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- SHORT COMMUNICATION -

Screening for antibacterial activity of combined extracts of *Euphorbia fusiformis* from Tamil Nadu, India: a new application

ANDIMUTHU RAMACHANDRAN¹, DEVARAJAN NATARAJAN^{2*}, CHOKKALINGAM MOHANASUNDARI³, KESAVAN SRINIVASAN⁴, SEBASTIAN SOOSAIRAJ⁵ and EKAMBARAM NATARAJAN⁶

¹ Centre for Climate Change and Adaptation, Anna University, Chennai 620 025, Tamil Nadu, India ² PG Department of Botany, Periyar E.V.R. College (Autonomous), Tiruchirappalli 620 023, Tamil Nadu, India ³ Department of Microbiology, Kandaswami Kandar's College, Velur, Namakkal 638 182, Tamil Nadu, India ⁴ Department of Biology, Eritrea Institute of Technology, Mai Nefhi, Asmara, Eritrea ⁵ Department of Plant Biology and Plant Biotechnology, St. Joseph's College (Autonomous), Tiruchippalli 620 002, Tamil Nadu, India ⁶ PG and Research Department of Botany, National College, Tiruchirappalli 620 001, Tamil Nadu, India

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Euphorbia fusiformis is a rare, endemic medicinal plant growing in Tamil Nadu (India) used in traditional medicine for fever, chronic wounds, headache, skin diseases, paralysis and liver disorders. The present investigation was undertaken to evaluate the combined antibacterial effects of aqueous and organic solvent (acetone, ethanol and methanol) extracts from the leaves and rootstocks of *E. fusiformis*, against selected Gram-positive and Gram-negative bacterial strains [*Bacillus subtilis, Staphylococcus aureus* (Gram^{+ve}), *Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhii* A & B (Gram^{-ve})]. All the extracts showed a broad spectrum of antibacterial activity. The acetone and methanol extracts were found to be the most effective. This study reveals the potential for replacement of synthetic drugs by herbal extracts.

Key words: Euphorbia fusiformis, antibacterial effects, extracts, bacterial pathogenic strains.

INTRODUCTION

Herbal medicine is the oldest form of health care known to mankind. Herbs have been used by all cultures throughout the history and they constitute an integral part of the development of modern civilization. In terms of using plant materials for traditional

* Corresponding author: tel.: +91 4327 245797, e-mail: mdnataraj@rediffmail.com; dnr_76@yahoo.com

medicine, it is estimated that local communities use over 7,500 species of plants (Anonymous, 1994; Arora, 1997). The varied systems of medicine, like Ayurveda, Unani and Homeopathy have utilized plants for their preparations and have recently gained great importance. The emergence of resistance to conventional antimicrobials is a serious problem, and emphasis should be given to the development of new agents/drugs which can inhibit the growth or kill the



FIG. 1. A. *Euphorbia fusiformis*, whole plant; B. aerial part with flowers and fruits.

resistant microorganisms. Medicinal and aromatic plants and their constituents are rich in antibacterial compounds which can be an alternative to the combat against certain bacterial diseases (Samy *et al.*, 1998; Meera *et al.*, 1999; Ramasamy & Manoharan, 2004; Natarajan *et al.*, 2005, 2007; Bisht *et al.*, 2006). The demand for medicinal plants is increasing in both the developing and developed countries due to the recognition that natural products are being non-toxic, having no side-effects and easily available at affordable prices.

