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## Research Article

## Biochemistry

### PHYTOCHEMICAL AND HPLC ANALYSIS OF *Ipomea obscura* LEAVES

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#### ABSTRACT

In the present study was to investigate the phytochemicals and inorganic elements in *Ipomea obscura* leaves. The results of this study clearly indicate that the preliminary phytochemical analysis of *Ipomea obscura* (leaves) revealed of flavonoids, phenolics, tannin, steroids, alkaloids, saponins, anthroquinones and terpenoids present in the all the extracts. Among the three extracts, methanol extract showed high concentrations than other extracts. Quantitative analysis revealed that *Ipomea obscura* leaf rich amount of total Phenol (220.0), alkaloids (42.0), tannin (72.12) saponin (62.0) and flavonoid (150.0) were presented. The inorganic elements of *Ipomea obscura* leaf showed that the presence of calcium, magnesium, sodium, potassium, sulphate, phosphate, chloride and nitrate were presented while iron was absent. HPLC profiles of *Ipomea obscura* were analysed and five phenolic compounds namely gallic acid, caffeic acid, apigenin, luteolin and quercetin having different elution times could be obtained when each compound was analyzed The results of the present study concluded that *Ipomea obscura* may be a good source of phytochemicals, minerals and HPLC. *Ipomea obscura* leaves may be useful for human health associated emerging diseases such as cardiovascular diseases, diabetes, hypertension and cancer.

**Keywords:** *Ipomea obscura*, Phytochemical, Inorganic elements and HPLC

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#### INTRODUCTION

Phytochemicals are a field of increasing attention, both in science and in commerce. As is now generally recognized, many plant compounds and pigments have effects on animals and human beings. There is a great effort now to study and understand at a fundamental level and significant health effects of these compounds. This field is maturing and the health effects of these compounds are now getting the careful study they warrant at both a chemical and a molecular biological level. Identifying bioactive compounds and establishing their health effects are active areas of scientific inquiry. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans (Velavan, 2015; Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant

chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Gibson et al., 1998; Mathai, 2000). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals fruits and herbs and each works differently (Arora et al., 1998). The plant kingdom is a rich source of potential drugs. In India, medicinal plants are widely used by all sections of the population, either directly in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicine. Research on natural resources has been encouraged by the World Health Organization since 1978. Most of the medicinal plants contain a number of chemical constituents such as flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, rotenoids etc. The phytochemical screening of the plants is a preliminary for verification and then these plants may be utilized as new sources of herbal drugs (BNF, 2003).

The mineral elements are separate entities from the other essential nutrients like proteins, fats, carbohydrates, and vitamins. Animal husbandry had demonstrated the need for minerals in the diet. In this century, biological assay methods clarified the significance and importance of mineral elements for human and animal nutrition and modern analytical techniques led to the detection of trace elements as essential nutrients and this is still an active area of current research. Micronutrient deficiencies are a major public health problem in many developing countries, with infants and pregnant women especially at risk (Batra and Seth, 2002). Infants deserve extra concern because they need adequate micronutrients to maintain normal growth and development (Rush, 2000). The micronutrient deficiencies which are of greatest public health significance are iron deficiency, causing varying degrees of impairment in cognitive performance, lowered work capacity, lowered immunity to infections, pregnancy complications e.g. babies with low birth weight, poor learning capacity and reduced psychomotor skills (Batra and Seth, 2002).

Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics and

About 150 phytochemicals have been studied in detail. Keeping in view, in the present study to investigate the phytochemical and inorganic elements in whole plant of *Ipomea obscura* (Tamil: Chirutali). *Ipomea obscura* is considered as stomachic, aperients and antiseptic. Flow and tender shoots are diaphoretic and given in fevers. An infusion of the plant given to promote lochial discharge. Oil in which roots are boiled is used as application for gout and rheumatism. An alcoholic extract of the plant showed antibacterial activity against *Escherichia coli*. The species is a herbal drug administered orally for the control of orchitis in practitioners of the Indian system of medicine. The drug was tested in comparison with synthetic anti-inflammatory drugs. It was found that the plant showed anti-inflammatory activity comparable to that of Indomethacin. It also showed inhibition of certain aspects of the inflammatory process, similar to hydrocortisone (Sadique et al., 2000).

## MATERIALS AND METHODS

### Plant materials:

The fully mature *Ipomea obscura* leaves were collected in January 2015 from Tamil University Campus, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rabinat Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamilnadu, India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

### Preparation of alcoholic extract

The leaf of *Ipomea obscura* was first washed well and dust was removed from the plant. Leaf was washed several times with distilled water to remove the traces of impurities from the plant. The leaf plant was dried at room temperature and coarsely powdered. The powder was extracted with Different extracts (Ethanol, methanol and aqueous) for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

### Preliminary phytochemicals screening

Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Harborne (1973), Trease and Evans (1989) and Sofowara (1993).

