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Phytochemical and Antimicrobial Evaluation of Plant Extracts of *Enicostemma hyssopifolium*

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ABSTRACT

Enicostemma hyssopifolium, a useful medicinal herb was subjected to antimicrobial as well as phytochemical investigations. The petroleum ether, chloroform, *n*-Butanol, ethanol and aqueous extracts of the whole plant of *Enicostemma hyssopifolium* were studied for *in vitro* antimicrobial activity against Human pathogenic microorganisms. The antimicrobial potential of *Enicostemma hyssopifolium* against human pathogenic microorganisms was investigated. Their isolated phytoconstituents were evaluated for their antimicrobial potential. Antimicrobial activity of various plants extracts was compared with commercially available antibiotics. The antimicrobial potential of the above plant extracts was seen against the test organism using agar gel diffusion susceptibility test by standard technique of Opara and Anasa(1993)[9]. The resulting zones of inhibition were measured in mm. The MIC (Minimum inhibitory concentration), MBC (Minimum Bactericidal Concentration) and MFC (Minimum Fungicidal Concentration) of the plant extracts was determined according to the Micro broth dilution technique (Murray *et.al.*1999)[7]. Phytochemical estimation was carried out according to the methods described by Trease and Evans (1989)[17]. Among all solvents used ethanol extract gave the highest zone of inhibition. With *Escherichia coli,* zone of inhibition was 20.0mm, with Coagulase (+) *Staphylococci* it was 27.3 mm., with Coagulase (-) *Staphylococci* 26.6 mm., with Pseudomonas aeruginosa 25.0 mm., with Enterococcus sp. 23.6 mm., with Candida albicans and Candida parapsilosis it was 22.0 mm and 20.5 mm respectively. Phytochemical analysis showed the presence of various phytoconstituents.

Keywords: Antimicrobial activity, phytochemical analysis, *Enicostemma hyssopifolium*

1. Introduction

Enicostemma hyssopifolium belongs to family Gentianaceae commonly known as Nava. Its common use in ayurvedic medicine is in antimalarial, abdominal discomfort, colic and for promoting digestion the collection were made from different area of Ajmer district i.e. Kishangarh (Site1), Beawar (Site2), Kekri (Site3), Pushkar (Site4) and Ajmer (Site5) for the study of antibacterial and antifungal activity. Medicinal plants have been used for the treatment of various human ailments since long. A revolution came in the medicinal world with the discovery of antibiotics, for treatment of various bacterial infections. However, their indiscriminate use has led to an alarming increase in antibiotic resistance among microorganisms, giving rise to multiresistant strains, which has become a global concern (Shariff, 2001) [13]. Thus, there is a renewed interest in exploring natural resources for such compounds. The need of the hour is to screen a number of new medicinal plants for promising biological activity and there *in vitro* propagation to conserve the biodiversity [6, 14, 15].

The antimicrobial potential of the above plant extracts was seen against the test organisms using the agar-gel diffusion susceptibility test. Sterile Mueller – Hinton plates were taken one plate/organism tested. Three wells of about 3.0 mm diameter were aseptically punched on each agar plate using a sterile cork borer, with at least 30 mm distance between adjacent wells and the periphery. According to the standard technique of Opara and Anasa (1993) [9] - 2-4 colonies of the test organisms were inoculated in sterile broth and these inoculums was swabbed using sterile swab on the surface of above punched Mueller - Hinton agar plates. A fixed volume (0.1 ml) of the plant extract was then introduced into the wells in the increasing concentration and then incubated at 37 ^{0}C for 24 hours. The resulting zones of inhibition were measured.

2.3 Determination of minimum inhibitory concentration (MIC):-

The MIC of the plant extracts was determined according to the micro broth dilution technique (Murray *et al.,* 1999) [7]. It was performed in 96-well microtiter plates for determining the minimum inhibitory concentration (MIC). Standardized suspensions of the test organisms(*Pseudomonas aeruginosa*, *Escherichia coli,* Coagulase positive *staphylococci,* Coagulase negative *staphylococci* , *Enterococcus* species, *Candida albicans* and *Candida parapsilosis*) were inoculated into a series of 96-well microtiter plate, including one growth and one sterility control .Brain Heart Infusion(BHI) and Sabouraud dextrose broth containing plant extracts in increasing concentration *viz* 2.5,5,7.5,10,12.5,15,17.5,20 mg/ml. and incubated at 37 ^{o}C for 24 hours. After overnight incubation these tubes were observed for turbidity. The microtiter plate showing the minimum turbidity was noted for MIC.

