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Review Article

Analysis of Leaf Extract of *E. hirta* for Antibacterial Sensitivity

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Abstract

Euphorbia hirta L. (Asthma Lata), a pantropic herbaceous wild plant which has been widely used as a medicinal plant throughout Bangladesh. The present study was designed to evaluate the antimicrobial activity of ethanolic and methanolic leaf extracts of E. hirta against six pathogenic bacteria viz. Bacillus subtilis, Sarcina lutea, Xanthomonas campestris, Escherichia coli, Klebsiella pneumonia and Pseudomonas sp. by disc diffusion method. Both crude extracts of E. hirta exerted the highest efficiency against S. lutea. The MIC value was lowest 128 µg/ml from ethanolic extract against S. lutea and K. pneumonia that produced 3±0.14 and 3±0.09 mm zone of inhibition respectively. The highest MIC value was 512 µg/ml from methanolic extract against S. lutea that produced 5±0.35 mm zone. The activity of ethanolic and methanolic extracts were compared with commercial antibiotic Cloxacillin.

Keywords*: euphorbia hirta, antimicrobial activity, evaluation, disc diffusion, zone of inhibition.*

INTRODUCTION

Despite tremendous progress in human medicine, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Plants are an important source of medicines and play a key role in world health [1]. Over 50% of all modern clinical drugs are of natural products origin [2] and natural products play an important role in drug development programs in the pharmaceutical industry [3]. Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases [4]. Herbal treatment is one possible way to treat diseases caused by multidrug resistant bacteria [5].

Euphorbia hirta L. belongs to the plant family Euphorbiaceae and genus Euphorbia. It is a slender-stemmed, annual hairy plant with many branches from the base to top, spreading up to 40 cm in height [6] and reddish or purplish in color. Leaves are opposite, elliptic – oblong to oblong – lanceolate, acute or subacute, dark green above; pale beneath, 1–2.5 cm long, blotched with purple in the middle, and toothed at the edge. The fruits are yellow, three-celled, hairy, keeled capsules, 1–2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds [7–9]. It is a potent medicinal plant and has established its sedative and anxiolytic activity [10], analgesic, antipyretic, anti-inflammatory, antidepressant for blood pressure [11], antihypertensive [12] and antioxidant [13].

MATERIALS AND METHODS

Plant Material

The leaves of *E. hirta* were collected from Kushtia district of Bangladesh during the month of July, 2011. This plant was then botanically identified by taxonomists and

the name of the plant, time, place, and date of collection were recorded. The leaves were initially rinsed with distilled water and dried on a paper towel in laboratory under shade and used for the present study.

Preparation of the Extract

Collected leaves were cleaned with deionized water and dried in shade and pulverized into fine powdered substances by a grinding machine. Each 30 g of powder was transferred into two separate 100 mL conical flasks. Then each 40 mL of methanol and ethanol (Merck-Limited, India) were added in the flasks respectively, closed by foil paper and placed on a shaker at 37 °C temperature for 24 h. The crude extracts were then filtered by passing the extracts through Whatman No. 1 filter paper (UK) and then concentrated under vacuum at 40 °C by using a rotary evaporator. The standard extract obtained was then stored in a refrigerator at 4 °C for further use [14].

Test Bacteria

Pure culture of three Gram-negative, i.e., *Escherichia coli, Pseudomonas sp, Klebsiella pneumoniae* and two Gram-positive, i.e., *Bacillus subtilis, Sarcina lutea* and *Xanthomonas campestris* bacterial isolates were obtained from the microbial type culture collection (MTCC) of Microbiology Laboratory of the Biotechnology and Genetic Engineering Department, Islamic University, Kushtia-7003, Bangladesh. The test bacteria were cultured on nutrient agar (Hi-Media, India) at 37 C for 24 h.

Bacterial Culture Media

For cultivation and maintenance of different bacterial culture and for the identification and microbial sensitivity, nutrient agar (Hi-Media, India) was used. Lactose broth (LB) media was used for culturing of the bacteria. Lactose broth is also used for the detection of coliform organisms in water, dairy products, and other materials [15–17].

