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# Preliminary Phytochemical screening of *Ipomoea obscura* (L) -A hepatoprotective medicinal plant

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**Abstract:** The Present paper deals with pharmacological aspects and phytochemical screening of *Ipomoea obscura* (L). Study includes phytochemical screening for different potent chemicals, antibacterial activity against human pathogenic strains [*Salmonella sp.* (MTCC); *Staphylococcus aureus, Bacillus subtillis. Escherichia coli, Pseudomonas sp.* etc].
**Keywords:** Phytochemical screening, antibacterial activity, *Ipomoea obscura.*

## Introduction:

Plant based drugs have been used world wide in traditional medicines for treatment of various diseases. India is the largest producer of medicinal herbs and appropriately called the Botanical garden of the world [1].The pharmacology provides an alternative approach for the discovery of antimicrobial (activity) agents, namely the study of medicinal plants with a history of traditional use as a potential source of substance with significant pharmacological and biological activities such as antioxidant, anticancerous & hepatoprotective [2]. The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multi resistant pathogenic bacteria and fungi. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry (phytochemicals) [3].

*Ipomoea obscura* (L.) commonly known as 'Laksmana' in ayurveda belongs to the family Convolvulaceae. It is small climbeing vine, with small cordate leaves and acuminate apex. Corolla composed of five fully fused petals. Plant grows on fences or low ground cover as substrate in disturbed areas [4]. Ayurveda has identified many medicinal properties of this plant and it is effectively used against dysentery, is applied to open sores and pustules. A paste of leaves is applied on ulcers, hemorrhoids and swellings [5]. Seeds and fruits are used as cleansing agents to improve difficult breathing, relive pain and to improve vision. It has also ornamental value as climber with attractive flowers. This plant also included as plants affecting central nervous system [6], and also actively used as an antioxidant [7].

The main aim of the present investigation was to study the antimicrobial activity and preliminary phytochemical screening of *Ipomoea obscura* leaf, stem and seed extract in different solvent like petroleum ether, absolute alcohol, chloroform, acetone and water and Qualitative and Quantitative analysis of some secondary metabolites, to ascertain ethnomedicinal claims of this widely used medicinal plant.

## MATERIALS AND METHODS

### Plant Material

Healthy plant material like leaves, stem and seeds of *Ipomoea obscura* were collected from Seminary hills, Nagpur, India. Referring the standard morphological characteristic features provides in the floras for the identification of the species.

### Preliminary phytochemical analysis.

Preliminary phytochemical screening of plant was done following the standard procedures adapted by the various workers.[8,9, 10].

### Preparation of extracts:

Fresh leaves, stem and seeds were washed thoroughly under running tap water, shade dried and used for extraction. Dried leaves stem and seeds were homogenized to a fine powder and stored in airtight bottles. 10gm of leaves, stem and seeds powders were extracted with 100ml of solvent (petroleum ether absolute alcohol, chloroform, acetone and water) for 24 hr. by using soxhlate apparatus. Extracts were used for different tests.

### Quantitative and Qualitative Phytochemical screening

Quantitative and Qualitative Phytochemical screening of plant was done according to standard procedures. Qualitative analysis of some phytochemicals such as alkaloids phenolics, flavonoids and saponins were done by employing Thin Layer Chromatographic technique [9].Where as quantitative chemical analysis of Alkaloids, Phenolics, Flavonoids and Saponins were done by different methods [11, 12].

### Antibacterial test

Antibacterial activity was carried out using different extracts and modified agar well diffusion method[13] against both human and plant pathogenic bacteria including *staphylococcus aureus, Bacillus subtillis; Rhodococci sp., Bacillus stearothermophilus (Gram+ve); Escherichia coli, Proteus vulgaris, Salmonella sp*. (MTCC), *Pseudomonas sp. (Gram-ve).*

### Test microorganisms

Selected pathogenic bacteria *Staphylococcus aureus, Bacillus substilis, Rhodococci (Gram+ve); Escherichia coli, Proteus vulgaris, Pseudomonas, salmonella,, E coli (Gram-ve)* were obtain from culture collection of center for Biotechnology SFS college, Seminary hills, Nagpur, India and Veterinary college, Nagpur, India. All bacterial species were maintained on nutrient agar medium for 36 hr. old bacterial culture were inoculated into nutrient broth and incubated at $37\pm2^0$C on rotary shaker at 100 rpm. After 36 hr. incubation, bacterial suspensions were used for further tests.

