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Assessment of Antibacterial properties of some herbaceous wild plants of family Euphorbiaceae

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ABSTRACT

Herbal medicines are of great demand in the developed as well as developing countries due to their wide medicinal and biological applications. Family Euphorbiaceae is one of the largest families of Dicotyledonous angiosperm which members are cosmopolitan in distribution, known for great medicinal importance. In present investigation the attempt has been made to confer antimicrobial properties of four herbaceous wild plants named as Dudhi / Asthma plant (*Euphorbia hirta)*, Chhotidudhi (*Euphorbia microphylla)*, Jungli Tulsi / Kala bhangra (*Croton bonplandianum)* and Black castnip (*Phyllanthus niruri)* belonging to Euphorbiaceae family . Toxic potentiality of these four plants have found as - Antibiotics > *Phyllanthus niruri* > *Croton bonplandianum* > *Euphorbia microphylla* > *Euphorbia hirta*. The present investigation also justifies the traditional uses of these herbaceous wild plants to treat various infectious diseases caused by microbes.

Keywords: *Antibacterial, Wild herb, Euphorbiaceae.*

INTRODUCTION

Medicinal plants are the richest bio-resources for the discovery of novel bioactive compounds, may considered as a gifts of nature to cure several diseases in human society. The curative potentiality of such plant is due to the presence of a wide array of complex chemical compounds known as secondary metabolites *viz*., alkaloids, flavonoids, saponins and phenolic compounds present in different parts namely root, stem and leaf which are used for antimicrobial and therapeutic purposes .There are several reports documenting the efficacy of plant extracts on microorganism by a number of researchers in different parts of the world (Dahanukar *et al*. 2000; Ganora, 2005; Abu-Shanab *et al*., 2004, Shrivastava *et al.*, 2013).

Although many of the plant species have been tested for their antimicrobial efficacy still the vast majority has not been evaluated thus far. Herbal medicines are in great demand in the developed as well as developing countries due to their wide medicinal and biological applications. The uses of plant extracts as antimicrobials have become popular because the effective life span of antibiotic is limited and over prescription as well as misuse of antibiotics are causing antimicrobial resistance.

In herbal medicines, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Barnes *et al*., 2007). Plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin and other small compounds (Cowan, 1999; Shakeri *et al*., 2012). Wide ranges of phytochemicals present in plants are known to inhibit bacterial pathogens (Cowan, 1999; Medina *et al*., 2005; Romero *et al*., 2005).

Euphorbiaceae family is one of the largest families of Angiosperms and the members are cosmopolitan in distribution, consisting large varieties of vegetative forms some of which are of great medicinal importance. Some of the Euphorbiaceae plant extracts are registered drugs and as such available on the market. Examples include Euphorbium (resiniferatoxin), from latex of *Euphorbia resinifera* (Appendino and Szallasi, 1997) marketed as 'Complexe Lehning Euphorbium N 88' and used as a nasal spray or compositum against viral infections, rhinitis of various origins, sinusitis, chronic nasal discharge, dry and inflamed nasal membranes as well as flu symptoms. *Euphorbia pilulifera* (the asthma weed) extract has been cited in Steadman's drugs list and can be applied against asthma, coryza and other respiratory infections and as an antispasmodic (www.drugs. com). The www.botanical.com website lists a number of Euphorbiaceae with varying curative features including: *Euphorbia peplus* L., *Euphorbia peploides, Euphorbia pilosa, Euphorbia palustris* being remedies for hydrophobia; *Euphorbia peplus, Euphorbia helioscopia, Euphorbia humistrata, Euphorbia hypericifolia, Euphorbia portulacoide*s L.

The present investigation report highlights the antimicrobial properties of four herbaceous wild plants belonging to Euphorbiaceae family.

MATERIALS AND METHODS

Four species of herbaceous wild plants were taken and identified, belonging to single family Euphorbiaceae commonly named as Dudhi, Asthma plant (*Euphorbia hirta),* Chhotidudhi (*Euphorbia microphylla),* Jungli Tulsi, Kala bhangra (*Croton bonplandianum)* and Stone breaker, Black castnip (*Phyllanthus niruri)* [Fig.- 1 to 4].