Inoculum Preparation

The OD (optical density) was measured with a spectrophotometer at a wavelength of 530 nm and bacterial population was confirmed to be within 10^7 mL^{-1} to 10^8 mL^{-1} and then plated out as inoculums [18].

Antibacterial Activity

The antibacterial activity of the test samples was tested by disc diffusion method [19–21]. The filter paper discs of 6 mm diameter were prepared using Whatman No. 1 filter paper (UK), soaked in extract and incubated for 17 h at room temperature for the purpose. The discs dipped in respective solvent were used as negative controls. The antibacterial agent cloxacillin was used as standard. 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 and 1 mL of bacterial culture was added. The impregnated discs were aseptically placed on the solidified agar media. The plain discs and standard were also placed on the solidified agar media. After 24 h of incubation at 37 °C temperature the culture plates were examined and the diameters of the inhibition zones were measured in mm unit. Minimum inhibitory concentration (MIC) was determined in the present study following the serial dilution technique [22].

Statistical Evaluation

The antibacterial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD (standard deviation).

RESULTS AND DISCUSSION

From Figure 1, it is seen that the leaf powder extract from ethanol of *E. hirta*

showed antibacterial activity against *S. lutea*, *E. coli* and *K. pneumoniae*. The crude extract of leaf powder produced 16 mm zone of inhibition against *S. lutea* and 11 mm zone of inhibition against *E. coli*.

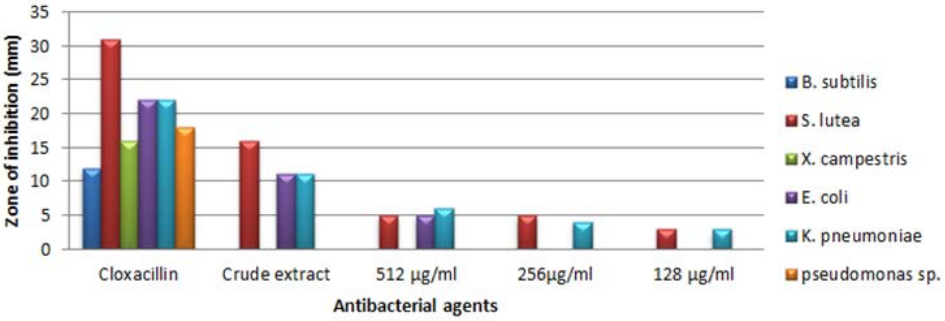

Fig. 1: *Comparative Antibacterial Activity of Commercial Disc (Cloxacillin 5 µg/µL)and Ethanolic Extract of E. hirta Leaf Powder.*

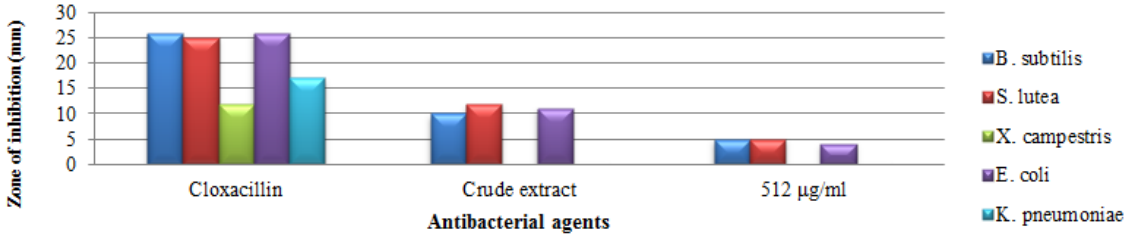

Fig. 2: *Comparative Antibacterial Activity of Commercial Disc (Cloxacillin 5 µg/µL)and Methanolic Extract of E. hirta Leaf Powder.*

It has been shown that the crude methanolic extract of *E. hirta* leaf powder produced the highest 12 mm zone of inhibition against *S. lutea* and 10 mm against *B. subtilis* and 11 mm zone of inhibition against *E. coli* (Figure 2).

