ABSTRACT
The investigation was carried out to determine the possible phytochemical components from the petroleum ether, chloroform, ethyl acetate and hydroalcoholic extracts of *Andrographis echioides*. Among the phytochemical screening of these extracts, hydroalcoholic extract illustrated that the whole plant was rich in flavonoids, saponins, tannins and phenols. This study was extended by scrutinizing the antimicrobial activity in the hydroalcoholic extract of *Andrographis echioides*. The analysis revealed that *Andrographis echioides* needs further research on toxicological aspects to develop safe drug.

Key Words: *Andrographis echioides*, Phytochemicals, Hydroalcohol, Antimicrobial activity.

INTRODUCTION
*Andrographis echioides* or *Indoneesiella echioides* L. Nees (False Water Willow) is an herb widely distributed in the dry districts of tropical India and Sri Lanka. In traditional medicine, the leaf juice of this plant is used as a remedy for fevers. The plant from genus *Andrographis* is used in goiter, liver diseases, fertility problems, bacterial, malarial and fungal disorders. Leaf juice boiled with coconut oil is used to control falling and graying of hair.
Phytochemicals in fruits, vegetable species and traditional herbal medicinal plants have been found to play protective role against many human diseases. Phytocomponents including phenolics, flavonoids and tannins and various plants or herbal extracts have been reported to be radical scavengers and inhibitors to lipid peroxidation. [6] Due to uniqueness of curing different ailments, the whole plant of *Andrographis echioides* was selected for the study. Hence the present investigation was carried out to determine the possible phytochemical components from *Andrographis echioides* and to analyze the antimicrobial activity against selected microorganisms.

**MATERIALS AND METHODS**

**Collection and identification of plant**

*Andrographis echioides or Indoneesiella echioides* used in the study was identified in the ABS Botanical Conservation and Training Centre, India-Southern Circle-Salem, Tamil Nadu, India. The reference material was kept under number [No: AUT/MCAS/035]. Fresh plants were collected randomly from the region of ABS Garden, Salem, Tamil Nadu.

**Preparation of plant extracts**

The plant material collected was washed with distilled water and dried. The dried plant material was ground in a grinding machine to fine powder and passed through a 24 mesh sieve. The plant extracts were prepared by soaking 25 g of the dried powdered plant material in 250 ml of petroleum ether, chloroform, ethyl acetate and hydroalcohol (methanol: water, 70:30v/v) at room temperature for 48 h. The extracts were filtered after 48 h first through a Whatmann filter paper No. 42 and then through cotton wool. The extracts were concentrated using a rotary evaporator with water bath set at 40°C. The extracts were weighed and stored at room temperature. The percentage yield of the extracts ranged from 7-15%w/w.

**Preliminary phytochemical screening**

Preliminary phytochemical analysis of *Andrographis echioides* was carried out as per standard methods described by Brain and Turner. [7]

**Detection of Alkaloids**

The extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.
Mayer’s test
Filtrates were treated with Mayer’s reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner’s test
Filtrates were treated with Wagner’s reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids
Lead acetate test
Extracts were treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Sulphuric acid test
Extracts were treated with few drops of sulphuric acid. Formation of orange colour indicates the presence of flavonoids.

Detection of Steroids
2 ml of acetic anhydride was added to 0.5 g of the extracts of each with 2 ml of sulphuric acid. The change of colour from violet to blue or green in samples indicate the presence of steroids.

Detection of Terpenoids
Salkowski’s test
0.2 g of the extract of the whole plant sample was mixed with 2 ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Detection of Phenols
Ferric chloride test
Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test
Extracts were treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of phenol.
Detection of Saponins
About 0.2 g of the extract was shaken with 5 ml of distilled water. Formation of frothing shows the presence of saponins.

Detection of Tannins
A small quantity of the extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Antimicrobial activity
The disc diffusion method was used to screen the antimicrobial activity.\cite{8} In vitro antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The various concentrations of 10 µg, 20 µg, 30 µg and 40 µg of the hydroalcoholic extract were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

RESULTS
The preliminary phytochemical screening of the various solvent extracts of Andrographis echioides is presented in Table 1. The phytochemical screening of petroleum ether extract of Andrographis echioides showed the presence of flavonoids and steroids. The chloroform extract of Andrographis echioides showed the presence of steroids and saponins. The ethyl acetate extract of Andrographis echioides showed the presence of steroids and the hydroalcoholic extract of Andrographis echioides showed that the whole plant was rich in flavonoids, phenols, terpenoids, tanins and saponins. These phytochemical components may be responsible for several medicinal activities of Andrographis echioides.

