Phytochemical and Antimicrobial activity of *Sphaeranthus indicus* Linn.

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**Abstract**
*Sphaeranthus indicus* Linn. (Asteraceae) is a common annual spreading herb found in a rice field throughout in India; five crude extracts were prepared from the whole plant *Sphaeranthus indicus* using different solvents by Soxhlet method. The extracts were subjected to screening to detect Preliminary phytochemical analysis and potential antimicrobial activity against *E. Coli, Staphylococcus aureus, Klebsiella pneumonia, Salmonella typhi* and fungal strain *Candida albicans* as standard by agar well diffusion method. The aim of our present study was to find out the Preliminary phytochemical analysis and antimicrobial activity of the different extracts of entire plant including flower heads of *Sphaeranthus indicus*. The different extracts such as Hexane, Methanol, Ethanol, petroleum ether and aqueous extracts exhibits comparable antimicrobial activity with the control. Presence of alkaloids and carbohydrate present in methanol extract, saponins are only present in distilled water and ethanol extract, phenolic compounds are only present in ethanolic extract while fixed oil and volatile oils are strongly present in all extract except in distilled water extract.

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1. Introduction

*Sphaeranthus indicus* Linn. belongs to family Asteraceae. The plant is commonly known as Gorakhmundi. It is an annual spreading herb, which grows approximately 15-30 cm in height. The plant is distributed throughout the plains and wet lands in India, Sri Lanka and Australia (Gogate, 2000). All the parts of the plant have medicinal uses. Plants have a great potential for producing new drugs of great benefit to mankind. Essential oil obtain by steam distillation of the whole herb contains ocimen, α- terpine, α- citral, geranion, α-ionone, β-ionone, d- cadinene, p- methoxy cinnamaldehyde (Basu et al., 1946) and an alkaloid spearanthine (Gupta et al., 1967). The alcoholic extract of powdered caputula contains stigmasterol, β- sitosterol, hentriacontane, sesque-terpine lactone, sphaeranthanolide flavone and isoflavone glycoside (Yadav et al., 1998). In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias (Kirtikar and Basu, 1978). There are several reports on the antimicrobial activity of
different extracts and biologically active compounds isolated from plant species used in herbal medicine (Yuldasheva et al., 2005). According to Ayurveda, this herb is used in medaroga, laxative, digestible, tonic, alterative, anthelmintic and alexipharmic (Gupta, 1984). It is used to treat vitiated conditions of hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. It is also used as a nerve tonic. *Sphaeranthus indicus* Linn showed good anti diabetic activity (Kharkar et al., 2013).

Considering the aforesaid, one of the traditionally used medicinal plants belonging was screened for their antimicrobial properties. *Sphaeranthus indicus* Linn. belongs to the family Asteraceae. It is used in homeopathic medicine for the treatment of insomnia, epilepsy, tetanus, muscle spasms and leaves presented anxiolytic activity (Ambavade et. al 2006; Amarasingam et. al. 1964). *Sphaeranthes indicus* Linn is an annual spreading herb used to treat hemicraniasis (Chopra et. al. 1958), jaundice, diabetes, hernia, haemorrhoids, helmenthiasis, skin diseases, nervous tone etc. the bark ground and mixed with whey is useful in treating piles. Leaf juice is boiled with milk and sugar prescribed for cough. An aqueous extract of the whole plant was slightly toxic to American cockroaches (Das and Bhattacharjee, 1970). *Sphaeranthus indicus* Linn was found to possess powerful medicinal properties to cure diseases of liver, bronchitis, jaundice and skin diseases. In view of the medicinal importance of *Sphaeranthus indicus* Linn. in the indigenous system, it was decided to work on the phytochemistry and antimicrobial investigation on *Sphaeranthus indicus* Linn.

2. Materials and Methods

A. Collection, identification and processing of plant material: Fresh plant material was collected from Ajaneri village paddy field and nearby area of Nashik. Plant was correctly identified with the help of Flora of Maharashtra (Singh et al., 2000) and Flora of Nashik District Lakshminararsimhan and Sharma, 1991). Plant material washed under running tap water dried under shad. It was then homogenized to fine powder with electric blender and store in airtight bottles. This sample was used for extraction of organic compound.

B. Extraction of organic crude material from *Sphaeranthus indicus* Linn.: 
50 gm of whole plant powder sample weighted and used for soxhlation.

**Solvent Used:** Depending on polarity the following solvent selected

1. Distilled water
2. Ethyl alcohol
3. Hexane
4. Petroleum eher
5. Methanol

(a) Phytochemical analysis of plant extract: The phytochemical are essential to metabolism and chemical processes of plant body. The phytochemical are studied are alkaloids terpenoids & steroids, flavonoids, glycosides, tannins & saponin.

