AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

The Study of Biochemical Activity and DNA Extraction from *Euphorbia hirta*

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ABSTRACT

Euphorbia hirta is native to India but is a pan tropical weed. It is used in traditional medicine for the treatment of boils, wounds and control of diarrhoea and dysentery. Therefore the Crude from different parts (leaf, and stem) of *Euphorbia hirta* (Euphorbiaceae) were extracted by different solvents and screened the antimicrobial activity by disc diffusion assay against five bacteria and 2 Fungi (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi , Bacillus subtilis, Klebsiella pnemoniae & Aspergillus Niger, Aspergillus flavus*). The Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) of stem and leaves extracts against each sensitive has also been evaluated. The maximum effect has shown in *Pseudomonas aeruginosa (7mm in leaf and 6 mm in stem)*. The leaf is having higher effect than stem. The basic photochemical analysis showed the presence of biologically active compounds in stem and leaf of *E.hirta*. The minimum effect of extract observed by soxhlet extraction *Pseudomonas aeruginosa (5mm in leaf and 3 mm in stem)*. The Alkaloids extracted by standard method and its activity also measured *Pseudomonas aeruginosa (0.5mm in leaf and 0.6 mm in stem)* , Then the crude, alkaloid & pure component of the plant parts (leaf, and stem) is compared. The crude, pure and alkaloids of leaves has higher inhibition than stem. The alkaloid is lower inhibition than crude and pure. In the two part of inhibiting activity *Pseudomonas aeruginosa* has the maximum effect. In alkaloid inhibiting activity *Salmonella typhi* has the maximum effect. DNA extracted from the leaf of the plant.

Keywords: *Euphorbia hirta*, MBC, DNA extraction, Alkaloids

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Received 13 February 2013, Accepted 12 March 2013

Please cite this article in press as: Sundari VV. *et al.*, The Study of Biochemical Activity and DNA Extraction from *Euphorbia hirta*. American Journal of PharmTech Research 2013.

INTRODUCTION

Natural products provide a rich source of bioactive molecules used for treating a wide range of different human diseases. Traditional medicinal systems such as Ayurveda, Unani, Homeopathy, Naturopathy, Siddha and others systems have been, for a long time, making use of plants as effective medicines to cure many detrimental diseases. India, considered to be the largest producer of medicinal herbs, is fittingly called the 'Botanical Garden of the World *Euphorbia hirta* the family of Euphorbiaceae. It is Terrestrial, annual, erect herb, up to 60 cm tall. Tab root white or brown. Stem rounded, solid, hairy, with abundant milk sap. Leaves simple, not lobed or divided, less than 2 cm long or wide. The stem and leaves produce white or milky juice when cut[9]. E. *hirta* is native to India and Australia. This Plant derived medicines have been a part of traditional health care in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases. Given the alarming incidence of antibiotic resistance of pathogenic microbes in particular, there is a constant need for discovering new and effective therapeutic agents. The aerial parts of the plant are harvested when in flower during the summer and dried for later use. Plant synthesized many compounds with complex molecular structures, as a result of secondary metabolism. Some of these compounds and their derivatives such as alkaloids, flavonoids, isoflavonoids, tannins, cumarins, glycosides, terpens and Phenolic compounds have antimicrobial properties. The alcoholic extract of the whole plant had an anticancer action in mice [7] .The plant has also been shown to have anti-helminthic activity

MATERIAL & METHODS

Plant Extraction

E. hirta was collected from various locations of Tanjore (Dist), Tamil Nadu. Leaves and stem powder were extracted with 25ml w/v of solvent (Acetone, Diethylether and chloroform) for 24hrs.

Selected Test Microorganisms

Pathogenic microorganisms selected for study of effect of *Euphorbia hirta* viz five bacteria, such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Bacillus cereus, Klebsiella pnemoniae and Fungi (Aspergillus flavus, Aspergillus Niger).* Bacterial strains were grown and maintained on "Nutrient Agar Medium" and for fungi maintained in PDA agar.They were stored at 40^0C until required for the study.[4,11]

Antimicrobial Assay for crude and pure sample of leaves and stem

Antimicrobial activity was evaluated by Disc Diffusion method (0.6mm in diameter of Disc).The

three solvent extracts were tested by sensitivity test. Microbial suspension was evenly placed with sterile Agar medium in Petri plates. Each extracted disc 0.5 gm concentrated .Plates were allowed to stand at room temperature for 1hr, for extract to diffuse into agar media and then incubated at 37°C for 24 - 48 hrs. The zone of growth inhibition around the disc was measured and area of inhibition was calculated [6]. The crude sample was subjected to soxhlet and analyzed the antimicrobial effects of acetone extract. The basic biochemical test also analyzed