The Antimicrobial activity of the hydroalcoholic extract of Andrographis echioides is shown in Table 2. The results for antimicrobial activity revealed that the plant extract inhibited the growth of all the test organisms.
Table 1: Phytochemical Analysis of Andrographis echioides

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemicals</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Ethyl acetate extract</th>
<th>Hydroalcoholic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phenols</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2: Antimicrobial activity of hydroalcoholic extract of Andrographis echioides against different pathogens

<table>
<thead>
<tr>
<th>Test microorganisms</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 µg</td>
</tr>
<tr>
<td>Gram Positive Bacteria</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>12</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>13</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>14</td>
</tr>
<tr>
<td>Gram Negative Bacteria</td>
<td></td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>13</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>-</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>12</td>
</tr>
<tr>
<td>Klebsiella aerogenes</td>
<td>17</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>11</td>
</tr>
</tbody>
</table>

Among gram positive bacteria, the hydroalcoholic extract showed the highest antibacterial activity against the growth of Escherichia coli having the zone inhibition of 20 mm, at 40 µg / disc. Besides this, the extract showed good activity against the growth of Staphylococcus aureus (16mm), Enterococcus faecalis (17mm) and Corynebacterium (16mm). Among gram negative bacteria, the hydroalcoholic extract showed the highest activity against the growth of Salmonella typhi (24mm) and Klebsiella aerogenes (24mm). Besides this, the extract showed good activity against the growth of Shigella flexneri (15mm), Pseudomonas aeruginosa (17mm), Proteus vulgaris (21mm) and Klebsiella pneumoniae (22mm).
DISCUSSION
The worldwide upsurge in the use of herbal preparations and active ingredients isolated from medicinal plants have provided the pharmaceutical industry with one of its most important sources of lead compounds, as up to 40% of modern drugs are derived from natural sources, using either the natural substance or a synthesized version. Furthermore, over a 100 new products are in clinical development, particularly as anti-cancer agents and anti-infectives. [9, 10]

*Andrographis echioides* is tested for phytochemical analysis and antimicrobial activities. Medicinal plants play a vital role in human health care; about 80% of the world populations rely on the use of traditional medicine, concomitantly based on plant materials. The results of the hydroalcoholic extract might be due to the presence of phytochemical constituents. The phytochemical analysis of the extract from the *Andrographis echioides* shows the presence of phytochemical constituents such as tannins, saponins, phenolic compounds, flavonoids and terpenoids. The presence of these secondary metabolites suggests that the plant might be of medicinal and industrial importance.

Plant products including phenols, flavonoids, tannins, saponins in the plant extract has been reported to be radical scavengers and inhibitors of lipid peroxidation. [11] The antioxidant properties of phenolic acids and flavonoids are due to their redox properties to chelate metals and quenching of singlet oxygen. [12] When phytochemical compounds react with a free radical it is the delocalization of the gained electron over the phenolic antioxidant and the aromatic nucleus that prevents the contamination of the free radical chain reaction but polyphenolic compound inhibit oxidation. [13]

Phytochemicals are natural and non-nutritive bioactive compounds produced by plants that act as protective agents against external stress and pathogenic attack. [14] Based on their biosynthetic origin, phytochemicals can be divided into several categories: phenolics, alkaloids, steroids, terpenes, saponins etc. Phytochemicals could also exhibit other bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties. [15] These plant-derived phytochemicals with therapeutic properties could be used as single therapeutic agent or as combined formulations in drug development. [16] Thus, the phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery. Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives and cheaper. [17]
Many plant species have been evaluated for their antimicrobial activity in the past 20 years. [18] Antibacterial effects of the hydroalcoholic extract of *Andrographis echioides* against gram positive bacteria like *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Corynebacterium* and gram negative bacteria like *Shigella flexneri, Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris, Klebsiella aerogenes* and *Klebsiella pneumoniae* suggest that they may possess remarkable therapeutic action in the treatment of gastro intestinal tract, skin diseases and other infectious disorder. The antimicrobial activity may be due to the presence of flavonoids and phenolic compounds present in the plant as secondary metabolites.

The antimicrobial activity revealed that the plant extract exhibited good potency against the growth of ten microorganisms. It is indicated that the plant extracts of *Andrographis echioides* contain compounds that largely inhibit the growth of gram positive and gram negative bacteria. In future studies we can formulate a new methodology for isolation and purification of the antimicrobial compounds present in *Andrographis echioides* which may act as a drug to control a broad range of microorganisms.

**CONCLUSION**

From the present study, it might be concluded that the maximum phytochemicals was observed in hydroalcohol than petroleum ether, chloroform and ethyl acetate extract which reveals that *Andrographis echioides* is highly valuable in medicinal usage for the treatment of various human ailments. With a wide spectrum of inhibition against both gram-positive and negative bacteria, hydroalcoholic extract of *Andrographis echioides* is worthy of further investigation as a natural antibacterial agent in the treatment of various infectious diseases. Antimicrobial activity of plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates.

**REFERENCES**