Fig. 1: (*Euphorbia hirta*)

Fig. 2: (*Euphorbia microphylla*)

Fig. 3: (*Croton bonplandianum*)

Fig. 4: (*Phyllanthus niruri*)

Leaf extracts of selected herbs were prepared following proper methods (Shrivastava *et al*., 2014). Firstly fresh leaves of wild herbs washed under running tap water and surface water was soaked with help of blotting paper then after leaf sample were dried completely in hot air oven at 40^{o}C for 24 hours and made its powder form for crude extract that stored separately in vial below 10^{o}C. To prepare the crude extract, ethanol, methanol and hot water were used as s solvent; 1g of powered were dissolved in 2ml, shake gently for 10 minute so it was dissolve properly. Treated as mother solution (100% con.) gradation of 75%, 50% and 25% concentrations were made in their respective solvent. Dilution stored in laminar air flow chamber to maintain aseptic condition.

To assess the toxic properties of crude extracts against pathogenic bacteria were taken during present study. Applying proper *in-vitro* techniques of antibacterial bioassay and antimicrobial efficacy of leaves crude extract were evaluated. Pure culture of *E. coli*- ATCC10536 and *Staphylococcus aureus*- ATCC 25923 were borrowed from recognised Pathology laboratory and culture was revived on NAM plate.

The antibacterial properties were evaluated by Zone of Inhibition. Overnight cultures (at 37^{o}C for 24 hrs.) of each bacterial strain (*E. coli* and *Staphylococcus aureus*) were spread with glass rod on the surface of Nutrient Agar plates. The antibacterial activity was screened using the cork borer (4mm in diameter) by well diffusion method, well were saturated with 50µl (1gm /2ml) of the leaf extract of *Euphorbia hirta*, *Euphorbia microphylla*, *Croton bonplandianum* and *Phyllanthus niruri* in under laminar air flow. Agar well diffusion method was used for determining antibacterial activity. Petri plates were prepared by pouring 25 ml of seeded nutrient agar and allowed to solidify. After solidification, bacterial culture were spread and on the surface of the agar plates, a standard cork borer of 4mm diameter was used to cut uniform well which was filled with the 2 ml extract of leaf. The plates were placed for incubation at 37^{o}C for 24hrs. After incubation, the diameter of clear zones around each well is measured and compared against zone of inhibition produced by solution of known concentration of standard antibiotic Gentamycin (10 mg) and Kanamycin (10 mg). Different concentrations (25%, 50%, 75% &100%) of extracts were used and results were observed.

RESULTS AND DISCUSSION

To analyze the effect of herbaceous wild plant extracts on the growth of the pathogenic bacteria, the inhibition of zone at different concentration of the extracts against specific test organism were measured. The extract restricted the growth of the bacteria on media around the well. The zones of inhibition as observed (Fig. 5) were measured have been computed in Table 1 & 2. [Fig. 6 -10)].

Toxicity of crude extract of herbaceous wild plant against bacterial growth has been found variously *in-vitro* culture plate. Crude extract of *Euphorbia hirta* inhibited the growth against both the bacteria during present experiment. In case of ethanol extract against *E. coli* was found 4mm in 25% , 9.2mm in 50% , 10mm in 75% and 11.5mm in 100% concentration, whereas methanol extract found 4.5mm in 25% , 6.5mm in 50% , 9mm in 75% and 10mm in 100% concentration and hot water extract found 5.5mm in 25% , 6.2mm in 50% , 10.5mm in 75% and 17.4mm in 100% concentration has been noticed in case of bacteria *Staphylococcus aureus* ethanol extract was found 4.2mm in 25% , 7.2mm in 50% , 8mm in 75% and 10mm in 100% concentration whereas methanol extract were found 4mm in 25%, 6.2mm in 50% , 8.4mm in 75% and 9mm in 100% concentration, hot water extract

were found 5mm in 25% , 8.5mm in 50% , 10mm in 75% and 10.5mm in 100% concentration and the maximum activity was shown by ethanol extract against *E. coli* bacteria. (Table-1).