Extraction of alkaloid

After preliminary detection of alkaloid. Different parts of E. hirta (stem &leaf) were taken for the extraction. Finely powered sample of plant parts were extracted with 10% acetic acid in ethanol for 4h. Extracts were concentrated and were make alkaline by NH4OH.Precipitate thus obtained was collected by centrifugation, washed with appropriate NH4OH, filtered, dried in and weighed. Extracts thus obtained were stored at 4°C in air tight glass vials for further use.[6] The effect of collected alkaloids were checked

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC)

Minimum inhibitory concentration (MIC) was determined for plant extract showing antimicrobial activity against test organisms in disc diffusion assay. Broth micro dilution method was followed for determination of MIC values. Bacterial suspensions were used as negative control, while broth containing standard drug was used as positive control. The micro titer plates were incubated at 37±2°C for 24h for bacteria. The MIC values were taken as the lowest concentration of the extracts in the well of the micro titer plate that showed no turbidity after incubation. The turbidity of the wells in the micro titer plate was interpreted as visible growth of microorganisms. The minimum bacterial concentration (MBC) was determined by sub-culturing 50µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on sub culturing was taken as MBC.[3]

Genomic DNA Extraction

The very young leaves are collected from *E.hirta*. Grind the 2g 0f tissue in 100ml of EEB until tissue is evenly dispersed. Incubate 60-70^{0}C for 20mins. Added chloroform equal to ½ of EEB volume, a complete emulsion of tissue were Centrifuged at maximum speed for 10mins at RT. Added two volume of 1% CTAB equal to volume of aqua's phase removed. Mixtures well then incubate at 30mins. Centrifuge at 9000 rpm for 5mins at RT. Added a volume of T50(pH8)N700E2 then added 2volume of EtOH. Then rest till 1hr at 65C.Add 2 volume of EtOH mix gently. Centrifuged at maximum speed for 5mins at RT. Decant the supernatant. 1 ml of 70% Et OH added. & rinse DNA pellet well. Centrifuge at maximum speed for 2-5mins at RT.

Decant the supernatant. Allow air dry for 1to2hrs. Resuspend 2.5g DNA in .2ml $T_{10(pH7.4)}E_{0.1}$. Incubate at 65C for until DNA fully resuspended. Then run the DNA in 1%of agarose gel at very low volt(20-30). [11]

RESULT & DISCUSSION

Antimicrobial Activity of Crude, Pure, Alkaloid

The Inhibition of pathogenic organism (*E. coli, Salmonella typhi , Bacillus subtilis, klebsiella pneumoniae, Pseudomonas aeroginosa, Aspergillus Niger and Aspergillus flavu*[16] by different organic solvent extraction and its tabulated for crude and pure(table 1,2,4) among 5 bacteria sps. *Pseudomonas* has shown maximum Inhibition(7mm in leaf and 6 mm in stem) in all extracts MBC,MIC also analyzed (table5,6,7,8) Figure 1

Table: 1 Antimicrobial activity of crude extract

S.No	Micro Organism	Solvents					
		Acetone		Chloroform		Di	Ethyl
		Leaf	Stem	Leaf	Stem	Leaf	Stem
1	*E. coli*	-	-	-	3	-	-
2	*Salmonella typhi*	6	4	3	-	1	-
3	*Bacillus subtilis*	3	1	-	-	-	2
4	*Klebsiella pneumoniae*	2	2	3	-	2	1
5	*Pseudomonas aeruginosa*	7	6	-	-	-	-
	Fungi						
1	*Aspergillus niger*	-	-	-	-	-	-
2	*Aspergillus flavus*	-	-	-	-	-	-

Table 2: Anti microbial Activity for Pure extract

Microorganism	Acetone	
	L	S
E. coli Salmonella typhi	3	2
Bacillus subtilis	1	0.6
Klebsiella pneumonia	0.9	0.4
Pseudomonas Aeruginosa	5	3

Table3. Preliminary phytochemical screening of ethanolic extract of Euphorbia hirta

