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PRELIMIARY PHYTOCHEMICAL AND ANTIBACTERIAL IVESTIGATIONS OF *EUPHORBIA NIVULIA* STEM EXTRACTS

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ABSTRACT

Euphorbia nivulia stem extracts (petroleum ether, chloroform, Ethanol and water) were used for analysing their potential phytochemical constituents and antimicrobial property. Results of the phytochemical screening revealed the presence of alkaloids, phenols, tannins, oil (Ethanol extract). Crude extracts (various solvents) of *Euphorbia nivulia* stem were used for studying their antimicrobial property. Antimicrobial activity results revealed that Ethanol extract showed the maximum activity, where as the petroleum ether, chloroform and water extract shows very less activity or no activity against the microbial strains used in this study.

Keywords: Antibacterial, *Euphorbia nivulia,* Ethanol, Phytochemicals.

INTRODUCTION

Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases [1-5]. The increase in resistance of microorganisms due to indiscriminate use of commercial antimicrobial drugs encouraged scientists to search for new antimicrobial substances from various sources including medicinal plants [6-11]. Phytochemicals such as nicotine, pyrethrins, rotenoids, brass inosteroids and azadirachtin obtained from plants have been evaluated and a few of them are also exploited commercially. The potential of higher plants as a source for new drugs is thus still largely unexplored [12-14]. Traditionally *Euphorbias* are used as purgative, analgesic, anti-inflammatory, antipyretic, antimicrobial, antiparasitic, in the treatment of cough, asthma, rheumatism, cancer and other maladies as folk remedy [15-17]. In the northeast region of Brazil, the latex of *Euphorbia nivulia*is used as an antimicrobial agent; a laxative agent; to control intestinal parasites; to treat asthma, cough, earache, rheumatism, cancer, epithelioma, sarcoma, skin tumors and as a folk remedy against syphilis [17]. Antibacterial activity has been reported for different euphorbia species [18-20]. Hence, the present study has been made to investigate the phytochemical constituents of *Euphorbia nivulia*stem extracts and their antimicrobial activities.

MATERIALS AND METHODS

Collection of plant materials

Fresh stems of *Euphorbia nivulia* were collected and washed thoroughly 2-3 times with running tap water and once with distilled water, and then air dried and finally dried in hot air oven at 60°C for 48 hours.

Solvent extraction

Dried stems of *Euphorbia nivulia* were powdered by using blender. 100 g of powdered stem were subjected to successive extraction by using different solvents in the increasing order of their polarity (petroleum ether, chloroform, Ethanol) in soxhlet apparatus, until the plant extract became colourless and then evaporated under the fan. The aqueous extracts were concentrated using hot air oven at 45°C till it dried.

Phytochemical analysis

Qualitative phytochemical analysis of the *Euphorbia nivulia* stem extracts was determined as follows: Alkaloids (50mg of solvent free extract with few

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ml of HCl, filtered); a 2ml of filtrate + 2 drops of Mayer's reagent / Wagner's reagent / Hager's reagent, white creamy precipitate / reddish brown precipitate / yellow precipitate indicated the presence of respective alkaloids. Carbohydrates (100mg extract + 5ml of distilled water, filtered); 1ml filtrate boiled over water bath + 2ml of Fehling's solutions (A and B) were added. 0.5ml of filtrate + 0.5ml of Benedict's reagent was added and heated over boiling water bath for 2 min. Appearance of red precipitate and characteristic precipitate indicated the presence of carbohydrates. Saponins (frothing test: 50mg extract + 20ml distilled water); The suspension was shaken in graduated cylinder for 15min. A two layer of foam indicated the presence of saponins. Tannins (50mg extract + 5ml distilled water); diluted extract + few drops of 5% FeCl3 dark green colour indicated the presence of tannins. Phenols: diluted extract + 3ml of 10% lead acetate white precipitate indicated the presence of phenolic compounds [21].

Bacterial Strains

Microorganisms were obtained from the National Collection of Industrial Microorganisms (NCIM), Pune, India. Amongst twelve microorganisms investigated, three Gram positive rods were *Bacillus cereus* NCIM2458, *Bacillus subtilis*NCIM2547 and *Bacillus pumilus*NCIM2189. Three Gram positive cocci were *Staphylococcus aureus* NCIM2672, *Staphylococcus epidermidis* NCIM2493 and *Streptococcus pyogenes* NCIM2608. Six Gram negative rods were *Escherichia coli* NCIM2809, *Escherichia coli* NCIM2810, *Enterobacteraerogenes*NCIM5139, *Klebsiella pneumonia* NCIM2707, *Proteusmirablis*NCIM2388 and *Citrobacter freundii* NCIM2489. All the microorganisms were maintained at 4 °C on nutrient agar slants.