Euphorbia microphylla extracts was showed toxic against *E. coli*, and may due to the crude extract of inhibited the growth against both bacteria during present experiment. In case of ethanol extract it was found 3.8mm in 25% , 5.5mm in 50% , 7mm in 75% and 8.5mm in 100% concentration, in methanol extract ZOI was found 4.5mm in 25% , 5mm in 50% , 6.5mm in 75% and 7mm in 100% concentration, whereas in hot water extract it found 3.6mm in 25% , 5mm in 50% , 6.3mm in 75% and 8mm in 100% concentration has been noticed in case of bacteria *Staphylococcus aureus;* for ethanol extract it was found 3mm in 25% , 5.4mm in 50% , 6.2mm in 75% and 7.4mm in 100% concentration, for methanol extract ZOI was found 3.2mm in 25% , 3.8mm in 50% , 4.8mm in 75% and 6.2mm in 100% concentration whereas in hot water extract it was found 3mm in 25% , 4.5mm in 50%, 6.5mm in 75% and 7.5mm in 100% concentration, the maximum toxicity was shown by ethanol extract against *E. coli* bacteria. (Table-1).

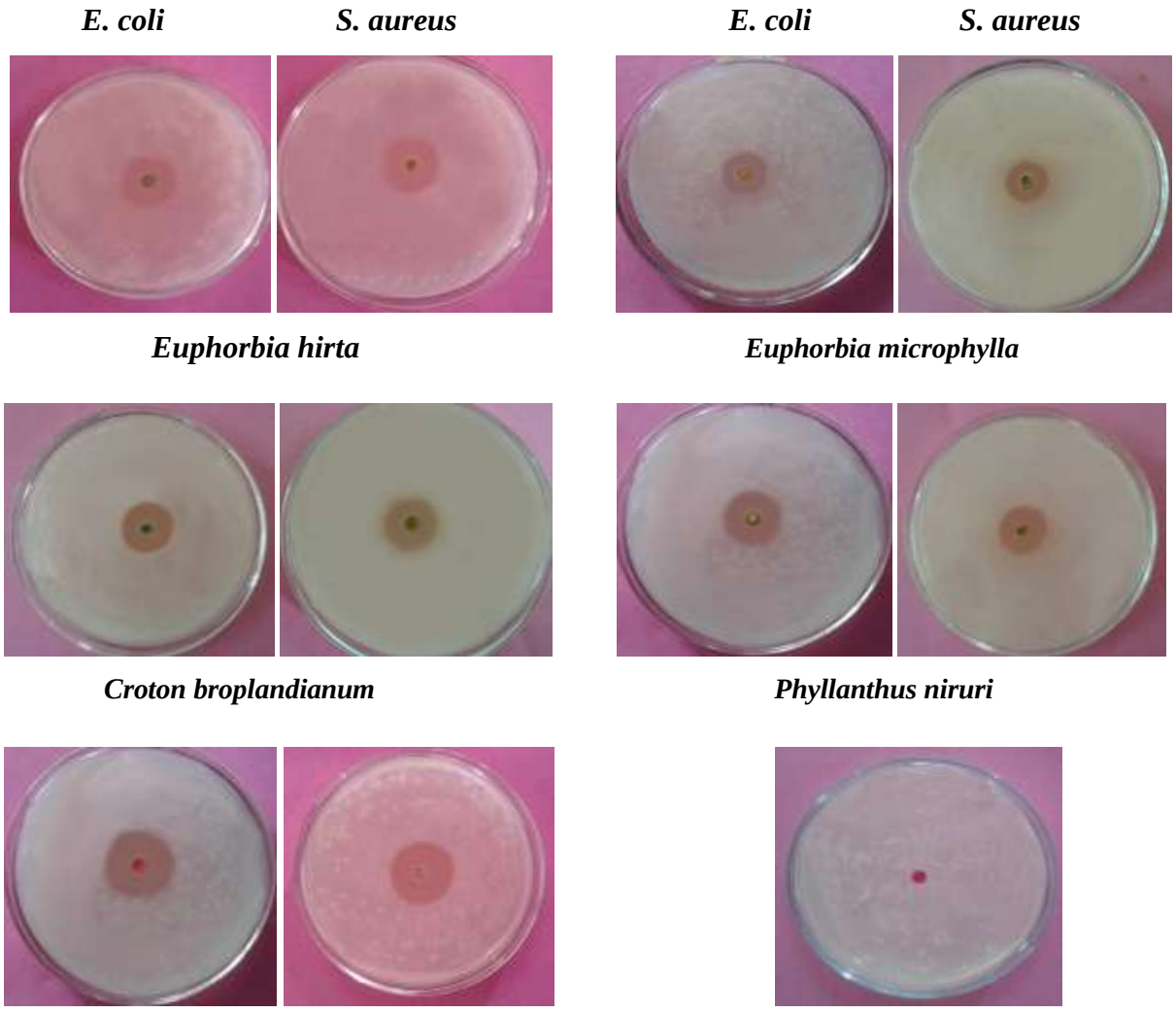

Fig. 5 (Showing ZOI)

Table 1
Inhibitory effect of leaf extracts on the growth of *E. coli* and *Staphylococccus aureus* and its comparison with standard antibiotics (Mean ± SD)

Leaf Extracts & Standard Antibiotics		Concentrations	Zone of inhibition (mm.)	
			E. coli	*S. aureus*
		0%	00	00
Leaf Extract of *Euphorbia hirta*	Ethanol	25%	4.0±0.22	4.2±0.25
		50%	9.2±0.52	7.2±0.42
		75%	10.0±0.55	8.0±0.45
		100%	11.5±0.65	10.0±0.55
	Methanol	25%	4.5±0.35	4.0±0.32
		50%	6.5±0.65	6.2±0.36
		75%	9.0±0.56	8.4±0.47
		100%	10.0±0.55	9.0±0.54