**Table no 1: Preliminary Phytochemicals Screening *I. obscura***

| Tests with all five extracts | | | | | | |
|---|---|---|---|---|---|---|
| **Chemical name** | **Part** | **P. ether** | **Chloroform** | **Acetone** | **Alcohol** | **Water** |
| **Alkaloids** | Leaf | + | + | + | + | + |
| | Stem | + | + | - | + | + |
| | Seed | + | - | + | - | + |
| **Steroids** | Leaf | - | + | + | + | - |
| | Stem | - | - | + | + | - |
| | Seed | + | + | + | + | - |
| **Triterpenoids** | Leaf | - | - | + | - | - |
| | Stem | - | - | + | - | - |
| | Seed | - | - | + | - | - |
| **Coumarins** | Leaf | + | + | + | + | + |
| | Stem | - | + | + | + | - |
| | Seed | + | + | - | - | - |
| **Flavonoids** | Leaf | + | - | - | - | - |
| | Stem | + | - | - | - | - |
| | Seed | + | - | - | - | - |
| **Phenolics** | Leaf | + | + | - | - | - |
| | Stem | - | + | + | - | + |
| | Seed | - | + | - | - | - |
| **Test with water extracts** | | | **Test with alcohol and water extracts** | | | |
| **Gums and mucilage's** | Leaf | + | **Chemical name** | **Part** | **Alcohol** | **Water** |
| | Stem | + | **Anthocyanins** | Leaf | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Seed | + | | Stem | - | - |
| **Saponins** | Leaf | + | | Seed | - | - |
| | Stem | - | **Anthocyanidins** | Leaf | - | - |
| | Seed | + | | Stem | - | - |
| **Phlobatanin** | Leaf | - | | Seed | - | - |
| | Stem | - | **Anthracene glycosides** | Leaf | - | - |
| | Seed | - | | Stem | - | - |
| **Chlorogenic acid** | Leaf | - | | Seed | - | - |
| | Stem | - | | | | |
| | Seed | + | | | | |
| **Tests with dry powder** | | | **Tannins** | Leaf | - | + |
| **Acubins** | Leaf | - | | Stem | - | + |
| | Stem | - | | Seed | - | + |
| | Seed | - | **Test with Petroleum ether extracts** | | | |
| **Irodoids** | Leaf | - | **Emodins** | Leaf | + | |
| | Stem | - | | Stem | + | |
| | Seed | - | | Seed | + | |
| **Cynogenic Glycosides** | Leaf | + | **Fatty acid and lipids** | Leaf | + | |
| | Stem | - | | Stem | - | |
| | Seed | - | | Seed | + | |
| **Anthraquinones** | Leaf | - | **Volatile Oils** | Leaf | + | |
| | Stem | - | | Stem | - | |
| | Seed | - | | Seed | + | |
| **Test with 70% ethanol extract** | | | **Carotenoids** | Leaf | + | |
| **Cardiac glycosides** | Leaf | + | | Stem | - | |
| | Stem | + | | Seed | - | |
| | Seed | + | | | | |

