Phytochemical Screening and Antimicrobial Activity of Ficus religiosa

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Abstract:
The leaves of Ficus religiosa was studied for invitro phytochemical screening and antimicrobial activity. The aqueous and methanolic solvent extract was used to screen the secondary metabolites and test the antimicrobial effect of extract on E.coli. The phytochemical analysis showed the presence of alkaloids, saponins phenols flavanoids protein tanins and terpenoids. The extracts were subjected for antimicrobial activity against E.coli using agar well diffusion method. Aqueous and methanolic extract showed a zone of inhibition of 10 mm and 12 mm respectively.

Keywords: Ficus religiosa, Solvent extraction, phytochemical screening, agar well diffusion, antimicrobial.

INTRODUCTION:
Natural products as source of therapeutics have always remained an edge over synthetic analagous. Secondary metabolites present in plants have multiple roles to be treated as therapeutics. In recent years plant based secondary metabolites has been used as ingredients in many Ayurvedic and traditional formulations. Herbal medicines are always considered to be safe that has led to its increase in demand. Ficus religiosa L. (Moraceae) has been extensively used in traditional medicine for a wide range of ailments of the central nervous system, endocrine system, gastrointestinal tract, reproductive system, respiratory system and infectious disorders[1]. The current study was aimed to carry out the phytochemical screening and to check invitro antibacterial activity against E.coli.

Classification:
Domain: Eukaryota
Kingdom: Plantae
Subkingdom: Viridaeplanta
Phylum: Tracheophyta
Order: Urticales
Family: Moraceae
Genus: Ficus
Species: F. religiosa

MATERIALS AND METHODS
Collection of Sample:
Leaves of Ficus religiosa was collected from the campus of IILM Academy of Higher Learning and thoroughly rinsed with distilled water and shade dried in the laboratory of Department of Biotechnology, IILM Academy.

Preparation of extracts:
Dried leaves were then grinded into fine particles with the help of grinder, further stored into air tight packets. Distilled water extract (aqueous extraction): 5gm of powdered leaves was taken in small conical flask. Then 50ml of distilled water added. Further flask was kept on the rotary shaker at 200 rpm for 24hrs. Distilled water extract (aqueous extraction): 5gm of powdered leaves sample in 2 different small conical flask is taken. Then 50ml of ethanold is added into both of the conical flask. Both the conical flask was kept on soxhlet till the solvent is vaporized completely.

Phytochemical screening:
Quantitative assay for presence of plant primary and secondary metabolites was performed using Standardized methods for the phytochemical analysis of the plant extracts.

Detection of alkaloids
One milliliter of aqueous extract was stirred and placed in 1% aqueous hydrochloric acid on a steam bath. Then, 1 mL of the filtrate was treated with Dragedorff’s reagent. Turbidity or precipitation with this reagent was considered as evidence for the presence of alkaloids [2].

Detection of carbohyrdates
Benedict's test–Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate [3].

Detection of saponins
About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion [4].

Detection of phenols
Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols [3].

Detection of flavonoids
A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids becomes colourless on addition of dilute acid, indicates the presence of flavonoids [4].

Detection of proteins
To the extract ninhydrin reagent (2,2-dihydroxyindene-1,3-dione) was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid. 

Detection of tanins
About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric
chloride was added and observed for brownish green or a blue-black colouration [5].

**Detection of terpenoids**

5 ml of each extract were mixed in 2 ml of Chloroform and 3 ml Concentrated sulphuric acid was carefully added to form a layer. A reddish brown colour at the interface indicates the presence of terpenoids [6].

**Antimicrobial Test:**
The antimicrobial activity was determined by agar well disc diffusion method. Inoculum preparation: E.coli sample was subcultured one day before of plate preparation. The culture was inoculated in luria broth and was kept in incubator overnight. The day OD was adjusted to 1.

Substrate preparation: Luria agar plate was prepared using pour plate method. Well was punched and 50µl of sample extract was loaded and was allowed to diffuse further it was kept in incubator at 37° C for overnight. Next day zone of inhibition was measured.

The assessment of the antibacterial activity was based on the measurement of the diameter of the inhibition zone formed around the well. The effects were compared with that of the standard antibiotic streptomycin.

**RESULT & DISCUSSION:**

**Phytochemical screening:** Phytochemical screening of aqueous and methanolic extract is shown in table 1. The aqueous extract showed the presence of carbohydrates saponins, phenols, flavonoids, tanins and terpenoid. While methanol extract showed presence of all phytochemical mentioned in table 1 except presence of alkaloid.

**Table 1. Phytochemical screening of crude extracts of Ficus religiosa.**

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Aqueous extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenols</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Protein</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Tanins</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Where + shows presence and – shows absence of phytochemical activities.

**Antimicrobial activity:** The control showed a zone of inhibition of 15 mm. the aqueous and methanolic extract showed inhibition of 10 mm and 12 mm respectively. The study showed the inhibition is concentration dependent. The extract can be used in development of formulation against E.coli.

**Table 2. Zone of inhibition of extract of Ficus religiosa.**

<table>
<thead>
<tr>
<th>Solvent extract</th>
<th>Zone of Inhibition (E.coli.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>10 mm</td>
</tr>
<tr>
<td>Methanolic</td>
<td>12 mm</td>
</tr>
<tr>
<td>Control (Streptomycin)</td>
<td>15 mm</td>
</tr>
</tbody>
</table>

**CONCLUSION:**
The use of plants and plant preparations has been in existent since prehistory. The World Health Organization (WHO) reported that about 80% of the world’s population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts [7]. The study showed the presence of different secondary metabolites present in extract of Ficus religiosa. Antimicrobial test showed the significant zone of inhibition that is comparable to standard antibiotic in terms of inhibition. Natural product has always grabbed attention of world in terms of its fewer side effects, cost effective and as better therapeutics. Further study will be required for quantitative estimation of mentioned metabolites and antimicrobial activity.

**REFERENCES:**