Euphorbia fusiformis Buch.-Ham. Ex. D.Don (Euphorbiaceae) is a rare endemic medicinal plant (Fig. 1) found in the central-eastern Ghats of Tamil Nadu, India (Britto et al., 2002; Natarajan et al., 2004). The ethnobotanical value of the plant refers to its recognized action as a remedy for several diseases like rheumatism, gout, paralysis and arthritis (Prakash & Singh, 2001). The dried root powder and the fresh rhizome are used to increase the secretion of the mother's milk, while the rhizome latex is applied to heal chronic wounds, skin diseases, liver disorders and diarrhea. A paste from the leaves is applied to the forehead to get relief from acute headaches (Raju et al., 2004). Caudifolin, methylellagic acid and euphol are the major chemical components of the drugs (Rastogi & Mehrotra, 1990-1995; Pullaiah, 2002). The reported biological activity of E. fusiformis, comprises anti-arthritic, anti-inflammatory (Singh et al., 1984) and antimicrobial properties (Natarajan et al., 2005, 2007). The combination of medicinal plant extracts exhibited better antibacterial properties than the use of individual extracts (Srinivasan et al., 2005). Hence, the present investigation was undertaken to screen the antibacterial activity of combinations of extracts from different plant parts (leaves and rootstocks) of *E. fusiformis* against human pathogenic bacterial strains.

MATERIALS AND METHODS

Fresh and healthy plant material was collected from the Chitteri hills (a segment of Eastern Ghats of Tamil Nadu), dried in shadow for 15 days and then used for the present investigation.

Extraction procedure and microorganisms used

Approximately 20 g of dried plant material (leaves and rootstocks) were crushed and blended to be used for each solvent. The blended materials were transferred to beakers and were soaked separately in 100 ml of sterile distilled water and organic solvents (acetone, chloroform, ethanol, and methanol) at room temperature. The extraction was done using rotary shaker (100 rpm for 3 days) and the extract was vacuum-dried to a concentration of 1/5 of the original volume. This stock was diluted in DMSO (dimethyl sulfoxide) before testing. Totally, eight bacterial strains (Bacillus subtilis, Staphylococcus aureus (Gram^{+ve}), Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Salmonella typhii A & B (Gram-ve) were used, obtained from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

Screening of antibacterial activity

The antibacterial activity of the combination of plant extracts, at a ratio of 1:1 (Singha & Gulati, 1990) was tested by the disc diffusion method (Bauer *et al.*, 1969) with some modifications. The inoculum was uniformly spread over the agar plated using a glass rod. A total of 0.2 ml of each extract was aseptically added to the discs. The plates were then incubated for 24 hrs at 37°C. After the incubation period, the diameter of inhibition around each disc was measured. The extracts were tested in triplicate and the standard deviation was calculated (Gupta, 1997). The standard antibiotic streptomycin (25 μ g ml⁻¹) acted as a positive control and sterile disc was dipped in the respective solvents as a negative control.

RESULTS

The results the effects of the different extracts from the leaves and rootstocks of *E. fusiformis* on the various microorganisms are presented in Table 1, and Figures 2, 3. The acetone and methanol extracts had

| TABLE 1. Antibacterial screet | iing of different solven | t extracts from the leaves | and rootstocks of <i>Euph</i> | orbia fusiformis | | |
|-------------------------------|--------------------------|----------------------------|--|---|------------------|--|
| Microorganism | | Diar | Combination of plaineter of inhibition zon | nt extracts (1+1) ne including disc (mm) | | |
| | A+B | C+D | E+F | G+H | I+J | Standard antibiotic Streptomycin (25 mg/disc) |
| Bacillus subtilis | I | 10.33 ± 0.47 | 10.66 ± 0.46 | 10.00 ± 0.00 | 11.66 ± 0.46 | 10.66 ± 0.46 |
| Staphylococcus aureus | I | Ι | 9.00 ± 0.00 | Ι | 9.33 ± 0.47 | 10.66 ± 0.46 |
| Proteus vulgaris | I | 8.66 ± 0.46 | I | 10.00 ± 0.00 | 9.66 ± 0.46 | 15.33 ± 0.47 |
| Escherichia coli | 10.00 ± 0.00 | 10.33 ± 0.47 | I | 9.66 ± 0.46 | 10.33 ± 0.47 | 10.00 ± 0.00 |
| Pseudomonas aeruginosa | I | 14.00 ± 0.81 | 10.33 ± 0.47 | 11.66 ± 0.47 | 11.33 ± 0.46 | 18.66 ± 0.46 |
| Klebsiella pneumoniae | I | 11.33 ± 0.47 | 12.66 ± 0.46 | 11.00 ± 0.00 | 11.00 ± 0.00 | 15.33 ± 0.47 |
| Salmonella typhii A | I | 15.00 ± 0.00 | 13.33 ± 0.47 | 11.00 ± 0.00 | 13.33 ± 0.47 | I |
| Salmonella typhii B | I | 12.66 ± 0.46 | Ι | 12.00 ± 0.00 | 8.66 ± 0.46 | I |
| Control* | I | Ι | I | I | I | I |
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All values are means of three experiments A+B= aqueous extract of leaf and rootstock; C+D= ethanol extract of leaf and rootstock; B+F= chloroform extract of leaf and rootstock; G+H= methanol extract of leaf and root-stock; I+J= acetone extract of leaf and rootstock; * Control= sterile disc soaked in respective solvents