S.No	Phytochemical	Result
1	Alkaloid	++
2	Flavonoids	+
3	Saponin	-
4	Coumarins	+
5	Ploy phenols	++
6	Cardiac glycosides	+
7	Triterpenes	+++
8	Cyanogenic glycosides	-

Table4. Antimicrobial activity of Alkaloids

Microorganism	L	S
E. coli	-	-
Salmonella typhi	3	1
Bacillus subtilis	1.5	0.3
klebsiella pneumoniae	1	0.8
Pseudomonas Aeruginosa	-	0.9

Table 5 MIC and MBC values of *Euphorbia hirta* against test pathogens (acetone)

Microorganism	L		S	
	MIC	MBC	MBC	MIC
E. coli	-	-	-	-
Salmonella typhi	0.32	0.64	0.33	0.66
Bacillus subtilis	0.34	0.68	0.36	0.72
Klebsiella pneumoniae	0.69	0.69	0.7	0.7
Pseudomonas Aeruginosa	0.31	0.15	0.315	0.65

Table 6. MIC and MBC values of *Euphorbia hirta* against test pathogens (Chloroform)

Microorganism	L		S	
	MIC	MBC	MIC	MBC
E. coli	-	-	0.35	0.69
Salmonella typhi	0.34	0.68	-	-
Bacillus subtilis	-	-	-	-
Klebsiella pneumonia	0.38	0.69	-	-
Pseudomonas Aeruginosa	-	-	-	-

Table:7 MIC and MBC values of *Euphorbia hirta* against test pathogens (Diethylether)

Microorganism	L		S	
	MIC	MBC	MIC	MBC
E. coli	-	-	-	-
Salmonella typhi	0.36	0.72	-	-
Bacillus subtilis	-	-	-	-
Klebsiella pneumonia	0.7	0.7	-	-
Pseudomonas Aeruginosa	0.71	0.71	-	-

Table 8: MIC and MBC values of *Euphorbia hirta* against test pathogens(Alkaloids)

Microorganism	L		S	
	MIC	MBC	MIC	MBC
E. coli	-	-	-	-
Salmonella typhi	0.44	0.88	0.48	0. 96
Bacillus subtilis	0.47	0.94	1.2	1.2
klebsiella pneumoniae	0.5	0.98	0.48	0.98
Pseudomonas Aeruginosa	-	-	0.9	0.9

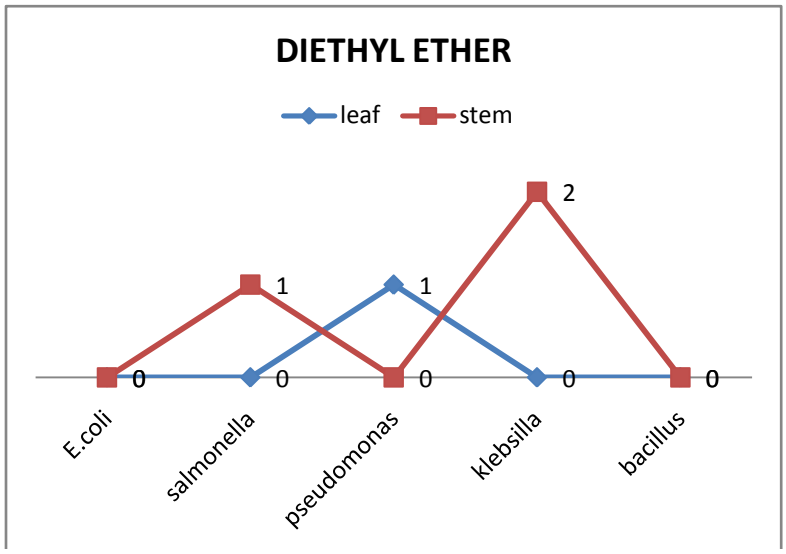

Figure 1 Antimicrobial activity of Crude in *Euphorbia hirta* by Disc Diffusion Assay (Di Ethyl Ether, Acetone, Chloroform)

Phytochemical Analysis

The photochemical activity of *E.hirta* leaf and stem showed positive result has indicate presence of biological active compound (table3)

Antimicrobial effect of extracted alkaloids:

0.5 gm of alkaloids were extracted and the effect of alkaloids were checked with the same bacterial species and the minimum, maximum inhibition shown as 1.5 mm in leaves and1mm in stem .(Table 4) (Figure 2)