**Table no- 2. Qualitative secondary metabolite screening by Thin layer chromatography**

| Chemical name | Solvent system | Plant part | Rf values | Total bands | Spray reagent |
|---|---|---|---|---|---|
| Alkaloids | Methanol: conc. NH4OH(200:3) | Leaves | 0.86, 0.94 | 2 | Dragendroff's Reagent |
| | | Stem | 0.86, 0.98 | 2 | |
| | | Seed | 0.30,0.86, 0.94 | 3 | |
| Flavonoids | Chloroform: methanol(19:1) | Leaves | 0.08,0.14,0.55, 0.76,0.97 | 5 | No Reagent, UV light |
| | | Stem | 0.08,0.55, 0.97 | 3 | |
| | | Seed | 0.55,0.97 | 2 | |
| Phenolics | Chloroform: methanol(27:0.3) | Leaves | 0.03,0.05,0.36, 0.63,0.74,0.85, 0.90 | 7 | Folin-cioaiteu's |
| | | Stem | 0.03,0.05,0.36, 0.63,0.74,0.85, 0.90 | 7 | |

| | | | | | |
|---|---|---|---|---|---|
| | | Seed | 0.03,0.05,0.36, 0.63,0.74,0.85, 0.90 | 7 | reagent |
| Saponins | Chloroform: glacial acetic acid: methanol: water(64:34:12:8) | Leaves | 0.57,0.70 | 2 | Iodine vapors |
| | | Stem | 0.55,0.65 | 2 | |
| | | Seed | 0.55,0.65 | 2 | |

**Table no- 3: Quantitative phytochemical analysis**

| Name of compound | Plant part (mg/gm Sample) | | |
|---|---|---|---|
| | Leaves | Stem | Seed |
| Flavonoids | 2.3 | 1.3 | 1.5 |
| Phenolics | 3.0 | 3.2 | 2.2 |
| Saponins | 140 | 120 | 149 |
| | % of alkaloids/ gm of sample | | |
| Alkaloids | 0.31 | 0.11 | 0.48 |

## Result and Discussion

### Phytochemical screening

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. A number of medicinal plants have been chemically investigated [14, 15] .The screening of *Ipomoea obscura* for medicinal value has been carried out by number of workers [16, 17].

A general screening conducted to characterize chemical composition of *Ipomoea obscura* leaf, stem and seed samples. The screening covered mainly nitrogenous compounds, isoprenoids, acetogenins, (Table no- 1), which are reported to have dramatic physiological activities mainly on central nervous system. All three samples leaf, stem and seed showed positive test with 3 different alkaloids on the basis of their Rf values in TLC. Out of which 2 observed in leaf and stem and 3 in seed sample (Table no- 2). 0.31%, 0.11% and 0.48% per gm of sample appeared in leaf, stem and seed respectively. (Table no- 3). Acetogenin screening included tannins, flavanoids, coumarins, emodins, anthocyanidins, anthocyanins, anthroquinones, anthracene derivatives, phenolics and fatty acid. Leaf, stem and seed all gave a positive test for tannins flavanoid, coumarins emodins and phenolics. On the basis of different Rf values, TLC showed abundant occurrence of few of these compounds, phenolics (7) while flavanoids (5) (Table no- 2). Rest of the acetogenic compounds were not found either of the sample (Table no-1). 3mg/gm, 3.2mg/gm and 2.2mg/gm total phenolics content appeared in leaf, stem and seed samples respectively.

Phenolics have attracted a great attention in relation to their potential for beneficial effects on health. Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolics compounds, especially their antimicrobial activity [18], antiviral, anti-inflammatory and cytotoxic activity [19]. It is a well documented fact that most medicinal plants are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant properties [20]. Phenolics are active in curing kidney and stomach problems as well as helpful as anti-inflammatory in action [21].

Total flavanoids in leaf, stem and seed was found to be 2.3mg, 1.3mg and 1.8 mg /gm of sample respectively (Table no- 2). Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism [22]. Tannins play important role such as potent antioxidant [23]. The screening for isoprenoids was confined to steroids, iridoids, triterpenoids, saponins, cardiac glycosides and carotenoids. Saponins are widely well known to have expectorant and antitussive activity. Total 4 saponins were found to be present, 2 of which were observed in leaf and other 2 which are common to stem and seed were found to have RF value 0.55 and 0.65 (Table no- 2). Total Saponins content in leaf, stem and seed was found to be 140mg, 120mg and 149mg per gm of samples respectively. Recent studies at Toronto, Department of Nutritional Sciences, Canada, have indicated that, dietary source of saponins offer preferential chemical preventive strategy in lowering the risk of human cancer. Saponins are found in many plants and animals. Several workers [24, 25] carried out an extensive phytochemical analysis of plants for the presence of saponin. Steroids and cardiac glycosides were found to be present in all samples i.e. leaf, stem and seed, where