Agar well-diffusion assay

In vitro antibacterial activity was examined for petroleum ether, chloroform, Ethanol and aqueous extracts from *Euphorbia nivulia* stem. The antibacterial activity of the extracts was determined by using the agar well diffusion technique [22]. Mueller- Hinton agar (Himedia, Mumbai) were seeded with 0.1 ml of overnight culture were allowed to set and well made by sterile cork borer (6 mm) and 40 µl of extract was added into each well. Then bacterial plates were incubated at 37°C 24 hours. Microbial growth was determined by measuring the diameter of zone of inhibition. DMSO (Dimethyl sulfoxide) was used todissolve the plant extract and also used as control.

RESULTS AND DISCUSSION

Different solvents were used (petroleum ether, chloroform, Ethanol, water) to extract the metabolites form the *Euphorbia nivulia* stem. The extracted samples were used for analyse the phytochemical constituents and their antibacterial activity.

Antibacterial activity of *Euphorbia nivulia* stem extracts

The antibacterial activity of various extracts of *Euphorbia nivulia* stem were analysed *invitro* by agar well diffusion method against twelve bacterial species. Among the twelve strains only five (*Bacillus cereus, Klebsiella pneumonia, Proteus mirablis, Staphylococcus aureus, Streptococcus pyogenes)* bacteria were inhibited to extracts. The activities of extracts against the five organisms were given in Table 1 and Figure 1. Among the four extracts, Ethanol extract showed antibacterial activity against five bacterial strains. The aqueous extract did not show any antibacterial activity. The petroleum ether and chloroform extract showed activity only against *Bacillus cereus.* The Ethanol extract of the investigated plant showed maximum (17mm) antibacterial activity against Gram negative bacteria *Proteus mirablis.* Recently many reports are published that Ethanolic extract of many plants showed very good activity. Especially in Euphorbia family [23] reported that the ethanol extract of *E. fusiformis* showed antibacterial activity against *S.aureus* and [24] reported that Ethanol extract of *E. hirta* and *Euphorbia nivulia* revealed antibacterial activity against *Staphylococcus epidermidis*. The sensitivity of test strains was, in decreasing order: *Proteus mirabilis* > *Staphylococcus aureus*>*Klebsiella pneumoniae*> *Streptococcus pyogenes*> *Bacillus cereus*. The basis for their differences in susceptibilitymight be due to the differences in the phenolic and alkaloid property of the *Euphorbia nivulia* composition [25]. *Bacillus cereus* was least sensitive compared to other test bacteria, whichmay be due to their ability to form highly resistant resting stages called endospores. Thisantibacterial study coincides with the previous study and showed activity against five neworganisms, which is not reported earlier.

In this study, it is not surprising that there are differences in the antimicrobial properties in different extracts of plants due to the phytochemical constituents. Some of the plant extracts may have contained potential antibacterial constituents, not in sufficient concentrations so as to be effective. It is also possible that compound may not soluble in petroleum ether, chloroform, Ethanol and water [26]. This is only a preliminary study of the occurrence of certain properties of *Euphorbia nivulia* stem an in-depth study will provide a good concrete base of all the phytochemicals functions mention above.

CONCLUSION

In the present study, we have found that most of the biologically active phytochemicals were present in the Ethanol extract of *Euphorbia nivulia* stem. Ethanol extract of this plant showed highest activity against the selected bacterial strains. Least activity was observed in

Table 1. Antimicrobial activity of *Euphorbia nivulia* stem extracts

Microorganisms	Solvents	Zone of inhibition in mm							
		Concentration of plant extract mg/ml							
		5	10	15	20	25	30	37.5	50
Bacillus cereus	PE	8	8	8	8	9	9	10	10
	CHCl4	9	9	10	11	11	12	12	12
	Ethanol	-	8	9	9	10	10	12	11
Klebsiella pneumonia	Ethanol	-	-	-	-	8	10	11	10
Proteus mirablis	Ethanol	8	11	14	14	15	12	16	18
Staphylococcus aureus	Ethanol	12	10	12	14	15	16	15	14
Streptococcus pyogenes	Ethanol	-	-	-	8	9	10	11	11

PE - Petroleum ether, CHCl4 – Chloroform

Table 2. The analysis of phytochemicals in the petroleum ether, chloroform, Ethanol and aqueous extract of *Euphorbia nivulia* stem

TESTS	Inference			
	Petroleum ether	Chloroform	Ethanol	Aqueous
Alkaloids	-	-	+	-
Carbohydrates	-	-	+	+
Saponins	-	-	-	-
Oils	+	+	-	-
Tannins	-	+	+	+
Phenols	-	-	+	-

chloroform extract followed by petroleum ether extract and no antibacterial activity was found in aqueous extract.

Euphorbia nivulia stem extracts possesses a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promising extracts open the possibility of finding new clinically effective antibacterial compound for a wide range of gram positive and gram negative microbes.