Minimum inhibitory concentration (MIC) was tested against all of the bacterial strains by using different concentrations of crude extracts. For the ethanolic extract, the MIC was 128 µg/mL against *S. lutea* and *K. pneumoniae* whereas it was 512 µg/mL against *E. coli*. For the methanolic extract, the MIC was 512 µg/mL against, *B. subtilis*, *S. lutea* and *E. coli* (Table 1).

Table 1: *Comparison Study of Minimum Inhibitory Concentration of Ethanol and Methanol Extract of E. hirta Leaf Powder.*

Bacterial strain	**Minimum inhibitory concentration (MIC) and zone of inhibition (DIZ)**			
	Ethanolic extracts (µg/mL)	**DIZ (mm)**	**Methanolic extract (µg/mL)**	**DIZ (mm)**
S. lutea	128	3 ± 0.14	512	5 ± 0.35
E. coli	512	5 ± 0.47	512	5 ± 0.08
K. pneumonia	128	3 ± 0.09	512	4 ± 0.11
B. subtilis	-	-	512	5 ± 0.18
DIZ = Diameter of zone of inhibition in millimeter scale.				

Impact of medicinal plant is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance [23]. Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management [21, 24–28], although over 250,000 undiscovered flowering plants with medicinal properties exist worldwide [21, 29]. The presence of antibacterial substances in the higher plants is well established [30]. Plants have provided a source of inspiration for novel drug compounds as plant-derived medicines have made significant contribution towards human health [31]. For these, the sensitivity screening study against some pathogenic bacterial strains of the plant *E. hirta* was evaluated.

As a medicinal plant, *E. hirta* has been used for female disorders but is now more important in treating respiratory ailments, especially cough, coryza, bronchitis and asthma [32]. In India, it is used to treat worm infestations in children and for dysentery, gonorrhea, jaundice, pimples, digestive problems and tumors [9]. So its traditional use has been investigated by several scientists. It was found that ethanol and methanol extracts of *E. hirta* leaf and whole plant were more effective and significant than aqueous and chloroform extracts in inhibiting the growth of the pathogenic bacteria, viz., *E. coli*, *K. pneumoniae* and *P. vulgaris* (Gram-negative) and *B. subtilis* and *S. aureus* (Gram-positive), but were less potent when compared to that of tetracycline used as positive control [33].

In the present study, the crude ethanolic and methanolic extracts of *E. hirta* showed potential antibacterial activity against both Gram-positive and Gram-negative pathogenic bacteria. For ethanolic extract, the MIC was 128 µg/mL against *S. lutea* and *K. pneumoniae* that produced 3 ± 0.14 and 3 ± 0.09 mm zone of inhibition respectively. For methanolic extract, the MIC was 512 µg/mL that produced 5 ± 0.18, 5 ± 0.35 and 4 ± 0.11 mm zone against *B. subtilis*, *S. lutea* and *E. coli* respectively. Ethanolic and methanolic extracts of *E. hirta* leaf showed the maximum degree of antibacterial activity properties. This may be due to the presence of alkaloids, tannins, saponins and flavonoids which are plant secondary metabolites known to possess antibacterial properties. Similar observations were also reported in various plant extracts with different concentrations [33, 34–38]. *X. campestris* and *Pseudomonas sp* showed slight resistance against the extract.

CONCLUSIONS

From the observed result of this project work it can be concluded that the *E. hirta* leaf extracts were found to be effective as a source of antibacterial agents against pathogenic bacteria and it was most effective against Gram-positive *S. lutea* than all other test bacteria. This study paves the way for further attention and research to identify the active compounds responsible for the antibacterial activity to be used as a potent drug to treat meningitis and pneumonia in children.