### Quantitative analysis of phytochemicals

Determination of total phenols (Edeoga et al.,). Tannin was determined by the method of Van-Burden and Robinson (1981). Saponin was determined by the method of Obdoni and Ochuko (2001). Alkaloid

determine by the method of Harborne (1973) Flavonoid was determined by the method of Bohm and Kocipai-Abyazan (1994).

#### Qualitative analysis of inorganic elements

Ash of drug material (500 mg) was prepared and treated with HNO<sub>3</sub> and HCl (3:1 v/v) for 1hour. After the filtration, the filtrate was used to perform the following tests (Khandelwal 2006).

#### HPLC analysis

Flavonoids were analysed using a HPLC method (Weerasak and Suwana, 2007).

### RESULTS AND DISCUSSION

Phyto is the Greek word for plant. There are many families of phytochemicals and they help the human body in the variety of ways. Phytochemicals may protect human from a host of diseases. Phytochemicals are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Narasinga,2003)

An assessment of the previous trends and impact of research into the phytochemistry on medicinal plants of the world is quite desirable before considering recent trends. After centuries of empirical use of herbal preparation, the first isolation of active principles alkaloids such as morphine, strychnine, quinine etc. in the early 19th century marked a new era in the use of medicinal plants and the beginning of modern medicinal plants research. Emphasis shifted away from plant derived drugs with the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation after 1945. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint during this period. Over the last decade, however, interest in drugs of plant and probably animal origin has grown steadily (Hamburger and Hostettmann,1991).

Utilization of medicinal plants has almost doubled in Western Europe during that period. Ecological awareness, the efficacy of a good number of phytopharmaceutical preparations, such as ginkgo, garlic or valerian and increased interest of major pharmaceutical companies in higher medicinal plants as sources for new lead structures has been the main reasons for this renewal of interest. With the development of chemical science and pharmacognosy

physicians began to extract chemical products from medicinal plants. A few examples of the products extracted from medicinal plants are - in 1920, quinine was isolated from Cinchona by the French pharmacist, Peletier & Caventou. In the mid- nineteenth century, a German chemist, Hoffmann obtained Aspirin from the bark of the willow. With the active principles in medicinal plants identified and isolated, plant-based prescriptions began to be substituted more and more with pure substances, which were more powerful and easier to prescribe and administer (Harvey, 2000).

Phytomedicine almost went into extinction during the first half of the 21st century due to the use of the 'more powerful and potent synthetic drug'. However, because of the numerous side effects of these drugs, the value of medicinal plants is being rediscovered as some of them have proved to be as effective as synthetic medicines with fewer or no side effects and contraindications. It has been proved that although the effects of natural remedies may seem slower, the results are sometimes better on the long run especially in chronic diseases.

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Ipomea obscura* whole plant investigated and summarized in Table-1. The phytochemical screening *Ipomea obscura* showed that the presence of flavonoids, phenolics, tannin, steroids, alkaloids, saponins, anthroquinones and terpenoids present in the all the extracts. Among the three extracts, methanol extract showed high concentrations than other extracts.

#### Quantitative analysis

Quantitative analysis revealed that the *Ipomea obscura* whole plant contain significant amount of phenols, alkaloids, saponin and terpenoids. Significant amount of Quantitative analysis revealed that *Ipomea obscura* leaf rich amount of total Phenol (220.0), alkaloids (42.0), tannin (72.12) saponin (62.0) and flavonoid (150.0) were presented (Table 2). The above phytoconstituents were tested as per the standard methods. This is because of the pharmacological activity of this plant is used to trace the particular compound.

#### Minerals

All human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water (Indrayan et al., 2005). Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body. Plants are the rich source of

**Table 1: Phytochemical screening of *Ipomea obscura***

S.No	Testing	Methanol extract	Ethanol Extract	Aqueous extract
1	Tannin	+	+	+
2	Phlobatannin	-	-	-
3	Saponin	+	+	+
4	Flavonoids	++	+	+
5	Steroids	+	+	+
6	Terpenoids	++	+	+
7	Triterponids	-	-	-
8	Alkaloids	+	+	+
9	Carbohydrate	-	-	-
10	Protein	-	-	-
11	Anthroquinone	+	+	+
12	Polyphenol	++	+	+
13	Glycoside	-	-	-

“+ ”present of the Compound, “-”Absence of the compound, “++” highly present

**Table 2: Quantitative analysis of *Ipomea obscura***

S.No	Secondary Metabolites	Methanol extract
1	Tannin	72.12
2	Saponin	62.00
3	Flavonoids	150.00
4	Total phenol	220.00
5	Alkaloids	42.00