	Hot Water	25%	5.5±0.65	5.0±0.56
		50%	6.2±0.54	8.5±0.55
		75%	10.5±0.66	10.0±0.58
		100%	11.0±0.72	10.5±0.60
Leaf Extracts of *Euphorbia microphylla*	Ethanol	25%	3.8±0.15	3.0±0.13
		50%	5.5±0.45	5.4±0.42
		75%	7.0±0.62	6.2±0.25
		100%	8.5±0.62	7.4±0.66
	Methanol	25%	4.5±0.73	3.2±0.52
		50%	5.0±0.70	3.8±0.65
		75%	6.5±0.55	4.8±0.15
		100%	7.0±0.60	6.2±0.56
	Hot Water	25%	3.6±0.52	3.0±0.50
		50%	5.0±0.13	4.5±0.73
		75%	6.3±0.43	6.5±0.45
		100%	8.0±0.62	7.5±0.30
Leaf Extracts of *Croton borplandianum*	Ethanol	25%	6.5±0.61	6.0±0.55
		50%	9.2±0.52	8.5±0.63
		75%	10.6±0.65	11.0±0.50
		100%	15.0±0.42	14.8±0.40
	Methanol	25%	6.4±0.60	6.2±0.58
		50%	8.6±0.65	8.0±0.50
		75%	10.0±0.51	10.5±0.63
		100%	12.5±0.65	12.0±0.70
	Hot Water	25%	6.0±0.55	6.4±0.60
		50%	8.4±0.64	7.5±0.45
		75%	9.0±0.55	8.2±0.62
		100%	11.0±0.73	10.5±0.70
Leaf Extracts of *Phyllanthus niruri*	Ethanol	25%	8.5±0.57	7.5±0.61
		50%	10.6±0.63	9.5±0.62
		75%	12.4±0.51	10.0±0.62
		100%	16.5±0.35	15.5±0.73
	Methanol	25%	6.5±0.50	6.0±0.55
		50%	8.3±0.40	8.6±0.45
		75%	10.2±0.65	9.6±0.52
		100%	12.5±0.73	11.0±0.60
	Hot Water	25%	7.5±0.61	6.0±0.50
		50%	10.6±0.42	8.4±0.45
		75%	12.5±0.73	11.0±0.50
		100%	13.0±0.15	12.0±0.70
Antibiotics	Gentamycin	10 mg. / disc	17.4±0.45	15.5±0.30
	Kanamycin	10 mg. / disc	20.00±0.35	16.5±0.73

Table 2
Effect of leaf extracts of *Euphorbia hirta, Euphorbia microphylla, Croton bonplandianum* & *Phyllanthus niruri* on the growth of *E. coli* & *Staphylococcus aureus* and its comparison with standard antibiotics 100% concentration (Mean ± SD).

