobial resistance is one of the biggest challenges facing global public health. Although antimicrobial drugs have saved many lives and eased the suffering of many millions, poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil conflicts and bad governance in developing countries have tremendously limited the benefits of these drugs in controlling infectious diseases. The development of resistance in the responsible pathogens has worsened the situation, often with very limited resources to investigate and provide reliable susceptibility data on which rational treatments can be based as well as the means to optimize the use of antimicrobial agents. The emergence of multi drug-resistant isolates in tuberculosis, acute respiratory infections, and diarrhea, often referred to as the diseases of poverty, has had its greatest toll in developing countries. The epidemic of HIV/AIDS, with over 30 million cases in developing countries, has greatly enlarged the population of immunocompromised patients. The disease has left these patients at great risk of numerous infections and even greater risk of acquiring highly resistant organisms during long periods of hospitalization.

Antibiotic resistance can occur via three general mechanisms: prevention of interaction of the drug with target, efflux of the antibiotic from the cell, and direct destruction or modification of the compound. The emergence of multi drug resistance in human and animal pathogenic bacteria as well as undesirable side-effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin.

Ahmad and Beg (2001) tested alcoholic extracts of 45 traditionally used Indian medicinal plants against drug-resistant bacteria and fungi (*C. albicans*) both related to the critical prognosis and treatment of infectious diseases in immunocompromised, AIDS and cancer patients. Of these, 40 plant extracts showed varied levels of antimicrobial activity against one or more test bacteria. Anticandidal activity was detected in 24 plant extracts. Overall, broad-spectrum antimicrobial activity was observed in 12 plants (*L. inermis, Eucalyptus* sp., H. *antidysentrica, H. indicus, C. equistifolia. T. belerica, T. chebula, E. officinalis, C. sinensis, S. aromaticum* and *P. granatum*). Several other studies have also demonstrated the importance of new bioactive phytocompounds against multidrug-resistant bacteria/fungi (Mendonca-Filho, 2006).

A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies. It would be advantageous to standardize methods of extraction and in vitro testing so that the search could be more systematic and interpretation of results facilitated. Also, alternative mechanisms of infection prevention and treatment should be included in initial activity screenings. Disruption of adhesion is one example of an anti-infection activity not commonly screened currently. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles.

2.11 Mode of action of bioactive phytocompounds

The mode of action of antimicrobial agents depends on the type of microorganism under consideration and is mainly related to their cell wall structure and the outer membrane arrangement. Gram-negative bacteria (*e.g. Pseudomonas aeruginosa*) display an intrinsic resistance to a wide variety of essential oils, which is associated with the hydrophilic surface of their outer membrane, rich in lipopolysaccharide molecules. A permeability barrier against toxic agents is formed. Small hydrophilic molecules are not prevented from passing through the outer membrane because of the action of abundant porin proteins. However, hydrophobic macromolecules, such as essential oils constituents, are unable to penetrate the barrier.

It has been proved that the effectiveness of the antibacterial agent generally increases with its lipophilic properties as a result of the action on cytomembranes. On the other hand, essential oils usually express low aqueous solubility, which prevents them from reaching a toxic level in cytomembranes, even if the oils have quite good affinity with the membranes. Some oil components of phenolic nature (e.g. carvacrol and thymol) cause a disruption of the lipopolysaccharide outer layer followed by partial disintegration of the outer membrane (Mendonca-Filho, 2006).

The mechanism of action of other bioactive phytocompounds and essential oils towards microorganisms is complex and has not yet been fully explained. .

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Glassware and chemicals

Good quality glassware and chemicals were used for all tests. All the glassware were of brand Borosil or Corning. They were washed with good detergent, rinsed in tap water and soaked in chromic acid clearing solution.

3.2 Clearing solution (Mahadevan & Sridhar, 1996)

Potassium dichromate	- 60 g
Conc $H_2 SO_4$	- 60 ml
Dist. water	- 1 L

Potassium dichromate was dissolved in warm water, cooled and sulphuric acid was added slowly. It was mixed thoroughly and used for cleaning glassware. Then, they were rinsed thrice in tap water, finally rinsed in distilled water and dried in hot air oven. Dried glassware and media were sterilized in an autoclave for 15 min at 15 lb/sq inch pressure. These sterilization and cleaning methods were used for further experiments.

3.3 Chemicals

Analytical grade chemicals supplied by Loba, Hi-Media, S.D. Fine Chemicals, E. Merck, Qualigens and Sigma Chemicals (U.S.A) were used in this study.

3.4 Plant collection and identification

The plant specimens were collected at flowering stage and identified as *Ipomoea mauritiana*. (Fig. 2.1) with the help of Flora of Madras Presidency (Gamble, 1967) and the flora of the Tamiladu and Carnatic (Matthew, 1991). The plant *I. mauritiana* was collected from Atakatti near Kerala, India and authenticated by Prof. N. Raaman, Director, Centre for Herbal Sciences, Centre for Advanced Studies in botany, University of Madras, and Chennai, India. When the plants were at flowering stage, the floral characters were used for identification of the plant with the help of floras (Gamble, 1967). These organs were photographed in closer views with the help of Nikon digital camera. The plant tuber part was collected, after a thorough investigation to check for any pathological disorders and from contamination of other plants, washed with distilled water and shade dried.