2.4 Minimum Bactericidal Concentration (MBC)

The MBCs were determined by first selecting tubes that showed no growth during MIC determination; a loop ful from each tube was sub cultured onto extract free agar plates, incubated for further 24 hours at 37 ^{o}C. The least concentration, at which no growth was observed, was noted as the MBC.

2.5 Minimum Fungicidal Concentration (MFC)

The MFC were determined by first selecting tubes that showed no growth during MIC determination; a loop full from each tube was sub cultured onto extract free agar plates, incubated for further 24 hours at 37 ^{o}C. The least concentration, at which no growth was observed, was noted as the MFC.

2.6 Phytochemical estimation: - This was carried out according to the methods described by Trease and Evans, (1989) [17]

3. Results:

All the bacterial and fungal pathogens used in this work demonstrated susceptibility to the Ethanol, n-Butanol, Chloroform, Distilled water and Petroleum ether using extracts of *Enicostemma hyssopifolium.* Ethanol extract gave the highest zone of inhibition (20.0mm) on *Escherichia coli*. Coagulase (+) *Staphylococci* showed the highest zone of inhibition i.e. 27.3 mm in diameter. Coagulase (-) *Staphylococci* showed the highest zone of inhibition i.e. 26.6 mm in diameter. With *Pseudomonas aeruginosa* the highest zone of inhibition was 25.0 mm in diameter. With *Enterococcus sp.* highest zone of inhibition recorded was 23.6 mm in diameter. With *Candida albicans and Candida parapsilosis* it showed the highest zone of inhibition i.e. 22.0 mm and 20.5 mm in diameter respectively. The observation that *Enicostemma hyssopifolium* has good inhibition against *Escherichia coli, Pseudomonas aeruginosa, Enterococcus sp.,* Coagulase *(+) Staphylococcus sp., Coagulase (-) Staphylococcus sp., Candida albicans and Candida parapsilosis* tends to prove worthy remedy to the problem of drug resistance against these pathogens which are already known to be resistant to the most of the standard antibiotics (Penicillin, Tetracycline, Erythromycin, Streptomycin, Fluconazole and Amphotericin B) Tetracycline showed no zone of inhibition against all tested microorganisms. *E.coli* developed resistance against most of the antibiotics except streptomycin. *Pseudomonas aeruginosa* and *Enterococci* showed resistance against all tested antibiotics. But *Enicostemma hyssopifolium* showed effective results against most of the resistant organisms. Bacteria developed resistance against most of the tested antibiotics. Leaf and Stem showed very effective results as compared to root of *Enicostemma hysopifolium*.Table1, 2 and 3 showed the anti-microbial effects of *Enicostemma hyssopifolium* against selected human pathogenic microorganisms.Table4 showed the commercially available antibiotics susceptibility against tested pathogenic microorganisms. Graph 1, 2, 3, 4 and 5 showed *Enicostemma hyssopifolium* Comparison of ethanolic, distilled water, n-Butanol, petroleum ether and chloroform plant parts extracts effects on test bacteria and fungi. Graph 6 showed Comparison of commercially available antibiotics with maximum zone found in *Enicostemma hyssopifolium*. Table 3 showed Preliminary phytochemical screening of plant parts of *Enicostemma hyssopifolium*. Table1 showed Minimum Inhibitory Concentration of different extracts of *Enicostemma hyssopifolium.* Table2 showed Minimum Bactericidal and Fungicidal Concentrations of different extracts of *Enicostemma hyssopifolium*. The results of these investigation showed that plant extracts of *Enicostemma hyssopifolium* possess appreciable and potential antimicrobial activity against commonly encountered microorganisms in humans. It is interesting to note that the action of the extracts of *Enicostemma hyssopifolium* is nontoxic.