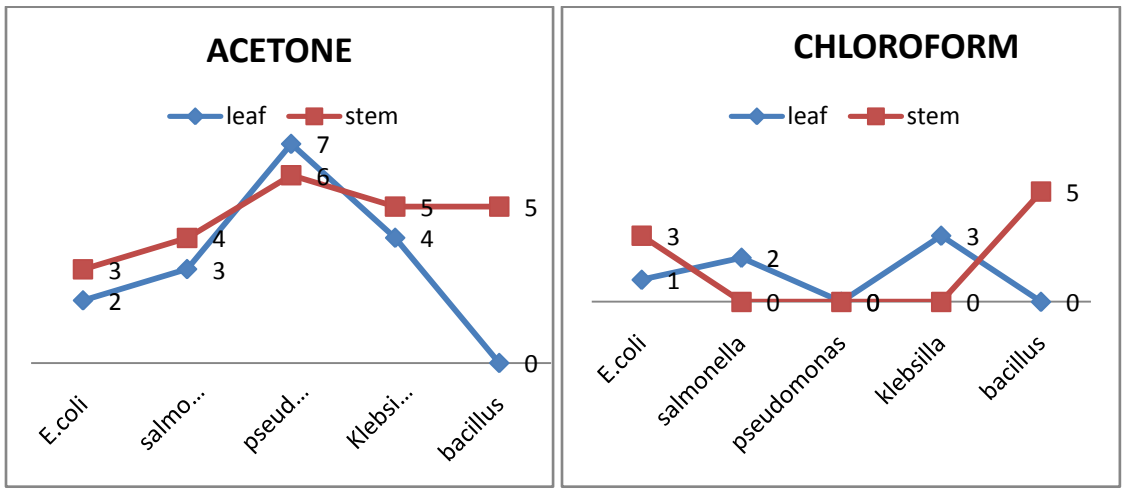

Figure2. Antimicrobial activity of Alkaloids of *Euphorbia hirta* by Disc Diffusion Assay

Genomic DNA Extraction

From the fresh leaves (0.2g) DNA has been extracted and electrophoresed with 1% agarose 2 different bands observed in same sample showed in figure 3.

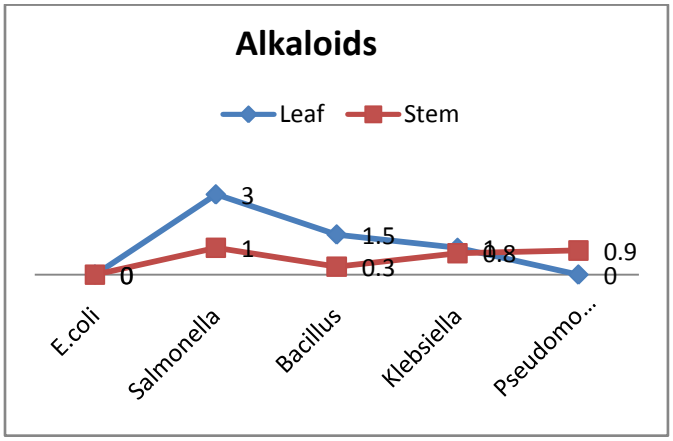

Figure: 3 Genomic DNA in 1% agarose Gel

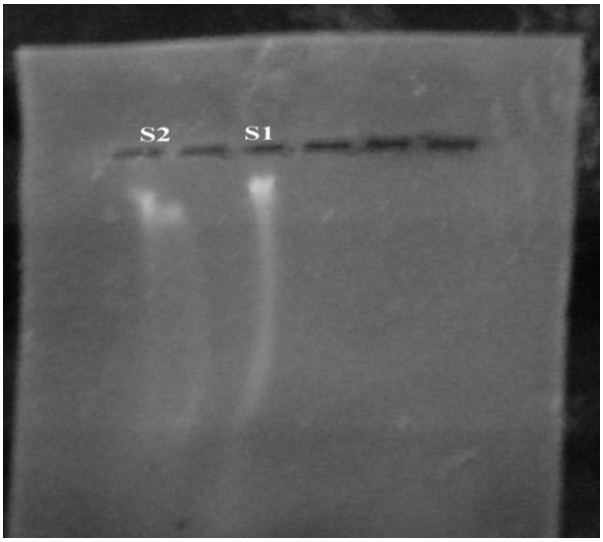

Figure 4:S1 - Stem DNA Sequence S2 – Leaf DNA Sequence

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