3.5 Preparation of extracts

A total of 5 kg of dry powder of the plant aerial parts was separated into 2 kg and 3 kg. The 2 kg powder was soaked at room temperature in methanol for 48 h. The extract was suction filtered using Whatmann filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and concentrated at 40°C to 45°C under reduced pressure using vacuum rotary evaporator type 350. The remaining 3 kg of plant powder was soaked separately at room temperature in hexane. The residual plant material was extracted successively by fractionized with increasing polarity chloroform and methanol in the same manner as followed for hexane (Harborne, 1998; Raaman, 2006). The concentrated chloroform, hexane and methanol crude extracts were subjected to column chromatography to isolate the active principles.

3.6 Determination of nutritive values

Since the plant under study is being used as staple food by some people, it was decided to determine the nutrient values of the tuber. The amount of carbohydrate, protein, fat, fibre, some minerals and few vitamins were determined per 100 g of dry powder of the tuber. The protein values were calculated from the nitrogen content and the factor used was 6.25. The fat contents were obtained from total ether extractives. The

carbohydrate content was the difference between 100 and the sum of moisture, protein, fat, fibre, ash contents. The moisture, fibre and fat contents were calculated as per standard procedures given in IS 1990. The food energy was calculated from the content of the proximate principles assuming that proteins, carbohydrates and fats yield 4, 4 and 9 K cals, respectively per gram.

The mineral elements were estimated by standard procedures. Calcium and magnesium were estimated. The elements were estimated with the help of Atomic Absorption Spectrophotometry. Four vitamins were also quantified. The values of Vitamin A, Vitamin B (Thiamine), Vitamin B2 (Riboflavin) and Vitamin C (Ascorbic acid) were estimated. It is usual practice to express Vitamin A value of foodstuff in terms of International Units. In vegetable food, the carotene content is usually given assuming that 0.6 g of carotene is equivalent to one I.U. of Vitamin A. All the values are given per 100 g of edible portion of the tuber.

3.6.1 Determination of crude protein (AOAC, 1990)

Principle

Sample was digested in H_2SO_4, using $CuSO_4$ as catalyst, converting nitrogen to ammonia, which was distilled and titrated.

Apparatus

Kjeldahl flask : 500 - 800 ml capacity

Distillation assembly - The assembly consisted of a round - bottom flask of a 1000 ml capacity fitted with a rubber stopper through one end of the connecting bulb tube. The other end of the bulb tube was connected to the condenser which was attached by means of a rubber tube to a dip tube, which dips into a known quantity of standard sulphuric acid solution contained in a conical flask of 500 ml capacity, to which 3 or 4 drops of methyl red indicator solution was added.

Reagents

1. Sodium sulphate anhydrous
2. Copper sulphate
3. Concentrated sulphuric acid
4. Sodium hydroxide solution - 500 g of sodium hydroxide was dissolved in 1000 ml of water
5. Standard sulphuric acid - 0.5 N
6. Standard sodium hydroxide solution - 0.1 N
7. Methyl red indicator solution - 1 g of methyl red was dissolved in 100 ml of methanol.

Procedure

One gram of prepared sample was transferred to Kjeldahl flask and 7.0 g of sodium sulphate anhydrous, 0.7 g of copper sulphate and 25 ml of sulphuric acid were added. The flask was placed in an inclined position and heated below the boiling point of the acid until the frothing ceased. The heat was increased until the acid boiled vigorously (for 3 h and 30 min). Water (200 ml) was added to Kjeldahl flask and after cooling the contents of the flask, 50% sodium hydroxide solution was added by the side of the flask so that it did not mix at once with the acid solution but forms a layer below the acid layer. The apparatus was assembled taking care to see that the tip of the dip tube extended below the surface of the 20 ml of standard sulphuric acid solution (0.5 N) in the receiver. Three drops of methyl red indicator solution was added to the acid. The contents of the flask were mixed by shaking and distillation was done till all the ammonia had passed over into the standard sulphuric acid solution till the colour in the receiver turned from red to yellow. Blank determination using all reagents in the same quantities but without the material tested was carried out.

Calculation

On an average, most proteins have 16% nitrogen in their composition. In other words, 1 mg of nitrogen equals to 6.25 mg of protein. Based on stoichiometric relationships involved in the titrations, 1 ml of 0.01 N HCl is equivalent to 140 mg of nitrogen as present in ammonia. Thus, from the volume of standard HCl used for titration, the amount of nitrogen in the sample was calculated and this value multiplied by 6.25.

$$\text{Percentage of protein} = \frac{\text{(Blank titre value - sample titre value) x strength of 0.1 N NaOH x 1.4007 x 6.25}}{\text{Weight of sample taken}}$$

where

6.25 = nitrogen factor

1.4007 = equal to protein in lysine Hydrochloric acid.

3.6.2 Determination of crude fat (IS, 1990)

Reagents

Petroleum ether of boiling range 40°C to 60°C

Hexane, food grade confirming to IS: 3470-1966.