Leaf Extracts & Standard Antibiotics		Concentrations	Zone of inhibition (mm.)	
			E. coli	*S. aureus*
Leaf Extracts	*Euphorbia hirta*	Ethanol	11.5±0.65	10.0±0.55
		Methanol	10.0±0.55	9.0±0.54
		Hot water	11.0±0.72	10.5±0.60
	Euphorbia microphylla	Ethanol	9.0±0.62	8.5±0.66
		Methanol	7.0±0.60	6.2±0.56
		Hot water	8.0±0.62	7.5±030
	Croton borplandianum	Ethanol	15.0±0.42	14.8±0.40
		Methanol	12.5±0.65	12.0±0.70
		Hot water	11.0±0.73	10.5±0.70
	Phyllanthus niruri	Ethanol	16.5±0.35	15.5±0.73
		Methanol	12.5±0.73	11.0±0.60
		Hot water	13.0±0.15	12.0±0.70
Antibiotics	Gentamycin	10 mg. / disc	17.4±0.45	15.5±0.30
	Kanamycin	10 mg. / disc	20.00±0.35	16.5±0.73

Toxicity of crude extract of herbaceous wild plant against bacterial growth has been found various link in *in-vitro* culture plate. Crude extract of *Croton bonplandianum* exhibited the toxicity against both the bacteria during present experiment. In case of ethanol extract against *E. coli* was found 6.5mm in 25% , 9.2mm in 50% , 10.6mm in 75% and 15mm in 100% concentration were as methanol extract found 6.4mm in 25% , 8.6mm in 50% , 10mm in 75% and 12.5mm in 100% concentration were as hot water extract found 6mm in 25% , 8.4mm in 50% , 9mm in 75% and 11mm in 100% concentration has been notice in case of bacteria *Staphylococcus aureus* ethanol extract was found 6mm in 25% , 8.5mm in 50% , 11mm in 75% and 14.8mm in 100% concentration were as methanol extract found 6.2mm in 25% , 8mm in 50% , 10.5mm in 75% and 12mm in 100% concentration were as hot water extract found 6.4mm in 25% , 7.5mm in 50% , 8.2mm in 75% and 10.5mm in 100% concentration, the maximum activity is shown by ethanol extract against *E. coli* bacteria. (Table 1).

Phyllanthus niruri extracts was showed activity against *E. coli*, and may due to the crude extract of inhibited the toxicity against both the bacteria during present experiment. In case of ethanol extract was found 8.5mm in 25% , 10.6mm in 50% , 12.4mm in 75% and 16.5mm in 100% concentration were as methanol extract found 6.5mm in 25% , 8.3mm in 50% , 10.2mm in 75% and 12.5mm in 100% concentration were as hot water extract found 7.5mm in 25% , 10.6mm in 50% , 12.5mm in 75% and 13mm in 100% concentration has been notice in case of bacteria *Staphylococcus aureus* ethanol extract was found 7.5mm in 25% , 9.5mm in 50% , 10mm in 75% and 15.5mm in 100% concentration were as methanol extract found 6mm in 25% , 8.6mm in 50% , 9.6mm in 75% and 11mm in 100% concentration were as hot water extract found 6mm in 25% , 8.4mm in 50% , 11mm in 75% and 12mm in 100% concentration, the maximum activity is shown by ethanol extract against *E. coli* bacteria.(Table - 1)