**Identification Test:** The test were done to find the presence of active chemical such as alkaloids, glycosides, terpenoids, steroid, flavonoids, saponin, tannin by the following procedure.

**Alkaloids:**
**Detection of alkaloids (Evans, 2002):** solvent free extract, 50 mg is stirred with ml of dilute hydrochloric Acid and filtered. The filtered is tested carefully with various alkaloid reagents as follows.

a. **Mayer’s test:** To a few ml of filtrate, a drop or two of Mayer’s reagent are added by the side of test tube. A white or creamy precipitate indicates the test as positive.

**Mayer’s Reagent:** Mercuric chloride (1.358 gm) is dissolved in 60 ml of water and potassium iodide (5.0 gm) is dissolved in 10 ml of water. The two solutions are mixed and up to 100 ml with water.

b. **Wagner’s (Wagner, 2004):** To a few ml of filtrate, few drops of Wagner’s reagent are added by the side of the test tube. A reddish-brown precipitate confirms the test as positive.

**Detection of Carbohydrates and Glycosides:** The extract (100 gm) is dissolved in 5 ml of water and filtered. The filtered is subjected to the following tests.

a. **Mayer’s test:** to 2 ml of filtered, two drops of alcoholic solution of a-naphthol are added, the mixture is shaken well and 1 ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

**Barfoed’s:** To 1 ml of filtered, 1 ml of Barfoed’s reagent is added and heated on a boiling water bath for 2 min. Red precipitate indicates presence of sugar.

**Barfoed’s reagent:** Copper acetate, 30.5 gm is dissolved in 1.8 ml of glacial acid.

a. **Benedict’s test:** To 0.5 ml of filtrate, 0.5 ml of Benedict’s reagent is added. The mixture is heated on a boiling water bath for 2 min. A
characteristic colored precipitate indicates the presence of sugar.

b. To 3 ml of the aqueous extract was added about 1 ml of Iodine solution. A purple color at the interphase indicates the presence of carbohydrate.

c. Keller Kiliani test:-
2 ml of extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The mixture was then poured into the test tube containing 1 ml of concentrated sulphuric acid. A brown ring at the interphase indicates the presence of deoxy sugar, characteristics of cardenolides.

Detection of Saponin: The extract (50mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 min. A two cm layer of form indicates the presence of saponin.

Detection of proteins and Amino acids : The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whitman No. 1 filter paper and the filtrate is subjected to tests for proteins and amino acid.

a. Million’s test: To 2 ml of filtrate, few drops of Million’s reagent are added. A white precipitate indicates the presence of proteins.

b. Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to two ml of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

c. Detection of phenolic compounds and Tannins:-

a. Ferric chloride test: The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compounds.

Detection of Gum and Mucilage: The extract (100 mg) is dissolved in 10 ml of distilled water and to this 25 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilage.

Glycoside: Glycosides are compounds which upon hydrolysis give rise to one more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

Terpenoid and steroids: Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color for steroids (Siddique and Ali, 1997).

Flavonoids: Four ml of extract solution was treated with 1.5 ml of 50 %methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color for flavones.

Tannins: To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and green black for catecholic tannins.

Fixed oils and fats: A small Quantity if extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

Saponification test:-
A few drops of 0.5 N alcoholic potassium hydroxide solutions are added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on water bath for two hours. Formation of soap particles neutralization of alkali indicates the presence of fixed oils and fats.

A. Antimicrobial Activity:-
Inoculum: The microorganism isolated and incubated at 35+2 C for 4 hrs. The turbidity of the resulting bacterial adjusted to turbidity equivalent to 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0 X 108 CFU/ml.

Bacterial strain used:-
To study antimicrobial activity following four bacterial strain and one fungal strain used.
1. Escherichia coli (ATCC25922)
2. Klebsiella pneumonia (ATCC 25922)
3. Salmonella paratyphi
4. Staphylococcus aureus (ATCC25923)
5. Candida albicans

Agar well diffusion method: The modified agar well diffusion method was employed Muller-Hinton agar plates were inoculated by streaking the swab over the entire sterile over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure even distribution of the inoculums. As a final step the rim of the agar was also swabbed. After allowing the inoculums to dry at room temperature, 6-mm-diameter wells were bored in the agar.
Each extract was checked for antimicrobial activity by introducing 100µl of 4000 µg/ml concentration into triplicate wells simultaneously and the dilution medium for the positive control was respective solvents. The plates were allowed to stand at room temperature for 1 hour for extract to diffuse into the agar and then they were incubated at 35 ± 2°C for 24 hr. solvent extract were showed area of inhibition that solvent extract further analyzed for find out minimum inhibition concentration (MIC) by using 25µl, 50µl, 75µl, 100µl, and 125µl against positive control used pure solvent 100µl. The plates were allowed to stand at room temperature for 1 hour for extract to diffuse into the agar and then they were incubated at 35 ± 2°C for 24 hr. zone of inhibition measured with scale and observations were noted in notebook.