Procedure

The prepared sample (5 g) was dried in a hot air oven at 105 ± 2°C for at least 2 h and was extracted with petroleum ether or hexane in a Soxhlet extractor. The extraction was done at a condensation rate of 5 to 6 drops per sec for 4 h and 2 to 3 drops per sec for 16 h. The extract was dried on a steam-bath for 30 min, cooled in a desiccator and

weighed. Alternate drying and weighing were done at 30 min intervals until the difference between two successive weighing was less than 1 mg and the lowest mass was noted.

Calculation

$$\text{Percentage of crude fat (on moisture free basis)} = \frac{100 \text{ X } (M1 - M2)}{M}$$

where

M1 = Mass in g of the extraction flask with dried extract

M2 = Mass in g of the extraction flask

M = Mass in g of the dried sample taken for the test.

3.6.3 Determination of the crude fibre (IS, 1990)

Reagents

Sulphuric acid - 0.255 N (1.25% v/v), accurately prepared

Sodium hydroxide solution 0.313 N (1.25% m/v), accurately prepared.

Procedure

The dried material (2 g) was extracted for fat content with petroleum ether or hexane, using Soxhlet extractor (alternatively, the residue from the crude fat determination can be used). The fat free dry residue was transferred to 1 L conical flask. Boiling dilute sulphuric acid (200 ml) was added to the flask with the fat free material and immediately the flask was connected with a reflux condenser and heated for 30 min. The

contents were filtered through fine linen held in a funnel. The residue was washed with boiling water. The residue on the linen was washed into 200 ml of boiling sodium hydroxide solution. Immediately the flask was connected with the reflux condenser and boiled for 30 min. The solution was filtered, washed with boiling water and transferred to a Gooch crucible prepared with a thin but compact layer of ignited asbestos. The residue was washed first with hot water and then with 15 ml of 95% (by volume) ethyl alcohol. The Gooch crucible and the contents were dried at 105°C in the hot air oven to constant mass. It was cooled and weighed. The contents of the Gooch crucible were incinerated at 600°C in a muffle furnace until all the carbonaceous matter was burnt. The crucible containing the ash was cooled in a desiccator and weighed.

Calculation

$$\text{Percentage of crude fat (on moisture free basis)} = \frac{100 \times (M1 - M2)}{M}$$

Where

M1 = Mass in g of Gooch crucible and contents before ashing

M2 = Mass in g of Gooch crucible containing asbestos and ash

M = Mass in g of the dried sample taken for the test.

3.7 Estimation of elements

Procedure

Analyses of minerals and trace elements of the tubers were done using Atomic Absorption Spectrophotometer (AAS). Quantification of elements such as calcium, iron, magnesium, copper, manganese, zinc were done.

Preparation of material for AAS study

The plant sample was taken after drying at 80°C for 12 h. It was ground finely and 1 g was taken in a 100 ml beaker and 5 ml of concentrated nitric acid and 2 ml of perchloric acid were added to the sample. It was covered with a watch glass and digested with heating to obtain a final volume of 3 - 5 ml. Evaporating the solution to dryness can cause loss of more volatile elements such as As, Se etc. Then, 10 ml of water was added to the beaker and the digested solution was filtered through an acid washed filter paper into a 50 ml volumetric flask. The filter paper was washed with water and the filtrate was made up to required volume with de-ionized water.

3.7.1 Estimation of Vitamin A (Indian Pharmacopoeia, 1996)

To 3 g of the sample, 5 ml of 50 % (w/v) potassium hydroxide solution and 50 ml of alcohol were added and refluxed in a water condenser for 1 h. The solution was cooled and transferred to a 500 ml separator, to which 50°ml of hexane was added and shaken vigorously for 5 min. When the layers separate, the organic layer was passed through sodium sulphate into a 200 ml volumetric flask. The aqueous layer was shaken 3 times with 30 ml of hexane for each time. All the organic layers were pooled together and diluted to 200 ml with the required hexane. The absorbance in UV spectrophotometer at 325 nm was taken. Using the factor 1830, the vitamin A content was calculated. The vitamin A is expressed as International Units, which refer to their biological potency. One IU is defined as the activity given by 0.03 g of vitamin A alcohol and 0.344 vitamin A acetate.

Calculation

$$\text{Vitamin A (IU)/100 g sample} = \frac{\text{Sample absorbance} \times 200 \times 1830 \times 100}{\text{Wt of sample taken} \times 100}$$

Conversion of vitamin A to carotene

Since one IU of vitamin A is equivalent to 0.6 g of carotene, the value of vitamin A can be converted as follows.

Vitamin A in I.U x 0.6 = µg of carotene

3.7.2 Estimation of vitamin C (Indian pharmacopoeia, 1996)

Preparation of meta phosphoric - acetic acid solution (MPAA)

To 15 g of meta phosphoric acid, 40 ml of glacial acetic acid was added and diluted to 100 ml with water.

Preparation of 2, 6 - dichlorophenol indophenol solution

2, 6 - dichlorophenol indophenol salt, 0.05 g was diluted to 100 ml with water and the solution was filtered.

Preparation of standard solution

Stock solution

To 0.05 g of L-ascorbic acid standard, 20 ml of MPAA solution was added and diluted to 250 ml with water.