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FIG. 2. Antibacterial activity of combined effect of leaves and rootstocks extracts of *Euphorbia fusiformis*. *Escherichia coli*, A. aqueous extract; B. ethanol extract; C. methanol extract; D. acetone extract. *Bacillus subtilis*, E. ethanol extract; F. chloroform extract; G. methanol extract; H. acetone extract. *Proteus vulgaris*, I. ethanol extract; J. methanol extract; K. acetone extract. *Klebsiella pneumoniae*, L. ethanol extract; M. chloroform extract; N. methanol extract; O. acetone extract. *Staphylococcus aureus*, P. chloroform extract.



FIG. 3. Antibacterial activity of combined effect of leaves and rootstocks extracts of *Euphorbia fusiformis*. *Staphylococcus aureus*, A. acetone extract. *Pseudomonas aeruginosa*, B. ethanol extract; C. chloroform extract; D. methanol extract; E. acetone extract. *Salmonella typhii* A, F. ethanol extract; G. chloroform extract; H. methanol extract; I. acetone extract. *Salmonella typhii* B, J. ethanol extract; K. methanol extract; L. acetone extract.

significant antibacterial properties against all tested pathogens, followed by the ethanol and chloroform extracts. In contrast the aqueous extract showed no activity against all tested bacterial strains except for *E. coli*.

DISCUSSION

Plants are sources of potent biochemicals which can be obtained from various parts (Gordon & David, 2001). Herbal remedies in traditional folk medicine provide a still largely unexplored field for the development of potentially new drugs for chemotherapy, which might help to overcome the growing problems of resistance and avoid the toxicity of the currently available commercial antibiotics. Therefore, the present research was focused on the above considerations and comprises the antibacterial screening of combinations of plant extracts from *E. fusiformis* against some human pathogenic bacterial strains. The results showed that extracts with organic solvents (especially acetone and methanol) are ideal to screen the biological activities of medicinal plants (Karaman *et al.*, 2003; Omer & Elnima, 2003). Similar conclusions were also drawn by Natarajan *et al.* (2005) using the same plant extracts. Another report has been focused on the antibacterial activity of individual or combined plant extracts of *Boswellia serrata* and *Citrus medica* against *S. aureus*, *E. coli*, *P. vulgaris*, *K. pneumoniae* and *P. aeruginosa* (Perumal *et al.*, 2004). The alcoholic extracts from the aerial parts of *Euphorbia hirta* (Sudhakar *et al.*, 2006) and *E. peplis* (Cateni *et al.*, 2003) were tested for their antimicrobial activity, particularly against *E. coli*, *P. vulgaris*, *P. aeruginosa* and *S. aureus*.

The overall performance of this investigation is that the Gram negative bacterial strains (*S. typhii* A & B, *P. aeruginosa* and *K. pneumoniae*) have greater sensitivity to most of the extracts than the Gram positive (*B. subtilis* and *S. aureus*) strains. It is concluded that the antibacterial screening of different solvent extracts from the leaves and rootstocks of *E. fusiformis* would be a promising alternative formula to treat various kinds of diseases caused by pathogenic bacteria.

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