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REFERENCES

1. Constabel F. *Planta Medica* 1990; 56: 421–425p.
2. Stuffness and Douros. *Am. J. Tro. Med. Hygien* 1982; 54: 67–71p.
3. Baker et al. *Food Science* 1995; 23(7): 97–98p.
4. Lakshmi N. et al. *Biosci. Biotechnol. Res. Asia* 2006; 03:1p.
5. Olukoya D. K. et al. *J. Ethnopharmocol* 1993; 39:69–72p.

6. Ogbulie J. N. et al. *Afri. J. Biotechnol* 2007; 6(13): 1544–1548p.
7. Williamson E. M. *Major Herbs of Ayurveda, China*. Churchill Livingstone: 2002.
8. Prajapati N. D. et al. *Agarbios* 2003.
9. Kirtikar K. R., Basu B. D. *Indian Medicinal Plants with Illustrations.* Dehradun, India: Oriental Enterprises. 2003.
10. Lanhers et al. *J. Ethnopharmacol* 1990; 29:189–198p.
11. Williams L. A. D. et al. *Phytotheraphy Res* 1997; 11: 401–405p.
12. Tona L. et al. *J. Ethnopharmacol* 1999; 68: 193–198p.
13. Wong C. et al. *Food Chem* 2006; 97(4): 705–711p.
14. Akneshi C. O. et al. *Nig. J. Bot* 2002; 15: 37–41p.
15. Vanderzant C., Splittstoesser D. F. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. American Public Health Association, Washington D.C. 1992.
16. Marshall R. T. (ed.). *Standard Methods for the Examination of Dairy Products*, 16th ed. American Public Health Association, Washington D.C.1992.
17. Eaton A. D. et al. *Standard Methods for the Examination of Water and Wastewater*, 19th ed. American Public Health Association, Washington D.C. 1995.
18. Gur S. et al. *W. J. Agri. Sci* 2006; 2: 439–442p.
19. Jorgensen J. H. and Turnidge J. D. *Susceptibility Test Methods: Dilution and Disk Diffusion Methods* 2007; 1152–1172p.
20. Luangtongkum T. et al. *J. Cli. Microbiol* 2007; 45: 590–594p.
39.
21. Mamun-or-Rashid A. N. M. et al. *Res. J. Med. Plant* 2012; 6: 334–340p.
22. Reiner R. *Antibiotica: An Introduction.* F Hoffman (Ed). Switzerland, La Roche and Co. Ltd. 1982; 23–30p.
23. Zampini I. C. et al. *J. Ethnopharmocol* 2009; 124: 499–505p.
24. Aiyelagabe O. O. *Fitoterapia* 2001;72: 544–546p.
25. Prashanth D. et al. *Fitoterapia* 2001;72: 171–173p.
26. Mounishwamy V. et al. *The Antiseptic* 2002; 99: 81–82p.
27. Woldemichael G. M. et al. *J. Natural Products* 2003; 66: 242–246p.
28. Sen M. K. et al. *Antibacterial Activity* of *Cissus quadrangularis Stem – A Preliminary In vitro Effort to Develop Antibiotic*, Saarbrucken (Germany): LAP-LAMBERT Academic Publishing: 2012; 24–27p.
29. Madureirap M. D. C. *Spore* 2008; 136: 16–17p.
30. Srinivasan D. et al. *J. Ethnopharmacol* 2001; 74: 217–220p.
31. Somchit et al. *J. Ethnopharma* 2003; 84: 1–4p.
32. Stuart M. *The Encyclopaedia of Herbs and Herbalism.* Orbis Publishing, London: 1979.
33. Kumar O. A. et al. *J. Phytol* 2010; 2(3): 08–12p.
34. Akujobi L. et al. *J. App. Sci* 2004; 7(3): 4328–4338p.
35. Esimone C. O. et al. *J. Pharam Res. and Dev* 1998; 3(2): 99–102p.
36. Cowan M. M. *Clin. Microbiol Rev* 1999; 12: 564–583p.
37. Nweze E. I. et al. *J. Biol. Res. Biotechnol* 2004; 2(1):39–46p.
38. Draughon F. A. *Food Technol* 2004; 58 (2): 20–28p.