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Antibacterial activity of crude extracts of different parts of Euphorbia hirta L. using different solvents against gram – negative bacteria - Escherichia coli.

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ABSTRACT

Crude extracts obtained from the aerial parts- leaves, buds and stems of *Euphorbia hirta* L. using different solvents (Methanol , Ethanol, Dimethyl sulfoxide and Aqueous) were used to test antibacterial activity against disease causing gram negative bacteria - *Escherichia coli* . The susceptibility of the test bacteria varies with the type of solvent of same plant parts used. Among treatments, maximum *in vitro* inhibition of tested bacteria *E. coli* was scored in methanol extracts of leaf of *E. hirta* which offered Zone of inhibition of 25 mm and Zone of Inhibition Area of 686.88 mm^2 .The potentiality of the plant parts against the test bacteria as evaluated by Diameter of Zone of Inhibition (DIZ) and Zone of inhibition Area (ZIA) indicated the presence of more active compounds in extracts. Methanol, dimethyl sulfoxide and aqueous extracts were found to be more active, whereas, ethanol possessed moderate effect on the test bacteria.

Key words: Antibacterial activity, solvents, crude extract, *Euphorbia hirta* L., *Escherichia coli.*

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INTRODUCTION

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines (Farombi, 2003). In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant (WHO, 2001, Aibinu et al., 2003; Aibinu *et al*., 2004). The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality (Williams, 2000). At present, there is an urgent and continuous need of exploration and development of cheaper and effective new plant based drugs with better bioactive potential and least side effects. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Pretorius *et al.,* 2003, Moreillion et al., 2005).

Euphorbia hirta Linn. is a perennial herb belonging to the family Euphorbiaceae. It is a common weed referred to as 'garden spurge'. It is an erect, small, ascending, annual plant which reaches a height of 50 cm. The stem is hairy and the leaves are oblong, elliptical, acute or subacute. Flowers are small, crowded and numerous seen together in thick cymes about 1cm in diameter. The fruits are yellow in colour, three celled, hairy and have keeled capsules which are around 1-2 mm in diameter. They contain three four sided, brown, wrinkled and angular seeds.

It is a potent medicinal plant and has established its sedative and anxiolytic activity , analgesic, antipyretic, anti-inflammatory,

antidepressant for blood pressure , antihypertensive and antioxidant. It is now more important in treating respiratory ailments, especially cough, coryza, bronchitis and asthma . In India it has been used for relieving worm infestations usually seen in children, diarrhea, dysentery, jaundice, gonorrhea, pimples, tumors and digestive problems. *Euphorbia hirta* has been widely used by Tribal as traditional medicine in a treatment against infectious pathogens.

Gram negative bacteria - *Escherichia coli* has been proved to be major causal organisms of various human infections such as food poisoning, nosocomial infections, wound infections and urinary tract infections and has been selected for the present study.

Antibacterial activity of crude extracts of *Euphorbia hirta* against some few bacteria associated with enteric infections was studied by some scientist.((El-Mahmood et al.,2009; Shanmugapriya et al.,2012 ; Ibrahim et al.,2012).Other workers have also shown that extracts of *Euphorbia hirta* inhibited the growth of various microorganisms (Suresh et al.,2008; Sunil Kumar et al.,2010).

The purpose of the present study was to investigate the antibacterial activity of different parts of *E.hirta* using different solvents against disease causing bacteria - *Escherichia coli.*

MATERIALS AND METHODS

Plant material

Euphorbia hirta was collected as whole plant from different locations of Ranchi district of Jharkhand ,India. Fresh leaves, buds and stems were initially separated and washed, completely shade-dried, powdered and used for extraction.

Preparation of the Extract

Collected Fresh leaves, buds and stems were cleaned with water and dried in shade and pulverized into fine powdered substances by a grinding machine. Each 15 g of powder was transferred into conical flask. Then 150 mL of methanol was added in the flasks, closed by foil paper and placed on a shaker at 37 °C temperature for 72 hr. The crude extract was then filtered by passing the extract through Whatman No. 1 filter paper and then concentrated . After complete solvent evaporation, extracts were weighed and stored in a refrigerator at 4 °C for further use . 500 mg of solvent residue was dissolved in 10 mL of solvents were used as the test extracts for antibacterial activity assay.

Test Bacteria

Pathogenic bacteria such as *Escherichia coli* was obtained from Birsa Agriculture University, Kanke , Ranchi, Jharkhand, India. The test bacterial species was maintained on nutrient agar media.