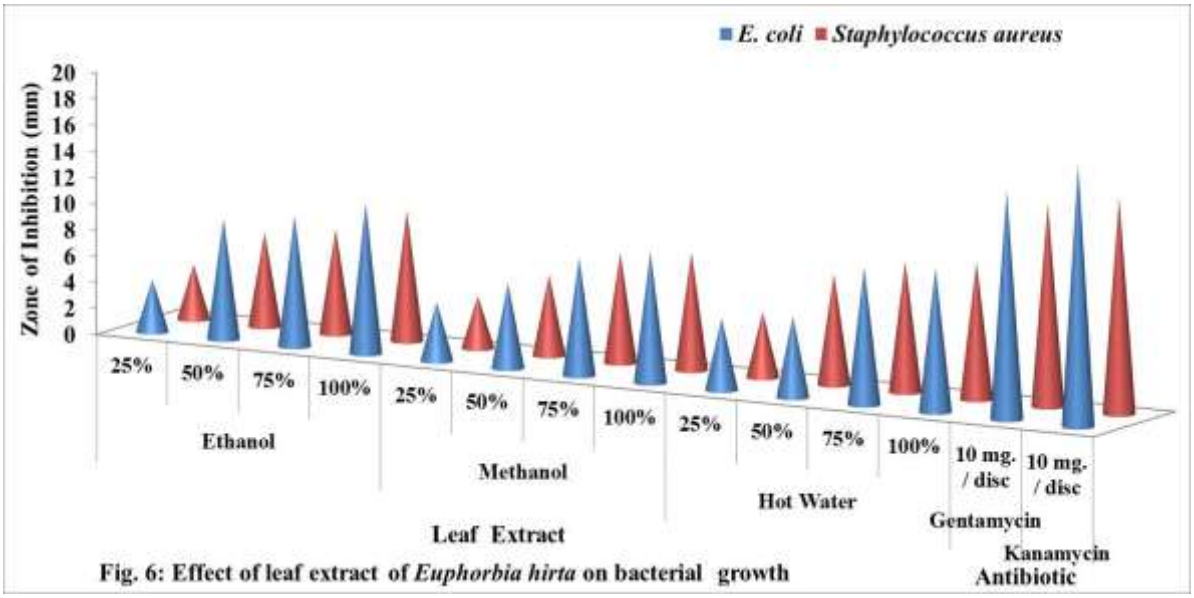

Fig. 6: Effect of leaf extract of *Euphorbia hirta* on bacterial growth

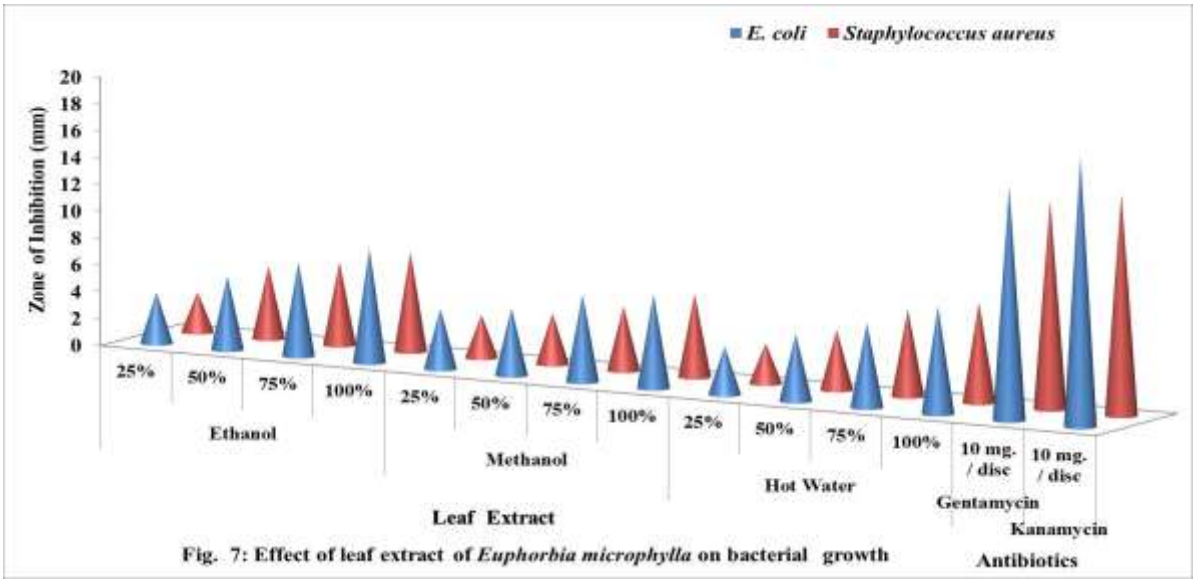

Fig. 7: Effect of leaf extract of *Euphorbia microphylla* on bacterial growth

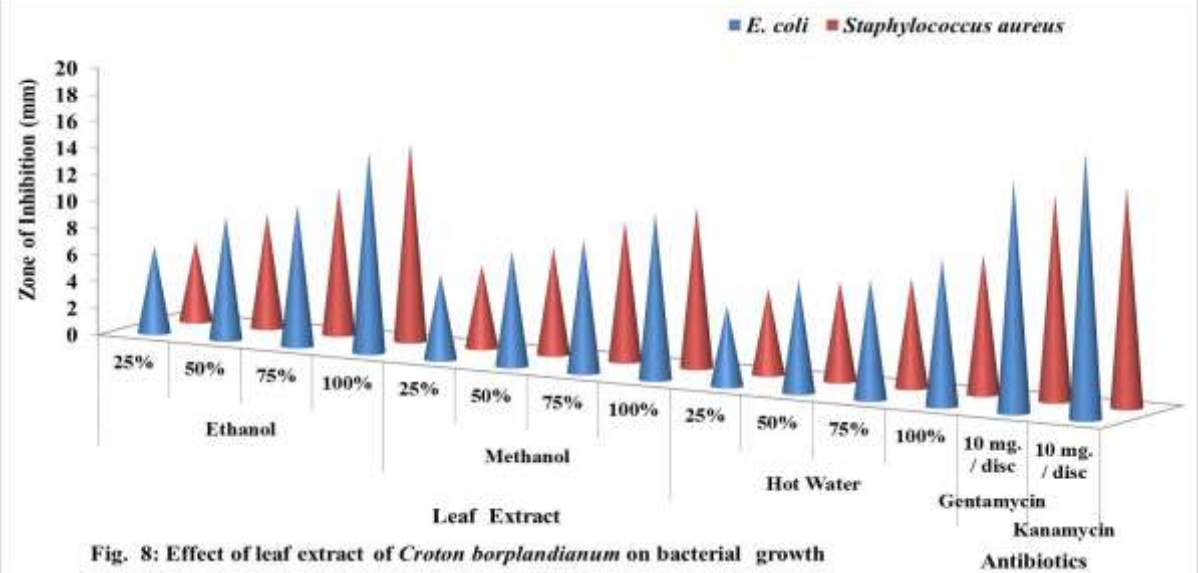

Fig. 8: Effect of leaf extract of *Croton borplandianum* on bacterial growth

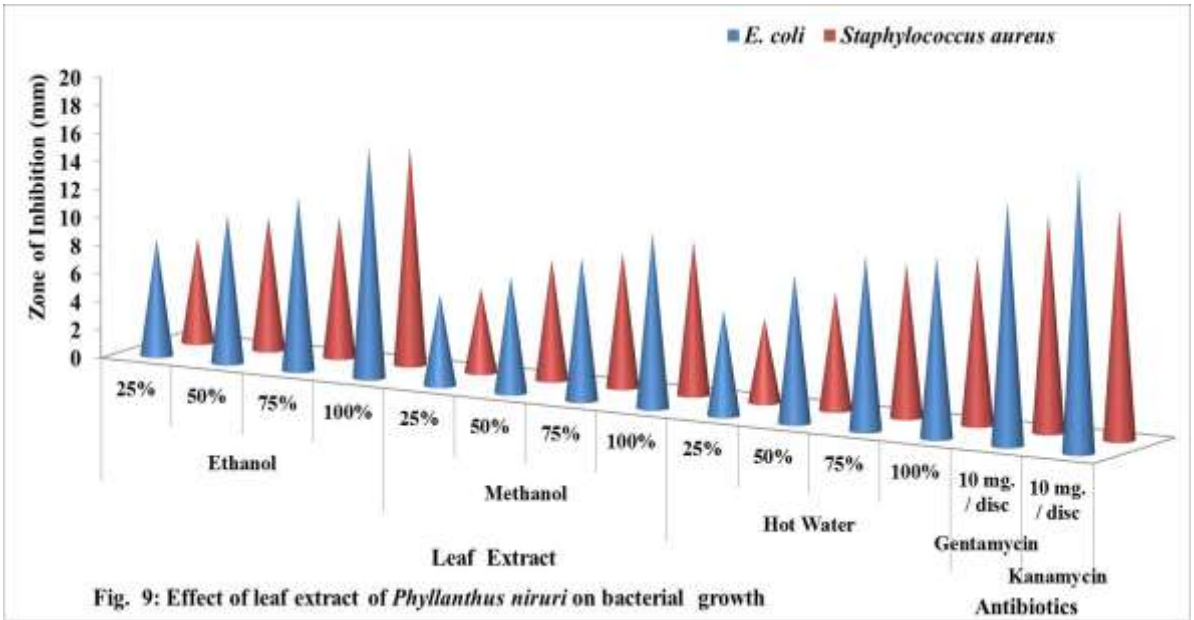

Fig. 9: Effect of leaf extract of *Phyllanthus niruri* on bacterial growth

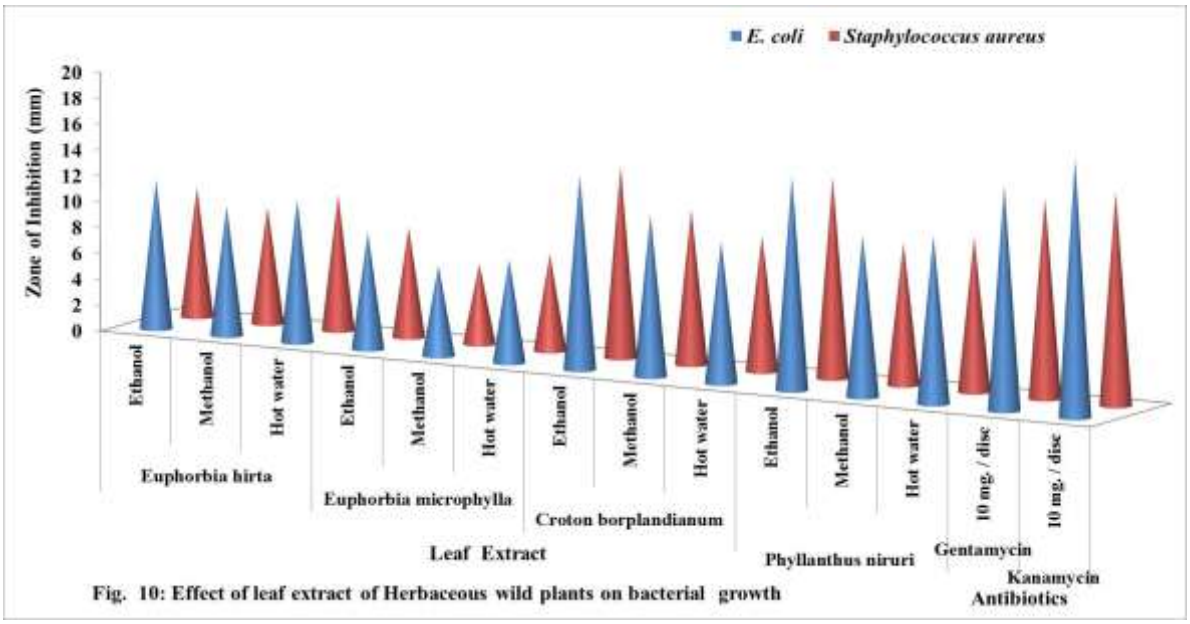

Fig. 10: Effect of leaf extract of Herbaceous wild plants on bacterial growth

CONCLUSION

The results of present investigation revealed that these wild herbaceous plants have good potentiality against bacterial growth. The extract prepared using the ethanol possessed greater antibacterial activity than other solvent. Toxic potentiality of these four plants of Euphorbiaceae have found as - Antibiotics > *Phyllanthus niruri* > *Croton bonplandianum* > *Euphorbia microphylla* > *Euphorbia hirta*. The present investigation also justifies the classified uses of the four herbaceous wild plants in the traditional system of medicine to treat various infectious diseases caused by microbes. Further chemical investigation may be carried out to isolate and identified the chemical constituents in the selected plants responsible for the antimicrobial activity. The plant may be screened for other potential biological activities as well. Such screening of various natural organic compound of plant origin and identifying active agent is the need of present therapeutic demand and development of new drug.

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