Preparation of sample solution

To 10 g of sample powder, 20 ml of MPAA solution was added and diluted to 500 ml with water. The solution was filtered.

Procedure

To 10 ml of standard stock solution, 5 ml of MPAA solution was added and titrated against 2, 6-dichlorophenol indophenol solution till pink color persisted for 10 sec. The titration was completed within 2 min. The titre value was noted. Sample solution (100 ml) was taken and the same procedure was repeated.

Calculation

$$\text{Ascorbic acid (mg)/100 g of sample} = \frac{\text{SAV x STV x 10 x 500 x 1 x STP}}{\text{STV x 250 x 1 x SAW x 100 x 100}} \times 100$$

Where

SAV = Sample titre value

STV = Standard titre value

STW = Standard weight

SAW = Sample weight

STP = Standard purity

3.7.3 Estimation of Vitamin B1 (Indian Pharmacopoeia, 1996)

Preparation of buffer solution

To 6.8 g of potassium di-hydrogen phosphate, 8 ml of 1 M sodium hydroxide solution was added and diluted to 1000 ml with water.

Preparation of dye solution

Bromothymol blue, 0.06 g was dissolved in 100 ml of chloroform.

Preparation of standard solution

Stock solution

Thiamine hydrochloride RS (100 mg) was dissolved in 100 ml of water.

Working standard solution

One ml of stock was diluted with 100 ml of buffer preparation of sample solution. To 10 g of sample powder, 100 ml of buffer was added and filtered.

Procedure

Sample solution 10 ml and 10 ml of working standard solution were taken in two different dry separating funnel. Ten ml of chloroform and 10 ml of dye solution were added to both the solutions and shaken for 2 min continuously. Then they were allowed to stand for 5 min with occasional shaking. The chloroform layer was collected by passing it through sodium sulphate anhydrous. The readings were taken at 420 nm using Shimadzu UV118 Spectrophotometer. The chloroform was used as blank.

Calculation

$$\text{Ascorbic acid (mg)/100 of sample} = \frac{\text{SAA x STW x 1 x 10 x 500 x 1 x STP x 100}}{\text{STA x 100 x 100 x 1 x SAW x 10 x 100}} \times 1000$$

where

SAA = Sample absorbance

STA = Standard absorbance

SAW = Sample weight

STP = Standard purity.

Determination of thiamine

$$\text{Thiamine (mg) / 100 g of sample} = \frac{\text{Thiamine HCl (mg)/100 g of sample x molecular weight of thiamine (300.77)}}{\text{Molecular weight of HCl (337.2)}}$$

3.7.4 Estimation of Vitamin B2 (Indian Pharmacopoeia, 1996)

To 5 g of sample powder, 150 ml of water and 5 ml of glacial acetic acid were added. The solution was boiled for 5 min and cooled. To this, 30 ml of 1.0 M sodium hydroxide solution was added and diluted to 500 ml with water. The solution was filtered and absorbance was measured at 444 nm in Shimadzu UV -1201 Spectrophotometer. Water was used as blank.

Calculation

$$\text{Riboflavin (mg)/100 g of sample} = \frac{\text{SA x 500 x 1 x 100 x 1000}}{\text{328 x SW x 100}}$$

where

328 - Molar extension coefficient

SA - Sample absorbance

SW - Sample weight

3.8 Qualitative phytochemical screening

The different qualitative chemical tests were performed for establishing the profile of given extract for its chemical composition. The following tests were performed on the extracts to detect various phytoconstituents present in them.

3.8.1 Detection of alkaloids (Evans, 1997)

Solvent free extract (50 mg) was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows:

A. Mayer's test (Evans, 1997)

To a few ml of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

Mayer's Reagent

Mercuric chloride (1.358 g) was dissolved in 60 ml of water and potassium chloride (5.0 g) was dissolved in 10 ml of water. The two solutions were mixed and made up to 100 ml with water.

B. Wagner's test (Wagner, 1993)

To a few ml of filtrate, few drop's of Wagner's reagent was added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

Wagner's reagent

Iodine (1.27 g) and potassium iodide (2 g) were dissolved in 5 ml of water and made up to 100 ml with dist water.

C. Hager's test (Wagner *et al.*, 1996)

To a few ml of the filtrate, 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

D. Dragendorff's test (Waldi, 1965)

To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added. A prominent yellow precipitate indicated the test as positive.

Dragendorff's reagent

Stock solution

Bismuth carbonate (5.2 g) and sodium iodide (4 g) were boiled for a few min with 50 ml glacial acetic acid. After 12 h, the precipitated sodium acetate crystals were filtered off using a sintered glass funnel. Clear, red-brown filtrate, 40 ml was mixed with 160 ml of ethyl acetate and 1 ml of water and stored in amber-coloured bottle.

Working solution

Ten ml of stock solution was mixed with 20 ml of acetic acid and made up to 100 ml with water.

3.8.2 Detection of carbohydrates (Ramakrishnan *et al.*, 1994)

The extract (100 mg) was dissolved in 5 ml of water and filtered. The filtrate was subjected to the following tests:

E. Molish's test

To 2 ml of filtrate, two drops of alcoholic solution of α-naphthol were added, the mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

F. Fehling's test

One ml of filtrate was boiled on water bath with 1 ml each of Fehling solutions I and II. A red precipitate indicated the presence of sugar.

