



## 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, anthelmintic, 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 polyphenolic extract of *E. hirta* has antiamebic [3] and antispasmodic activity [4] Quercitrin, a flavanoid glycoside, isolated from the herb showed an antidiarrheal 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, suberect, 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, gonorrhoea, rheumatism and cures disease of heart, spleen and blood. The pharmacological studies have shown that *Tephrosia purpurea* possess following biological activity such as antiulcer [8], antimicrobial, antibacterial, anti viral, anti asthmatic, hepatoprotective [9], antihyperglycemic [10] and antihyperlipidemia,

immunomodulatory activity, antioxidant, wound healing property, antiallergic 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, Maharashtra, 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, gonorrhoea, 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:

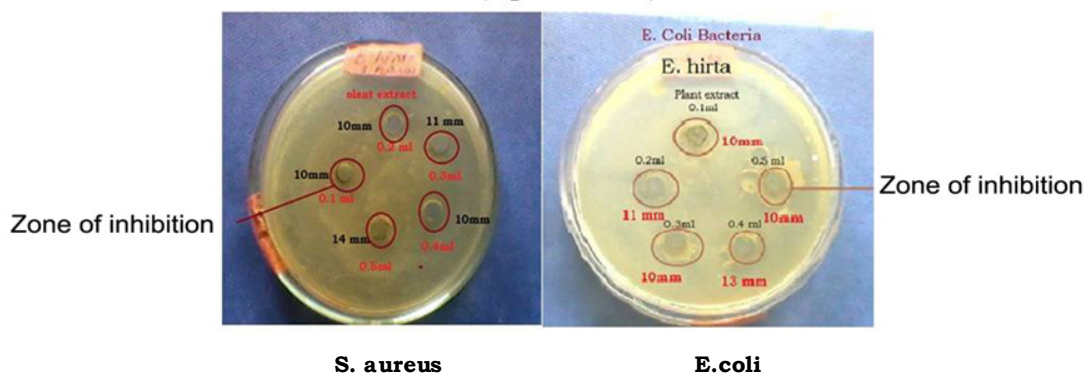
Phyto compounds	Tests	Euphorbia hirta	Tephrosia purpurea
Sterols	Salkowaki's reaction	-	-
	Lieberman's test	-	-
Alkaloids	Mayer's reagent	+	+
	Wagner's Reagent	+	+
Saponnins	Foam test	+	-
Flavanoids	With conc. HCl	+	-
Prote ins	Xanthoproteic test	+	-
Amino acids	Ninhydrin test	-	-
Tannins	Ferric Chloride test	+	-
Phenol	Ferric Chloride test	+	+
Anthraquinone	Bomtrage'r's test	-	-

**Table 2.** Antimicrobial activity of plant extract:

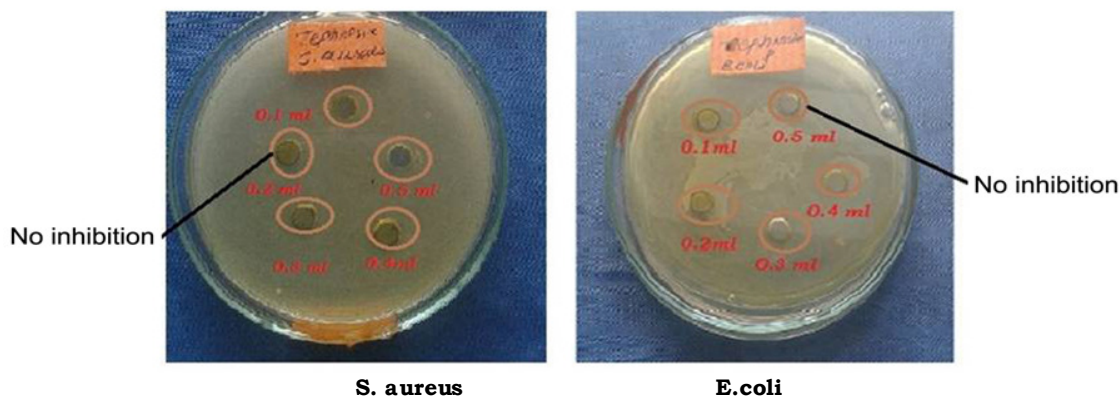
Plant species	Plant part used	Concentration (mg/ml)	Zone of inhibition (mm)	
			S. aureus	E.coli
Euphorbia hirta	Whole plant	0.1 ml	10 mm	10 mm
		0.2 ml	10 mm	11 mm
		0.3 ml	11 mm	10 mm
		0.4 ml	10 mm	10 mm
		0.5 ml	14 mm	13 mm
Tephrosia purpurea	Whole plant	0.1 ml	NA	NA
		0.2 ml	NA	NA
		0.3 ml	NA	NA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

NA: No Activity

ANTIMICROBIAL ACTIVITY OBSERVATIONS (Figure 1 and 2)



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



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

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