Antibacterial Activity

Antibacterial activity of leave, bud and stem extracts using different solvents : methanol, ethanol and dimethyl sulfoxide and aqueous were determined by disc diffusion method on nutrient agar medium . The filter paper discs of 5 mm diameter were prepared using Whatman No. 1 filter paper , soaked in extract . The discs dipped in respective solvent were used as negative controls. The petri-dishes were sterilized in hot air oven and nutrient agar medium was sterilized by autoclaving. This media was poured in the sterile petri-dishes . Test bacteria was spread on the solid plates with a sterile swab moistened with the bacterial suspension. Plates were incubated at 37°C for 24hrs. Antibacterial activities were evaluated by measuring zone of inhibition by using Himedia zone scale in mm unit.

RESULTS

The antibacterial activity of extracts of leave, bud and stem of *E. hirta* in different solvents (Methanol, Ethanol, Dimethyl sulfoxide and Aqueous) against human pathogenic gram negative bacteria *Escherichia coli* showed varied level of inhibition (Table -1). Among treatments, maximum *in vitro* inhibition of tested bacteria *E. coli* was scored in

methanol extract of leaf of *E. hirta* which offered Zone of inhibition of 25 mm and Zone of Inhibition Area of 686.88 mm² . Further, bud extract of *E. hirta* in methanol and aqueous solvent and also leaf extract of same plant in dimethyl sulfoxide were effective against *E. coli* which recorded same significant Zone of inhibition of 20 mm and Zone of Inhibition Area of 471.00 mm² (Fig. - 1).

A significant inhibition zone of clinical bacteria *E. coli* was found in aqueous and ethanol extracts of leaf of *E.hirta* showing 15 mm zone of inhibition. From Table -1, it is seen that the extracts from different parts of *E. hirta* in different solvents such as methanol ,ethanol, dimethyl sulfoxide and aqueous showed antibacterial activity against *E. coli* .Methanol extract of *E. hirta* leaf showed the maximum degree of antibacterial activity properties. But crude ethanol extract of *E. hirta* bud and stem powder produced the minimum 13 mm and 10 mm zone of inhibition respectively and Zone of Inhibition Area of 234.72 mm² and 157.00 mm² respectively against *E.coli* .

These results may suggest the distribution of antibacterial potential in leave , bud and stem part of *Euphorbia hirta* L. that can be explored further for the isolation and characterization of the compound agent in pharmaceutical industries.

Table 1: Antibacterial activity of *Euphorbia hirta* against *Escherichia coli.*

Solvent	**Leaf**		**Bud**		**Stem**	
	DIZ (mm)	ZIA (mm²)	DIZ (mm)	ZIA (mm²)	DIZ (mm)	ZIA (mm²)
Methanol	25	686.88	20	471.00	15	294.38
Ethanol	15	294.38	13	234.72	10	157.00
Dimethyl	20	471.00	15	294.38	10	157.00
sulfoxide						
Aqueous	15	294.38	20	471.00	15	294.38

DIZ = Diameter of zone of inhibition in millimeter scale.
ZIA = Zone of Inhibition Area in millimeter square.

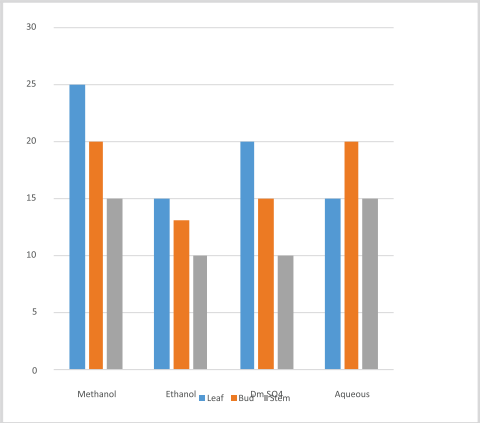

Fig 1 : Antibacterial activity of *Euphorbia hirta* against *Escherichia coli* .

The result of this study showed that extracts of different parts of *E. hirta* have varied antibacterial activities against the tested organism. This suggests that the extracts of these plants are broad spectrum in their activities. The result of this study, confirms its use traditionally in treating antibacterial infections like dysentery, wound infection. These primary extracts open the possibility of finding new clinically effective antibacterial compounds.

Out of all the extracts from *E. hirta* , the methanol extract was the most active. It showed marked antibacterial activities against *E.coli* . This may be due to the presence of alkaloids, tannins, saponins and flavonoids , which are secondary metabolites of plants. These secondary metabolites are actually the defensive mechanisms of the plants against pathogens. However the present study of *in vitro* antibacterial evaluation of *E.hirta* forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs.

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