4. Discussion and Conclusion

Enicostemma hyssopifolium (Chhota chirayata) is a common weed of grassland and more frequent in open fields. The herb contains glycoside and ophelic acid and used as laxative, stomachic, tonic and blood purifier .The juice of whole plant is recommended by the tribal and rural people to treat malarial fever. There is scanty literature available on the phytochemical estimation and antimicrobial effect of *Enicostemma hyssopifolium* till date. Gami *et al*., (2011) [3] worked on Screening of methanol & acetone extract of some medicinal plants species *viz*. *Alstonia scholaris* (stem bark), *Achyranthes aspera* (whole plant) *Moringa oleifera* (leaves), *Tinospora cordifolia* (stem), and *Enicostemma hyssopifolium* (stem).These were screened for their antibacterial activity. They also carried out phytochemical estimation of selected plant species. They showed the presence of protein, carbohydrate, saponins, tannin, glycoside, alkaloids, flavonoids, terpenoids, steroids and fixed oil. Our results agree with their finding in response to *Enicostemma hyssopifolium.* Patel (2010) [12] analyzed the antibacterial activity of methanolic & acetone extract of some medicinal plants species. They concluded that the secondary metabolites of plants were more soluble in methanol than that of acetone. Present investigation agrees with their statement. Patel *et al*., 2009 [11] showed aldose reductase inhibitory activity of C-glycosidic flavonoids derived from *Enicostemma hyssopifolium. They concluded that* C-glycosidic flavonoid from *Enicostemma hyssopifolium* has significant activity against sugar-induced cataract *in vitro*. Patel *et al.*, 2009 [11] worked on kinetic study for *in-vitro* intestinal uptake of monosaccharide across rat reverted gut sacs in the presence of some anti- diabetic medicinal plants. Little work has been reported on antibacterial activity of *Enicostemma hyssopifolium* the best of our knowledge till date. However extensive work on antibacterial activity of *Enicostemma axillare, Enicostemma littorale* in India has been done by many researchers. Gopal *et al*., (2011) [4] analyzed the *in-vitro* antifungal activity of various extracts of *Enicostemma littorale.* They examined the

ethanol extract pronounced activity against *Aspergillus niger* and *Candida albicans*. Same results were obtained in the present study by using *Enicostemma hyssopifolium.* Vaijanathappa *et al*., 2008 [18] worked on antioxidant activity of *Enicostemma axillare*. They concluded that the whole plant parts of *Enicostemma axillare* showed moderate total antioxidant capacity. Pavithra *et al*., (2010)[12] worked on antibacterial activity of plants *viz*. *Delonix elata*, *Enicostemma axillare, Merremia tridentata, Mollugo cerviana* and *Solanum incanum* used in Indian herbal medicine. They concluded that the methanol extract of the tested plants was most effective in inhibiting the bacterial growth. They suggested that polar solvent methanol was most successful in extracting secondary metabolites responsible for the antibacterial property than chloroform and hexane solvents. Present investigation agrees with their statement.

Baranisrinivasan *et al.,* (2009) [1] worked on hepatoprotective effect of *Enicostemma littorale* Blume and *Eclipta alba* during ethanol induced oxidative stress in albino rats. Murali *et al*., (2002) [8] studied the effect of chronic treatment with *Enicostemma littorale* in non-insulin-dependent diabetic rats .They suggested that aqueous extract of *E. littorale* is a potent herbal anti- diabetic. It produces an increase in insulin sensitivity, normalizes dyslipidemia and provides nephroprotection in diabetic rats. Deore *et al*., 2008 [2] worked on *in vitro* antimicrobial and antioxidant studies on *Enicostemma axillare* raynal leaves. They concluded that active phytoconstituents was responsible for prominent antioxidant and antimicrobial activity showed by *Enicostemma axillare.* Present investigation agrees with this statement. Soni, *et al.*, 2009[16] analyzed the *in vitro* anti plasmodial activity of *Enicostemma littorale*. They represented that the potential antimalarial action of plant and its active phytoconstituent, may give new lead to researchers in field of antimalarial drug discovery. Gupta *et al*., 2007 [5] worked on hepatomodulatory role of *Enicostemma littorale* blume against oxidative stress induced liver injury in rats. These results suggested the hepatomodulation by *E. littorale* Blume against oxidative stress mediated through interference with free radical generation and reduction in fat metabolism. The consequences of the following research have declared that many active bioconstituents of *Enicostemma hyssopifolium* acquire high quality in its working action. Thus it should be experimented upon by scientists in the progress of human medicines and drug. *Enicostemma hyssopifolium* research suggested the room for the enhancement of discovering remedies for many strains of enteric microorganisms which are now resistant to many usable and similar antibiotics in our countries. *Enicostemma hyssopifolium* is an iconic cure for human diseases as have been performed in this work. The results defined that *Enicostemma hyssopifolium* is a syllable important plant for more experiments in medical microbiology.