3. Results and Discussion

The plant material was subjected to successive extraction with Hexane, Methanol, Ethanol, petroleum ether and distilled water. Result of physiochemical properties are showed in (Table 1). Phytochemical studies of different crude extract revealed presence of alkaloids, carbohydrates, steroids, saponin, tannins and phenols etc. in (Table 2). All the plant extract detected presence of alkaloids. Carbohydrates are present strongly present in methanol extract, saponins are only present in distilled water and ethanol extract, phenolic compounds only presence ethanolic extract, fixed oil and volatile oils are strongly present in all extract except distilled water. The phytochemical compounds identified in the presence study this are bioactive and it shows various pharmacological activities like of hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. It is also used as a nervine tonic. *Sphaeranthus indicus* Linn showed good anti diabetic activity (Kharkar et al., 2013).
### Table 3: Antimicrobial Activity of *Sphaeranthus indicus* Linn.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Microorganism strain</th>
<th>Extract of <em>Sphaeranthus indicus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Zone of inhibition in cm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>1.</td>
<td><em>E. coli</em></td>
<td>Conc. Of Extract in µl</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
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</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>3.00</td>
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<tr>
<td></td>
<td>Pet. Ether</td>
<td>Nil</td>
</tr>
<tr>
<td>2.</td>
<td><em>S. aureus</em></td>
<td>Distilled water</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Pet. Ether</td>
<td>Nil</td>
</tr>
<tr>
<td>3.</td>
<td><em>K. pneumonia</em></td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>Methanol</td>
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</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Pet. Ether</td>
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</tr>
<tr>
<td>4.</td>
<td><em>S. paratyphi</em></td>
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<tr>
<td></td>
<td>Ethanol</td>
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</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>Hexane</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Pet. Ether</td>
<td>Nil</td>
</tr>
<tr>
<td>5.</td>
<td><em>C. albicans</em></td>
<td>Distilled water</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Pet. Ether</td>
<td>Nil</td>
</tr>
</tbody>
</table>

(MIC of Hexane Extract on *E. Coli*)  
(MIC of Ethanol Extract on *S. aureus*)
**Ph**

487

(MIC of Methanol Extract on *S. aureus*)

(MIC of Hexane Extract on *C. albicans*)

*Sphaeranthus indicus* Linn whole plant five extract (d.w, hexane, methanol, ethanol, and petroleum ether) tested against human pathogenic bacteria and fungi. Out of this five extracts, hexane, ethanol, methanol, showed high in vitro antibacterial activity against *S. aureus*, hexane extract showed strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*. *Candida albicans* fungal pathogen strongly inhibited by ethanol and hexane extract. none of antimicrobial activity found against bacteria *Klebsiella pneumonia*, distilled water extract and pure solvent used as control not showed any activity against micro organism (Table - 3). Further studies carried out isolation and purification of medicinally important compounds is useful for various pharmacological activities and it’s also help curing the various diseases.

**Research Highlights**

In the present work, we focused on the preparation and phytochemical screening of the aqueous, ethanol, methanol, hexane and petroleum ether extracts of *Sphaeranthus indicus*. Antimicrobial activity of these solvent extracts was tested against four bacteria and only one fungal organism.

Out of this five extracts, hexane, ethanol, methanol, showed high in vitro antibacterial activity against *S. aureus*, hexane extract showed strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*. *Candida albicans* fungal pathogen strongly inhibited by ethanol and hexane extract, none of antimicrobial activity found against bacteria *Klebsiella pneumonia*.

All the plant extracts detected presence of alkaloids and carbohydrates are present strongly present in methanol extract, saponins are only present in distilled water and ethanol extract, phenolic compounds only presence ethanolic extract, fixed oil and volatile oils are strongly present in all extract except distilled water.

**Limitations**

The study was carried to test the influence of whole plant extract as the susceptibility of the pathogen rather than concentrating on a specific component of extract.

**Justification of Research**

Plant extracts have been used in traditional medicine since the time of immemorial to control various types of infectious diseases in humans. The present research work focused on four bacterial and one fungal pathogens causing diseases. Therefore with the help of plant extracts we can control the human diseases by herbal medicine.

**Conclusion**

The aim of our present study was to find out the Preliminary phytochemical analysis and antimicrobial activity of the different extracts of entire plant including flower heads of *Sphaeranthus indicus*. The different extracts such as Hexane, Methanol, Ethanol, petroleum ether and aqueous extracts exhibits comparable antimicrobial activity with the control. Presence of alkaloids and carbohydrate present in methanol extract, saponins are only present in distilled water and ethanol extract, phenolic compounds are only present in ethanolic extract while fixed oil and volatile oils are strongly present in all extract except in distilled water extract.

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