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REFERENCES

1. Rajasekharan PE, Ganeshan S. Conservation of medicinal plant biodiversity in Indian perspective. *J Med Aromatic Pl Sci.*, 24, 2002, 132-147.
2. Schultes RE. The Kingdom of plants In: W.A.R. Thomson (ed.), Medicines from the earth, McGraw Hill Book Co., New York., 1978, 208.
3. Leeja L, Thoppil JE. Antimicrobial activity of Ethanol extract of Origanum majorana L. (Sweet marjoram). *J. Environ. Biol*., 28, 2007, 145-146.
4. Tonk, Shobhita, Roli Bartarya, Maharaj Kumari K, Bhatnagar VP, Srivastava SS. Effective method for extraction of larvicidal component from leaves of Azadirachta indica and Artmisia annua Linn. *J. Environ. Biol*., 27, 2006, 103-105.
5. Setia N, Batish DR, Sing HP, Kohli KK. Phytotoxicity of volatile oil from Eucalyptus citriodora against some weedy species. *J. Environ. Biol*., 28, 2007, 63-66.
6. Piddock KJV, Wise R. Mechanisms of resistance to quinolones and clinical perspective. *J AntimicrobChemother*., 23, 1989, 475-483.
7. Singh M, Chaudhry MA, Yadava JNS, Sanyal SC. The spectrum of antibiotic resistance in human and veterinary isolates of Escherichia coli collected from 1984-1986 in Northern India. *J AntimicrobChemother*, 29, 1992, 159-168.
8. Mulligen ME, Murry-Leisure KA, Ribner BS, et al. Methicillin resistant Staphylococcus aureus. *Am. J. Med*., 94, 1993, 313-328.
9. Davis J. Inactivation of antibiotics and the dissemination of resistance genes. *Science*, 264, 1994, 375-382.
10. Robin EH, Anril W, Alexander, M, Loeto, M, Keith K. Nasopharyngeal carriage and antimicrobial resistance in isolates of Streptococcus pneumoniae and Heamophilus influenzae Type b in children under 5 years of age in Botswana. *IJID*, 3(1), 1998, 18-25.
11. Karaman I, Sahin P, Gulluce M, Oguten, et al. Antimicrobial activity of aqueous and Ethanol extracts of Juniperus oxycedrus L. *J Ethanopharmacol*., 2837, 2003, 1-5.

12. Dubey NK, R Kumar, P Tripathi. Global promotion of herbal medicine: Indian's opportunity. *Current Sci.*, 86, 2004, 37-41.
13. Patwardhan B, ADB Vaidya, M Chorghade. Ayurveda and natural products drug discovery. *Current Sci*, 86(6), 2004, 789-799.
14. Kumar P. Valuation of medicinal plants for pharmaceutical uses. *Current Sci*., 86(7), 2004, 930-937.
15. Hohmann J, Molnar J. Euphorbiaceae diterpenes: Plant toxins or promising molecules for the therapy? *Acta Pharm Hung*, 74, 2004, 149-157.
16. Newall CA, Anderson LA, Phillipson JD. Herbal medicines. London. The Pharmaceutical Press, 1996, 109.
17. Betancur-Galvis L, Morales GE, Forero JE, Roldan J. Cytotoxic and antiviral activities of Colombian medicinal plant extract of the Euphorbia genus. *Mem Inst Oswaldo*., 97, 2002, 541-546.
18. Annapuna J, Chowdary I, Lalitha G, Ramakrishnan S, Iyengar, D. Antimicrobial activity of Euphorbia nivulia leaf extract. *Pharm Biol*., 42, 2004, 91-93.
19. Goncalves B, Ramos V, Soares D, Mashrouah C, Silveira D. Antimicrobial and cytotoxic activities screening of some Brazilian medicinal plants used in governadorvaladares district. *Rev Bras Cienc Farm*., 42, 2006, 195-202.
20. Sudhakar M, CV Rao, PM Rao, DB Raju, Y Venkateswarlu. Antimicrobial activity of Caesalpiniapulcherrima, Euphorbia hirta and Asystasiagangeticum. *J. Fitoterpia*., 77, 2006, 378-380.
21. Raaman N. (2004). *Phytochemical Techniques*, 2004, 1-275.
22. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45, 1996, 493-496.
23. Natarajan D, Britto S, Srinivasan K, Nagamurugan N, Mohanasundari C, Perumal G. Antibacterial activity of Euphorbia fusiformis.A rare medicinal herb. *J Ethanopharmacol*., 102, 2005, 123-126.
24. Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and Ethanol extracts of some medicinal plants for potential antibacterial activity. *Turk J. Biol*., 29, 2004, 203-210.
25. Grosvenor PW, Supriono A, Gray DO. Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2, Antibacterial and antifungal activity. *J Ethnopharmacol*., 45, 1995, 97- 111.
26. Stainer RY, JL Ingraham, ML Wheelis. General Microbiology, 5th ed. London: The MacMillan Press Ltd, 1986.