Table 1: Minimum Inhibitory Concentration of different extracts of Enicostemma hyssopifolium

Chloroform Extracts (mg/ ml)	R	15	10	10	15	ND	ND	ND
	S	10	10	10	10	10	10	10
	L	12.5	10	10	10	10	10	10
Aqueous Extracts (mg/ ml)	R	ND	10	10	12.5	ND	ND	ND
	S	10	10	10	10	10	12.5	20
	L	20	15	15	17.5	17.5	12.5	12.5
Ethanol Extracts (mg /ml)	R	12.5	7.5	10	10	ND	ND	ND
	S	10	5	5	7.5	7.5	10	10
	L	10	7.5	7.5	10	7.5	7.5	7.5
n-Butanol Extracts (mg/ ml)	R	12.5	7.5	10	10	ND	ND	ND
	S	10	5	7.5	7.5	10	10	10
	L	10	7.5	7.5	10	7.5	7.5	10
Petroleum Ether Extracts (mg /ml)	R	12.5	7.5	10	10	ND	ND	ND
	S	10	5	5	7.5	7.5	10	10
	L	10	7.5	7.5	10	7.5	7.5	7.5
Test Organism		**E. Coli**	**Coagulase (+) Staphylococci**	**Coagulase (-) Staphylococci**	**Pseudomonas aeruginosa**	**Enterococci**	**Candida albicans**	**Candida parapsilosis**

L' = Leaves, 'S'= Stem, 'R'=Root 'ND' = Not Detectable

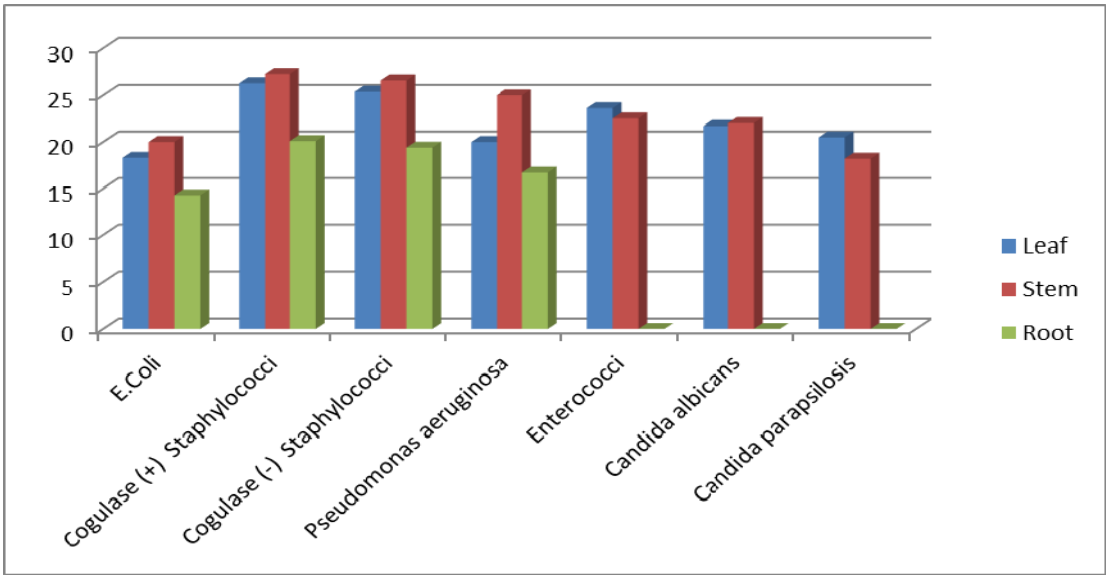
Table 2: Minimum Bactericidal and Fungicidal Concentrations of different extracts of *Enicostemma hyssopifolium*