**Table 3: Qualitative analysis of inorganic element in *Ipomea obscura***

S.No	S.No	Inorganic Elements	Observation
1	Calcium	White precipitate	Presence of calcium
2	Magnesium	White Crystal	Presence of Magnesium
3	Sodium	Yellow precipitate	Presence of Sodium
4	Potassium	Yellow precipitate	Presence of Potassium
5	Iron	Blue Color	Absent of Iron
6	Sulphate	White Precipitate	Presence of Sulphate
7	Phosphate	Yellow precipitate	Presence of Phosphate
8	Chloride	White Precipitate	Presence of Chloride
9	Nitrate	Brown Color ring	Presence of nitrate

all the elements essential for human beings. There is a relationship between the element content of the plant and its nutritional status. Some elements are essential for growth, for structure formation, reproduction or as components of biologically active molecules while others have some other beneficial effects (New Wall et al., 1996). The vitamins of the *Ipomea obscura* investigated and summarized in Table-4. The inorganic

elements of *Ipomea obscura* showed that the presence of The inorganic elements of *Ipomea obscura* leaf showed that the presence of calcium, magnesium, sodium, potassium, sulphate, phosphate, chloride and nitrate were presented while iron was absent.

The following elements were found in *Ipomea obscura*. They are calcium, sodium, potassium, sulphate, phosphate and chloride were

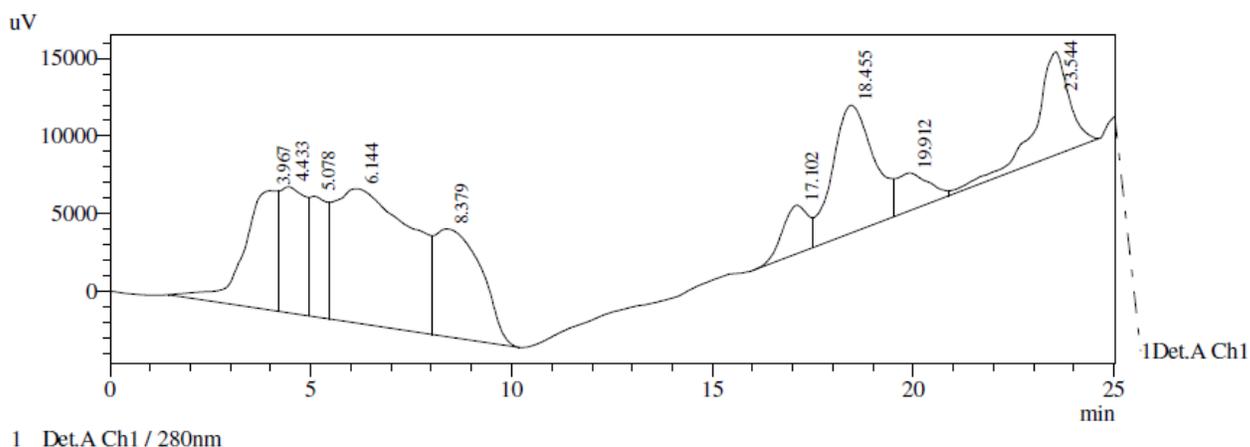
presented. Magnesium and carbonate were absent in *Ipomea obscura* extract (Table -4). Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body. Every form of living matter requires these inorganic elements or minerals for their normal life processes. Minerals may be broadly classified as macro (major) or micro (trace) elements. The third category is the ultra trace elements. The macro- minerals include calcium, phosphorus, sodium and chloride, while the micro- elements include iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum,

fluoride, chromium, selenium and sulfur. The macro-minerals are required in amounts greater than 100 mg/dl and the micro-minerals are required in amounts less than 100 mg/dl. The ultra trace elements include boron, silicon, arsenic and nickel which have been found in animals and are believed to be essential for these animals. Evidence for requirements and essentialness.

**HPLC**

HPLC profiles of *Ipomea obscura* were analysed and three phenolic compounds namely Gallic acid, Caffeic acid, Apigenin, Luteolin and Quercetin having different elution times could be obtained when each compound was analyzed individually using the mobile gradient phase consisting of ethanol and 1% acetic acid in water during 30 minutes run time.

**Fig 1 HPLC chromatogram of *Ipomea obscura* leaves**



**Table 6 HPLC chromatogram of *Ipomea obscura* leaves**

PEAK#	Ret. Time	Area	Height	Area%	Height%	Compounds
1	4.433	354889	8123	8.847	13.627	Gallic acid
2	5.078	236881	7754	5.906	13.008	Apigenin
3	6.144	1162421	8665	28.979	14.536	Luteolin
4	18.455	593592	8233	14.798	13.812	Quercetin
5	19.912	133943	2432	3.339	4.081	Caffeic acid
Total		4011197	59609	100.000	100.000	

**CONCLUSION**

The results of this study clearly indicate that the preliminary phytochemical analysis of *Ipomea obscura* (whole plant) revealed presence of flavonoids, phenolics, tannin, steroids, alkaloids, saponins, anthroquinones and terpenoids present in

the all the extracts. Among the three extracts, methanol extract showed high concentrations than other extracts. Quantitative analysis revealed that *Ipomea obscura* leaf rich amount of total Phenol (220.0), alkaloids (42.0), tannin (72.12) saponin

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