Fehling's solution

Fehling's solution I: Copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 ml with dist water.

Fehling's solution II: Potassium sodium tartarate (173 g) and sodium hydroxide (50 g) was dissolved in water and made up to 500 ml

G. Barfoed's test

To one ml of filtrate, 1 ml of reagent was added and heated on a boiling water bath for 2 min. Red precipitate indicated the presence of sugar.

Barfoed's reagent

Copper acetate, 30.5 g was dissolved in 1.8 ml of glacial acetic acid.

3.8.3 Detection of coumarins

H. Benedict's test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic coloured precipitate indicated the presence of sugar.

Benedict's reagent

Sodium citrate (173 g) and sodium carbonate (100 g) were dissolved in 800 ml of distilled water and boiled to make it clear. Copper sulphate (17.3 g) dissolved in 100 ml dist water was added to it.

3.8.4 Detection of glycosides

50 mg of extract was hydrolysed with concentrated hydrochloric acid for 2 h on a water bath, filtered and the hydrolysate was subjected to the following tests.

I. Borntrager's test (Evans, 1997)

To 2 ml of filtrate hydrolysate, 3 ml of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

J. Legal's test

Fifty mg of the extract was dissolved in pyridine, sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide. Presence of glycoside was indicated by pink colour.

3.8.5 Detection of saponins

K. Foam test (Kokate, 1999)

The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A two cm layer of foam indicated the presence of saponins.

3.8.6 Detection of proteins and amino acids (Fisher, 1968; Ruthmann, 1970)

The extract (100 mg) was dissolved in 10 ml of dist water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to tests of proteins and amino acids.

L. Millon's test (Rasch and Swift, 1960)

To 2 ml of filtrate, few drops of Millon's reagent was added. A white precipitate indicated the presence of proteins.

Millon's reagent

Mercury (1 g) was dissolved in 9 ml of fuming nitric acid. When the reaction was completed, equal volume of dist water was added.

M. Biuret test (Gahan, 1984)

An aliquot of 2 ml of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink colour in the ethanolic layer indicated the presence of proteins.

N. Ninhydrin test (Yasuma and Ichikawa, 1953)

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to two ml of aqueous filtrate. A characteristic purple colour indicated the presence of amino acids.

3.8.7 Detection of phytosterols (Finar, 1986)

O. Libermann-Burchard's test

The extract (50 mg) was dissolved in 2 ml of acetic anhydride. To this, one or two drops of concentrated H_2SO_4 was added slowly along the sides of test tube. An array of colour changes showed the presence of phytosterols.

3.8.8 Detection of fixed oils and fats (Kokate, 1999)

P. Spot test

A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.

Q. Saponification test

A few drops of 0.5 N alcoholic potassium hydroxide solution was added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 2 h. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

3.8.9 Detection of phenolic compounds

R. Ferric chloride test (Mace, 1963)

The extract (50 mg) was dissolved in 5 ml of dist water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

S. Gelatin test (Evans, 1997)

The extract (50 mg) was dissolved in 5 ml of dist water and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. White precipitate indicated the presence of phenolic compounds.

T. Lead acetate test

The extract (50 mg) was dissolved in dist water and to this, 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

3.8.10 Detection of flavonoids

U. Alkaline reagent test

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavanoids.

V. Magnesium and hydrochloric acid reduction (Harbone, 1998)

The extract (50 mg) was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) were added. The presence of flavanol and glycosides were inferred by the development of pink to crimson colour.

3.8.11 Detection of gums and mucilages (Whistler and Miller, 1993)

The extract (100 mg) was dissolved in 10 ml of dist water and to this, 25 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilages.

3.9 Thin layer chromatography

Principle

Pre-coated silica gel thin layer chromatogram sheet (E. Merck) was used for TLC. The crude extracts and isolated compounds were spotted at 2 cm from the edge of the sheet. The chromatogram was developed in a mixture of suitable solvent system and dried at room temperature. The spots were visualized with UV light at 254 and 35 nm. The dried TLC plates were then sprayed with 10% H_2SO_4 and heated at 110°C for 5 min (Waldi, 1965). Alternatively, the developed TLC plates were placed in iodine chamber (Harborne, 1998; Raaman, 2006). The R_f values of the coloured spots were recorded.

$$\text{Relative fronts} = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

RESULTS & DISCUSSION

4. RESULTS AND DISCUSSION

The major challenges in investigating the giant potato are

(1) The unknown content of the nutritional values, which urges the completeness of extraction.

(2) Screening of bioactive phytoconstituents for new potent drug discovery from medicinal plant sources

4.1 PREPARATION OF PLANT POWDER EXTRACT

The root tubers collected from the Western coastal areas were chopped and shade dried. It was then grounded to a form in between coarse and fine. The plant powder of 1kg was soaked in organic solvents of 5L with increasing polarity. The powdered samples were extracted by cold percolation method using fractionized solvents like hexane, chloroform and methanol. The powder sample was soaked for about 72 hours in the first phase and for 24 hours in the second and third phase at room temperature and regular agitation was given. The crude extract was filtered using Whatmann No.1 filter paper and then condensed in the Rotary vaccum flash evaporator. Finally the condensed solvent free crude extracts were obtained for hexane, chloroform and methanol.

