ANTIMICROBIAL AND PHYTOCHEMICAL INVESTIGATION OF EUPHORBIA HIRTA
AND TEPHROSIA PURPUREA

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Abstract:
The phytoconstituents from extracts of collected plant material of Euphorbia hirta and Tephrosia purpurea were subject to phytochemical screening and for biological activity viz; antimicrobial activity. The phytochemical analysis of Euphorbia hirta exhibit Phenol, Tannins, Saponins, Flavonoids, Alkaloids and Proteins. E.hirta possesses antibacterial properties. Tephrosia purpurea were rich in Phenol and Alkaloids. There is no antibacterial activity was observed in methanol extract of Tephrosia purpurea.

Keywords: Antimicrobial, phytochemical, Euphorbia hirta, Tephrosia purpurea

Introduction
Medicinal plants represent rich source of antimicrobial agents. E. hirta belongs to the plant family Euphorbiaceae and genus Euphorbia. It is characterized by the presence of white milky latex which is more or less toxic. E.hirta possesses antibacterial, antihelmintic, antiasthmatic, sedative, antispasmodic, antifertility, antifungal, and antimalarial properties. E. hirta is used in the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, etc.), bronchial and respiratory diseases (asthma, bronchitis, hay fever, etc.), and in conjunctivitis. The stem sap is used in the treatment of eyelid styes and a leaf poultice is used on swelling and boils [1] Decoction of dry herbs is used for skin diseases. Root decoction is also beneficial for nursing mothers deficient in milk. Roots are also used for snake bites [2] The phenolic extract of E. hirta has antiamoebic[3] and antispasmodic activity [4] Quercitrin, a flavanoid glycoside, isolated from the herb showed an anti diarrheal activity [5-6] The alcoholic extract of whole plant shows hypoglycemic activity in rats [7] Tephrosia purpurea belongs to the family Fabaceae, subfamily Faboideae is commonly known in Sanskrit as 'sharapunkha'. It is highly branched, herbaceous, sub erect, perennial herb widely grown in India. Whole plant and various parts of the plant are useful as ayurvedic medicines. Medicinal uses of drugs are tonic, laxative, diuretic, bronchitis, bilious febrile attack, boils, pimples, diarrhea, gonorrhea, rheumatism and cures disease of heart, spleen and blood. The pharmacological studies have shown that Tephrosia purpurea posses following biological activity such as antiuicer [8], antimicrobial, antibacterial, anti viral, anti asthmatic, hepatoprotective [9], antihyperglycemic [10] and antihyperlipidemia, immunomodulatory activity, antioxidant, wound healing property, anti allergic activity.

The objective of the present studies is to collect the Plant material of E. hirta and Tephrosia purpurea from different places in Ballarpur (Maharashtra) & to carry out the extraction and phytochemical screening of the phytoconstituents from selected extract. The extracts studied for biological activity viz; antimicrobial activity.

Materials and Methods

COLLECTION OF PLANT MATERIALS
The study period was from December 2014 to March 2015. The fully matured plants of Euphorbia hirta & Tephrosia purpurea were collected from nearby the forest area of Ballarpur, Chandrapur district. Maharshtra, India. During December 2014 and were washed thoroughly with tap water to remove dust particles then with sterile distilled water and shade dried.

EXTRACTION OF PLANT MATERIAL
The whole dried plants were ground into a fine powder and the total mass was subjected to extraction by a hot percolation method with Water, Ethanol and Methanol in Soxhlet apparatus. Each solvent extraction step was carried out for 24 hrs. After extraction the extracts were concentrated by evaporation and stored at 4°C for further study. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

QUALITATIVE PHYTOCHEMICAL ANALYSIS
The Phytochemical Analysis of the extracts was done using standard procedures. The following qualitative tests were carried out.

1) Test for Sterols: Salkowaki’s reaction, Liberman’s test
2) Test of Alkaloids: Mayer’s reagent, Wagner’s Reagent
3) Test for Saponins: Foam Test
4) Test for Anthraquinone: Bomtrager’s test
5) Test for Tannins: Ferric Chloride solution, Lead Acetate test
6) Test for Flavonoids:
7) Test for Phenols: Ferric chloride test, Nitric acid test
8) Test for Proteins: Xanthoproteic test
9) Test for amino acids: Ninhydrin test
10) Test for Sugars: Molisch’s test
11) Test for Fats/Lipids:

ANTIMICROBIAL ACTIVITY

SELECTION OF BACTERIAL CULTURES
Two bacterial cultures Escherichia coli and Staphylococcus aureus were selected for the present investigation.