as fatty acid and lipids, volatile oil were found in leaf and seed samples only. Steroids have been reported to possess anti-inflammatory activities [26]. Carotenoid was present in leaf sample only. Iridoids and acubins were gave negative test in all three samples.

**Antibacterial activity**

Leaf, stem and seed samples were tested against 9 different bacterial strains which are pathogenic to humans. Four Gram +ve bacteria *Staphylococcus aureus*, *Bacillus subtillis*, *Bacillus streaothermophilus* and *Rhodococci sp*. and five Gram–ve viz. *Escherichia coli, Escherichia coli (Positive strain), Proteus vulgaris, Pseudomonas sp.* and *Salmonella sp*.(MTCC). It is found that the leaf stem and seed extracts of *Ipomoea obscura* inhibited growth of all bacteria confirming their antibacterial activity.

However, stem sample was found to have more potential activity than leaf and seed samples. All the three samples in case of *Salmonella sp.* (MTCC) inhibit the growth with greater range of zone of inhibition diameter i.e. 16mm, 18mm and 14mm. (Table no- 4). As *Salmonella* is the causative agent of lever disorders i.e. dysentery and diahhorea, it can be safely say that this plant have hepatoprotective activity.

Present investigation reported, this plant is warehouse of chemo-diversity which will be useful in screening for medicines like steroids, alkaloids, phenolics, flavanoids and some other chemicals. Antibacterial activity conclude that this plant stop bacterial growth. The results are encouraging but scientific scrutiny is absolutely necessary before being put in practice.

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**Table no- 4: Screening of *I. obscura* for antibacterial activity**

| Bacterial strain | Zone of inhibition in mm along with well diameter (5mm) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaves extracts | | | | | Stem extracts | | | | | Seed extracts | | | | |
| | 1a | 2a | 3a | 4a | 5a | 1a | 2a | 3a | 4a | 5a | 1a | 2a | 3a | 4a | 5a |
| ***E. coli (mixed)*** | -- | -- | 13 | 14 | -- | -- | 11 | 12 | 16 | -- | -- | -- | 9 | 19 | -- |
| ***B. subtilis*** | -- | -- | 7 | 7 | -- | -- | 12 | 7 | -- | -- | -- | -- | 9 | 9 | -- |
| ***Pseudomonas sp.*** | -- | -- | 7 | 9 | -- | -- | -- | 8 | 8 | 13 | -- | -- | 9 | 10 | -- |
| ***S. aureous*** | 13 | -- | 9 | -- | -- | -- | -- | 8 | -- | 20 | -- | -- | 7 | -- | -- |
| ***P. vulgaris*** | -- | -- | -- | 9 | -- | -- | -- | -- | 11 | -- | -- | -- | -- | 9 | -- |
| ***Salmonella sp.*** | 12 | 8 | 16 | 13 | -- | -- | 7 | 11 | 9 | 18 | -- | 9 | 14 | 14 | -- |
| ***E. coli(positive strain)*** | -- | -- | -- | 12 | -- | -- | -- | -- | 13 | -- | -- | -- | -- | 11 | -- |
| ***Rhodococci*** | -- | -- | 12 | 9 | -- | -- | -- | 8 | 13 | 17 | -- | -- | 12 | 15 | -- |
| ***B. stearothermopelus*** | -- | -- | 7 | 9 | -- | -- | -- | -- | 9 | 16 | -- | -- | 12 | 11 | -- |

**1a- petroleum ether , 2a- chloroform, 3a- acetone, 4a – ethanol and 5a – water extract**
**--: Not Observed**

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