Chloroform Extracts (mg/ ml)	R	17.5	12.5	12.5	17.5	ND	ND	ND
	S	12.5	12.5	12.5	12.5	12.5	12.5	12.5
	L	15	12.5	12.5	12.5	12.5	12.5	12.5
Aqueous Extracts (mg/ ml)	R	ND	12.5	12.5	15	ND	ND	ND
	S	12.5	12.5	12.5	12.5	12.5	15	22.5
	L	22.5	17.5	17.5	20	20	15	15
Ethanol Extracts (mg /ml)	R	15	10	12.5	12.5	ND	ND	ND
	S	12.5	7.5	7.5	10	10	12.5	12.5
	L	12.5	10	10	12.5	10	10	10
n-Butanol Extracts (mg/ ml)	R	15	10	12.5	12.5	ND	ND	ND
	S	12.5	7.5	10	10	12.5	12.5	12.5
	L	12.5	10	10	12.5	10	10	12.5
Petroleum Ether Extracts (mg /ml)	R	15	10	12.5	12.5	ND	ND	ND
	S	12.5	7.5	7.5	10	10	12.5	12.5
	L	12.5	10	10	12.5	10	10	10
Test Organism		**E. Coli**	**Coagulase (+) Staphylococci**	**Coagulase (-) Staphylococci**	**Pseudomonas aeruginosa**	**Enterococci**	**Candida albicans**	**Candida parapsilosis**

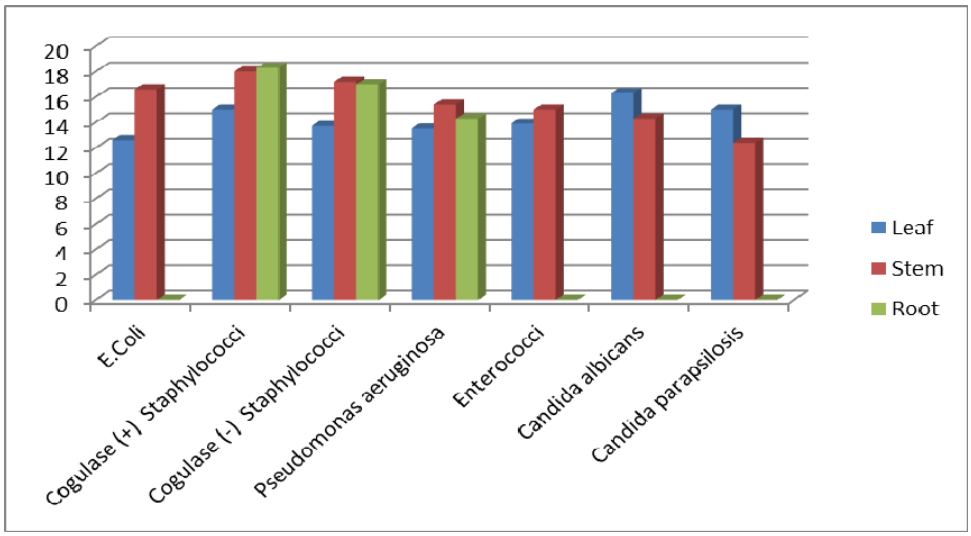
L' = Leaves , 'S'= Stem, 'R'=Root 'ND' = Not Detectable

Table 3: Preliminary phytochemical screening of plant parts of *Enicostemma hyssopifolium*