PREPARATION OF MICROBIAL INOCULUMS
The fresh microbial cultures were prepared and used during the research period. The Nutrient Broth (NB) was prepared and poured into several tubes. Then pure microbial cultures were collected from the institute and inoculated in the tubes by using inoculation needles or loops. After these tubes were incubated (37°C for 24-28 hrs for bacteria). After incubation the cultures were used for the experiments.

PREPARATION OF NUTRIENT AGAR MEDIUM
1000ml of Nutrient agar medium is prepared; pH was adjusted to 6.8. The medium is sterilized by using autoclave at 121°C for 15 lbs pressure for 15 minutes and allowed to cool.

SCREENING FOR ANTIBACTERIAL ACTIVITY
(Agar well diffusion method)
The antibacterial activities of the plants were tested against the selected bacterial cultures. The 20 ml of sterilized Nutrient agar medium was poured into each sterile petriplates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5 mm are made in the medium by using a sterile cork borer, 150ul of extracts were transferred into separate wells. After these plates were incubated at 37°C for 24-28 hours. After incubation period, the results were observed and measure the diameter of inhibition zone around each well.

ANTIBIOTIC SENSITIVITY TEST ON BACTERIA
(POSITIVE CONTROL)
The antibiotic sensitivity test using standard antibiotics (kanamycin, methicillin and ampicillin) were analyzed by the method of Bauer et al., (1996). The sterilized nutrient agar medium was poured into each sterile petriplates and allowed to solidify. By using a sterile cotton swabs, a fresh bacterial culture with known population count was spread over the plates by following spread plate technique. Then the selected standard antibiotic disc was placed on the bacterial plates. Then the plates were incubated for 24 hours at 37°C. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the isolates.

Results and Discussion

The phytochemical analysis of Euphorbia hirta exhibit Phenol, Tannins, Saponins, Flavonoids, Alkaloids and Proteins appeared only in the Methanol extract. Tephrosia purpurea were rich in Phenol & Alkaloids. (Mentioned in table1) Which have been found to have invitro antimicrobial properties. The Methanol extract of plant Euphorbia hirta was active against strains of Staphylococcus and was less active against E.coli. In our study extract of Euphorbia hirta exhibited zone inhibition against S. aureus & E.coli. There is no antibacterial activity was observed in methanol extract of Tephrosia purpurea. (Mentioned in table 2).

The present study suggests the bioactive compounds can be used for future studies and ethno botanical survey reveals the usage of these plants extracts in treating the various diseases such as female disorders, respiratory ailments, worm infestations in children, dysentery, jaundice, pimples, gonorrhea, digestive problems, skin diseases, Jaundice, filariasis, anemia, fever, boils, pimple and hemorrhoids etc.

From our study and previous literature survey, we can come to conclusion that the whole plants of Euphorbia hirta is found to be rich in Phenols, Tannins, Saponins, Flavonoids, Alkaloids and Proteins. Tephrosia purpurea is found to be rich in Alkaloids and Phenols. There is no antibacterial activity observed in methanol extract of Tephrosia purpurea but Euphorbia hirta shows the antimicrobial properties therefore, we can conclude that these plants are useful for the medicinal purpose.
Table 1. Phytochemical Analysis:

<table>
<thead>
<tr>
<th>Phyto compounds</th>
<th>Tests</th>
<th>Euphorbia hirta</th>
<th>Tephrosia purpurea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterols</td>
<td>Salkowaki's reaction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Liberman's test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Mayer's reagent</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner's Reagent</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>With conc. HCl</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>Xanthoproteic test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Ninhydrin test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric Chloride test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>Ferric Chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>Bomtrage's test</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial activity of plant extract:

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Plant part used</th>
<th>Concentration (mg/ml)</th>
<th>Zone of inhibition (mm)</th>
<th>S. aureus</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphorbia hirta</td>
<td>Whole plant</td>
<td>0.1 ml</td>
<td>10 mm</td>
<td>10 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 ml</td>
<td>10 mm</td>
<td>11 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3 ml</td>
<td>11 mm</td>
<td>10 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4 ml</td>
<td>10 mm</td>
<td>10 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ml</td>
<td>14 mm</td>
<td>13 mm</td>
<td></td>
</tr>
<tr>
<td>Tephrosia purpurea</td>
<td>Whole plant</td>
<td>0.1 ml</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 ml</td>
<td>NA</td>
<td>NA</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.3 ml</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td></td>
<td></td>
<td>0.4 ml</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ml</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

NA: No Activity

ANTIMICROBIAL ACTIVITY OBSERVATIONS (Figure 1 and 2)

S. aureus     E. coli

Figure 1-PICTURE OF ANTIMICROBIAL ACTIVITY OF E. HIRTA EXTRACT

S. aureus     E. coli

Figure 2-PICTURE OF ANTIMICROBIAL ACTIVITY OF TEPHROSIA PURPUREA EXTRACT
References