Fig. 4.1 Hexane extract from giant potato

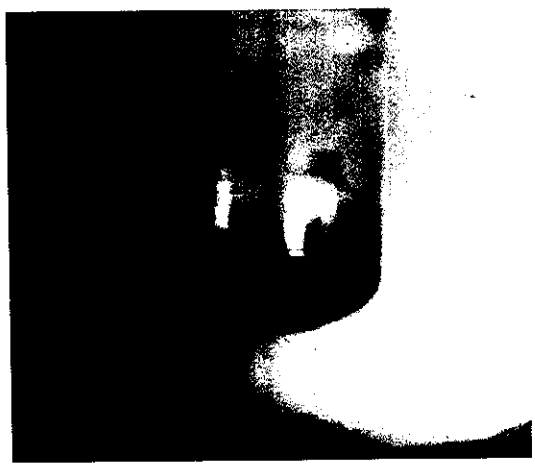
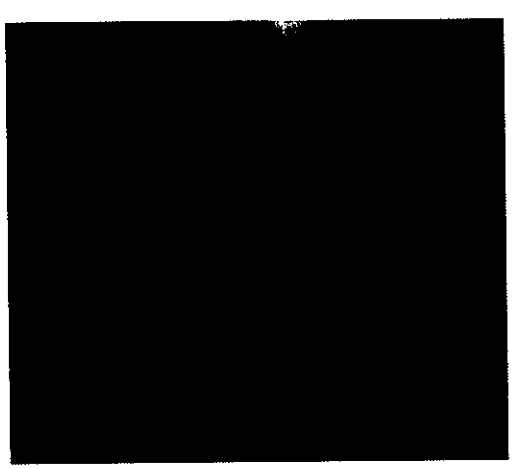


Fig. 4.2 Chloroform extract from giant potato

Fig. 4.3 Methanol extract from giant potato



Separate plant powder sample of 250g was taken for water extraction and extract for the sample was obtained by soaking fresh plant powder sample in distilled water and kept in a water bath at temperatures of 55°C and 85°C respectively for about 30 minutes. After which the water extract was filtered and lyophilized to a powder form. The lyophilized powder was then used for further analysis.

These crude extracts of hexane, chloroform, methanol and water were obtained for the reason that they would contain flavonoids, saponins, oils, fats, fibres and many other components. The tests later on with these crude samples proved to be positive through nutraceutical and qualitative phytochemical analysis.

4.2 NUTRACEUTICAL VALUE ANALYSIS

4.2.1 Proximate Analysis

The physical appearance of the plant powder was found to be pale brown in colour and coarse to touch. The sample showed the presence of crude fibre content of about 1.5%, crude protein content of 5.27%, reducing sugar content of about 35.45% and the total ash content was found to be 5.73% as shown in table 4.1.

Table 4.1 Proximate Analysis of the components present in the extracts of giant potato.

S.No	Components	Test results
1.	Crude fibre	1.5%
2.	Crude protein	5.27%
3.	Total fat	0.58%
4.	Reducing sugar	35.45%
5.	Total ash	5.73%
6.	Acid value	0.67%
7.	Saponification value	200.41
8.	Iodine value	2.581

9.	ph of 1% w/v suspension	6.15
10.	Loss on drying at 105° c	10.68%
11.	Carbohydrate	76.24%
12.	Vit.A, Vit.C, Vit.B1, & Vit.B2	Below detectable level

The total acid value was found to be 0.67 and the total fat content neared to the value of 0.58% and the iodine value of the plant sample reached to about 2.581 as in table 4.1. The saponification value of the sample was found to be 200.41

The pH of 1% w/v suspension was tested as 6.15. The loss on drying at 105° C is 10.68% and the calculated value of carbohydrate was found to be 76.24%. Having the above compositions the plant may be used for edible purposes as a basic ingredient.

Since the proximate analysis of the sample shows the content of rich fibre and protein the sample contain rich nutrients. Proteins which are said to be the body builders are very essential to our body. Those proteins are rich in the plant samples of *Ipomoea mauritiana.*

The saponin content of the plant as shown in table 4.1 may contribute significantly to the role played by tubers as anti-oxidant, anti-diabetic and anti-viral food.

4.2.2 TRACE ELEMENTS

Since the results showed certain contents of sugar, ash, and fibre, the research was further extended to find the presence of trace elements. It was found that the iron content was about 38.03 parts per million. Hence it proved to be a major source of iron. Then found to contain sodium of about 0.063%, potassium content to about 0.31%.

Table 4.2 Trace Elements present in the extracts of giant potato

S.No	Trace Elements	Test results for 100g/ dry weight
1.	Iron	38.03ppm
2.	Sodium	0.06%
3.	Potassium	0.31%
4.	Calcium	0.91%
5.	Phosphorous	0.49%
6.	Magnesium	0.27%
7.	Zinc	0.01%

ppm - parts per million

The calcium content was found to be in a major concentration. This enables for the support of bones and teeth, however the amount was quite considerable as it was found to be nearly 1% in 100g as shown in table 4.2. The phosphorous content of the sample reported to be 0.49% and the metal ion zinc was found to be 0.014% which proved to be present in minute quantity. These results showed that the trace elements have a greater role in determining the properties of the plant sample as these minerals present in minute quantities are vital for the human body.