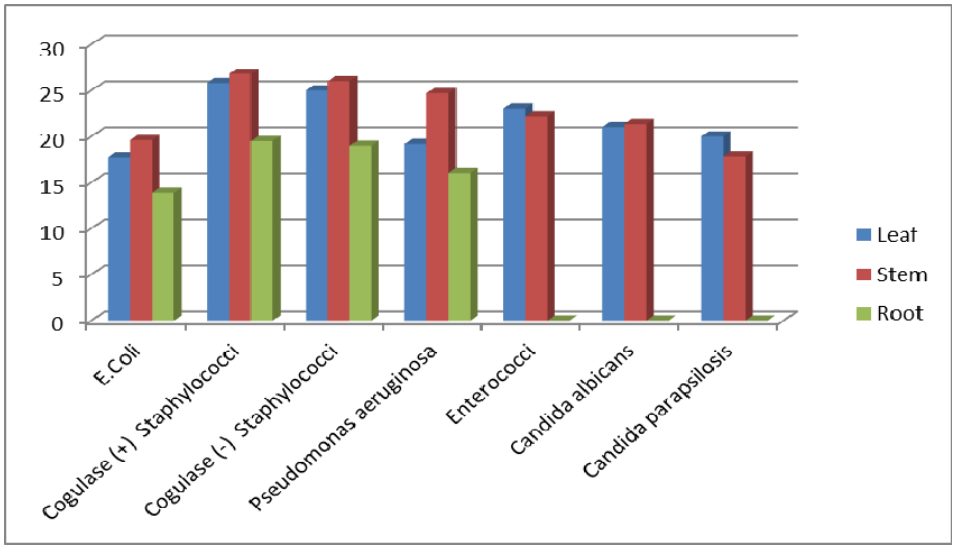
Phyto chemicals	**Distilled Water**			**Chloroform**			**Petroleum Ether**			**n-Butanol**			**ethanol**		
	L	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**
Alkaloids	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
Glycosides	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+
Saponins	+	+	+	+	+	_	+	-	-	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	-	+	+	-	-	-	-	+	+	-	+	+	-
Tannins	-	+	-	-	+	-	-	+	-	+	+	+	+	+	+
Flavonoids	+	+	-	+	+	-	+	-	-	+	+	+	+	+	+
Tritepenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reducing	-	-	-	+	+	-	-	-	-	+	+	-	+	+	-



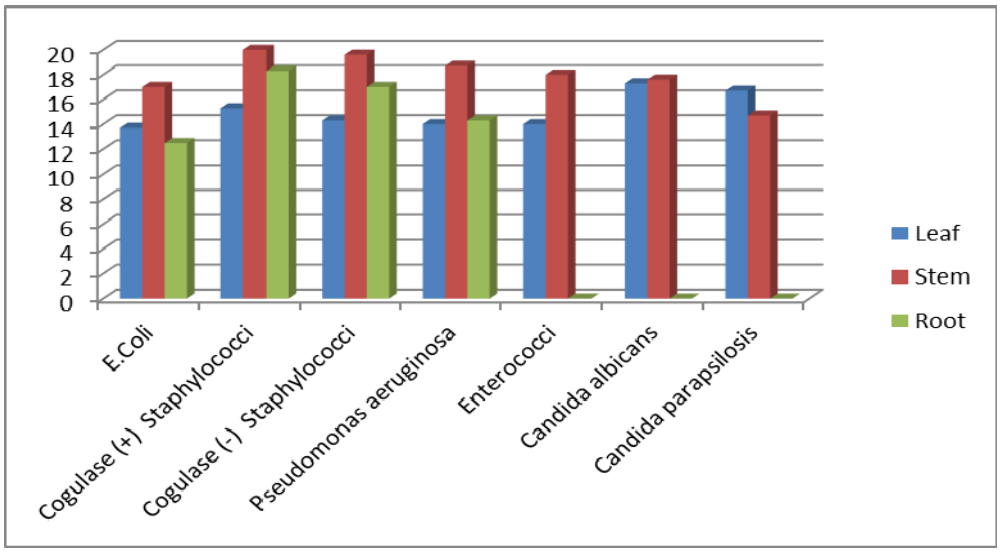
Graph 1: Comparison of *Enicostemma hyssopifolium* ethanol plant parts extracts effects on test bacteria and fung