4.2.3 CALORIC VALUES

The caloric value for Carbohydrates was 304.96 which was found to be comparatively higher, the fat and protein caloric values was calculated to be 5.22 and 21.08 respectively.

Table 4.3 Caloric values for the tuber of *Ipomoea mauritiana*

S.No	Components	Test results
1.	Carbohydrates	304.96
2.	Fat	5.22
3.	Protein	21.08
4.	**TOTAL CALORIES/ 100 g**	**331.26**

The total caloric content per 100 g was 331.26. Vitamins are organic substances present in small amounts in many foods. They are required for carrying out many vital functions of the body. The four vitamins tested to be below detectable level. Having the above caloric values this plant may have a high potential as an ingredient for various food formulations

4.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

When considering the qualitative phytochemical analysis, table 4.4 the alkaloids shows a greater presence in all the four types of extracts as shown in table 4.4. This shows that the plant is a rich source of alkaloid which is a medicianally important compound.

Table 4.4 Qualitative phytochemical screening of extracts from *Ipomoea mauritiana*.

S. No	Phytochemical Test	Hexane	Chloroform	Methanol	Water
1.	Alkaloids	++	++	++	++
2.	Carbohydrates	+++	+++	+++	++
3.	Coumarins	++	++	++	++
4.	Glycosides	–	–	++	++
5.	Saponins	–	+	+	+
6.	Proteins and amino acids	++	+	+	++
7.	Phytosterols	–	–	–	–
8.	Fixed oils and fats	–	–	+	+
9.	Phenolic compounds	+++	+++	+++	+++
10.	Flavonoids	+++	++	++	++
11.	Gums and mucilages	–	–	–	–

- Negative; + positive with low concentration; ++ positive with normal concentration; +++ positive with higher concentration.

The presence of carbohydrates and coumarins was also detected to be good enough in all the four types of extract from table 4.4 which promises that the plant sample is a very good source of carbohydrate. Hence the tuber can also be an edible part of the plant which becomes a greater advantage of the plant.

Oils and fats are present in a lower concentration in table 4.4 which indicates that the plant may be devoid of cholesterol. Hence this plant can be given for cholesterol patients which may not affect their health.

Since the flavonoids and phenolic contents are quite high in concentration as in tabe 4.4, it suggests that the plant may be used for wound healing and anti-inflammation because of its anti-oxidative property. Flavonoids are important in human health because of their high pharmacological activities as radical scavengers. (Hertog, *et al*, 1993).

Our results from table 4.4 showed a positive value of saponins in the foam test which may contribute a significant role in acting as an anti-oxidant, anti-diabetic and anti-viral food.

4.4 THIN LAYER CHROMATOGRAPHY

The greater speed of thin layer chromatography is due to the more compact nature of the adsorbent when spread on a plate and is an advantage when working with labile compounds. The sensitivity of thin layer chromatography is such that separations on less than microgram amounts of material can be achieved. Another reason for still using plates coated in the laboratory is that the moisture content of the silica gel can be controlled for some critical seperations. Nowadays, however, it is usual to employ precoated plates of commercial manufacture in most work, since these are more uniform and provide more reproducible results.

Table 4.5 Thin layer chromatographic profiles of *Ipomoea mauritiana.*

FRACTIONATED HEXANE EXTRACT		
The band separated from the starting point	**Distance traveled by the compounds (cm)**	**R*f* values Solvent front = 17 cm**
1	3.4	0.2
2	6.3	0.37
3	8.8	0.51
4	9.6	0.56
5	12.4	0.73
6	13.3	0.78
7	15.4	0.90

Fig 4.4 Iodine vapour exposed thin layer chromatographic plates

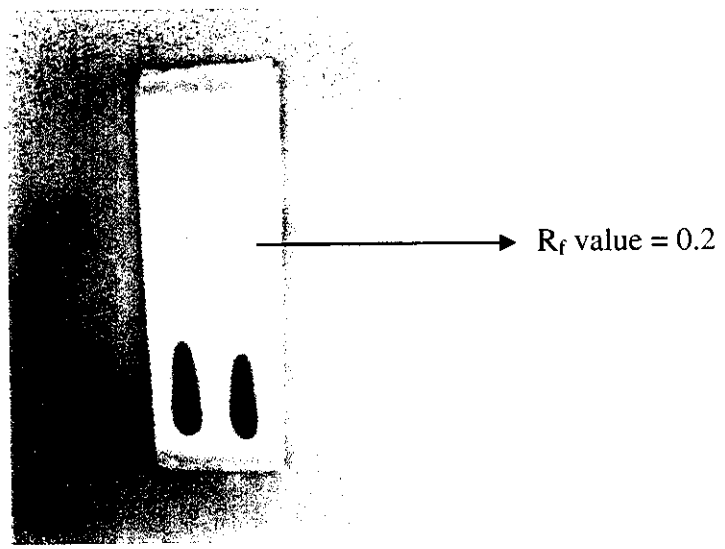
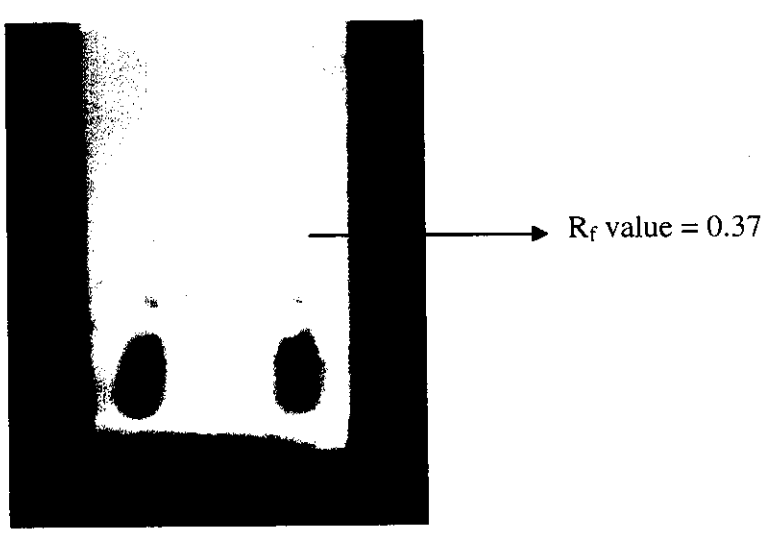


Fig 4.5 Iodine vapour exposed thin layer chromatographic plates



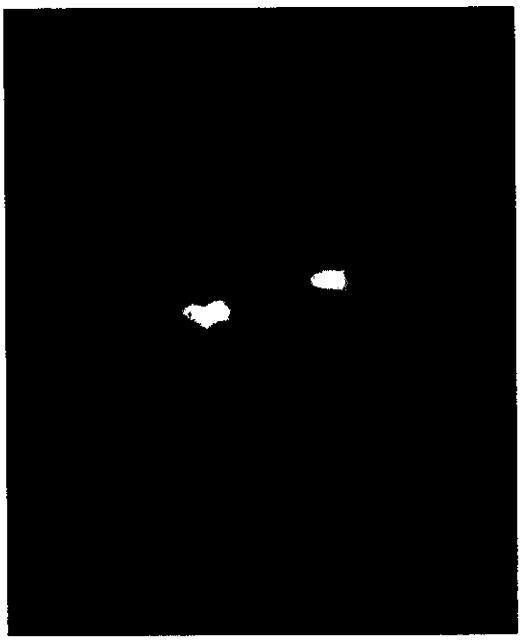


Figure 4.6 Compounds emitting fluorescence under ultra -violet chamber of 356 nm

Thin Layer Chromatography was performed on silica gel plates for the various extracts of Hexane, Chloroform and Methanol. Silica gel was mixed with water in the ratio of 2.1 and coated with the glass plates. Depending on the particle size of the adsorbent calcium sulphate hemihydrates (15%) may have to be added to help bind the adsorbent on to the glass. Plates after spreading have to be air dried and then activated by heating in an oven at 100-110°C for 15 minutes. The samples are then spotted and the plate is kept in the mobile phase. The mobile phase is prepared by mixing the solvents in various ratios. As the mobile phase rises the compounds are separated as bands. Detection of compounds on thin layer chromatographic plates was done by exposing them to iodine vapour. The Rf values are then calculated based upon the distance travelled by the solvent and solute front.

Flavonoids are phenolic and hence change in colour when treated with base thus they are easily detected on chromatograms. The phyto chemical constituents present in the extracts of hexane show intense absorption bands in the ultra-violet and visible regions of the spectrum.

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The tubers of *Ipomoea mauritiana Jacq* species were collected from the western coast of India and identified. They were then dried and powdered. The residual plant material was extracted successively by fractionation with increasing polarity of solvents like hexane, chloroform and methanol. The water extracts was also prepared using fresh plant powder separately.

Further various nutraceutical studies, qualitative phtochemical analysis and the standardization of thin layer chromatography were conducted and the results from the present studies show that giant potato could be a good food supplement. The underutilized giant potato with good nutrient composition can be a good substitute for the conventional food stuffs in health food formulations and can find applications in therapeutic foods. Tubers of *Ipomoea mauritiana* has great potential for producing healthy and functional food products. The gene transfer, micropropagation and the widespread cultivation of these tubers as normal species of potato could happen in the mere future as it has a good caloric content of carbohydrates, proteins, fats and fibres. Medicinally, important alkaloids were also present in the sample. The presence of phenols, flavonoids, proteins and trace elements like calcium was also detected to be good in the extract. Hence the tuber can also be an edible part of the plant which becomes a greater advantage of the plant. Oils and fats are present in a lower concentration which indicates that the plant may be devoid of cholesterol. The flavonoid contents were high in concentration, which suggests that the plant is a rich anti-oxidant source and may be used for wound healing and anti-inflammation purposes. Flavonoids are important in human health because of their high pharmacological activities as radical scavengers.

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