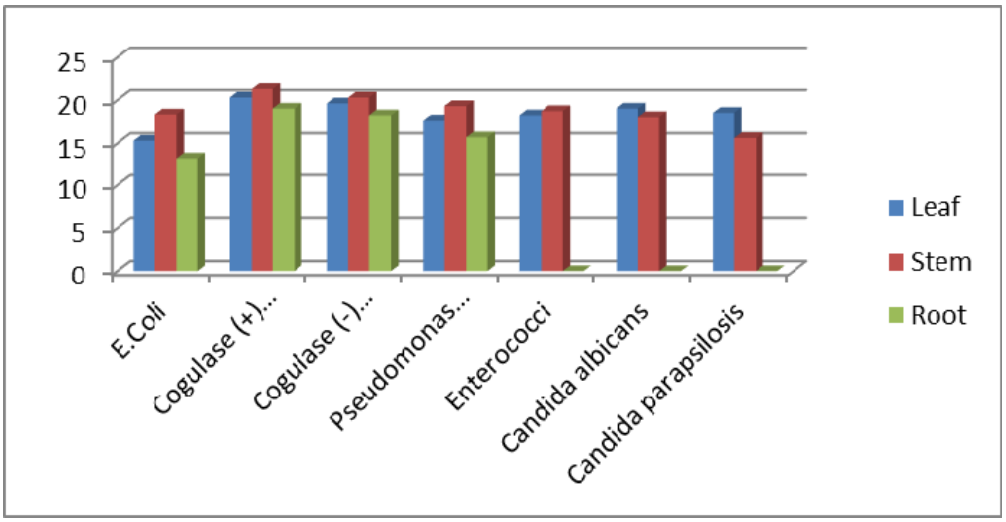
Graph 2: Comparison of *Enicostemma hyssopifolium* Distilled water plant parts extracts effects on test bacteria and fungi



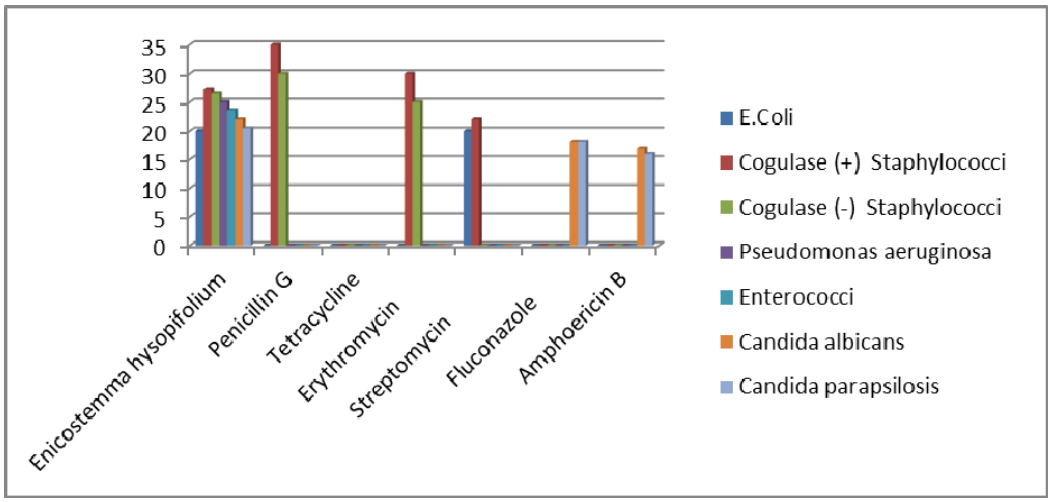
Graph 3: Comparison of *Enicostemma hyssopifolium* n-Butanol plant parts extracts effects on test bacteria and fungi



Graph 4: Comparison of *Enicostemma hyssopifolium* Petroleum ether plant parts extracts effects on test bacteria and fungi



Graph 5: Comparison of *Enicostemma hyssopifolium* Chloroform plant parts extracts effects on test bacteria and fung



Graph 6: A **c**omparison of commercially available antibiotics with maximum zone found in *Enicostemma hyssopifolium*

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