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Research Article

SYNERGISTIC ANTIBACTERIAL EFFECT OF METHANOLIC EXTRACT OF CERTAIN INDIAN MEDICINAL PLANTS

Munireddy Durgadevi*, Mannarreddy Prabu, Manavalan Tamilvendan, Puthupattu Thangavelu Kalaichelvan and Venkatesan Kaviyarasan

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India.

*Corresponding Author e-mail: munireddy.durga@gmail.com

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Abstract

Aerial parts of *Eclipta alba* (L.), *Centella asiatica* (L.) and *Phyllanthus amarus* (L.) are used in treating various ailments skin, antinociceptive, stomach pain, anti-inflammatory, antiviral, rejuvenator, antimytotoxic, antihepatotoxic, antihyperglycemic, antioxidant and cancer. In this study, three plants are used individually to check their antimicrobial activity against *Salmonella Typhi*, *Escherichia coli* and *Bacillus subtilis*. *P. amarus* has exhibited maximum inhibition activity against *E.coli* (30±0.5 mm) followed by *B. subtilis* (28± 0.32 mm) and *S. Typhi* (24±0.16 mm). For the first time, we report the synergistic potential of these plants in the ratio of (1:1:1) against the test pathogens, which showed excellent antibacterial activity when compared with individual plant extract.

Keywords: Antibacterial activity, *Eclipta alba, Centella asiatica, Phyllanthus amarus*

INTRODUCTION

Indian medicinal plants represent a rich source of antibacterial agents (Mahesh and Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann *et al*., 2008). Plant products used in traditional medicine still remain the principal source of pharmaceutical agents (Ibrahim, 1997; Ogundipe *et al*., 1998). In common, with most traditional phytotherapeutic agents *C. asiatica* is claimed to possess a wide range of pharmacological effects, being used for human wound healing, mental disorders, atherosclerosis, fungicidal, antibacterial, antioxidant and anticancer purposes. *C. asiatica* has also been reported to be useful in the treatment of inflammations, diarrhea, asthma, tuberculosis and various skin lesions and ailments like leprosy, lupus, psoriasis and keloid. In addition, numerous clinical reports verify the ulcer-preventive and antidepressive sedative effects of *C. asiatica* preparations, as well as their ability to improve venous insufficiency and microangiopathy (Zheng and Qin, 2007).

Various biological activities are possessed by *E. alba*, such as memory disorder treatment, general tonic, edema, fever, rheumatic joint pain treatment, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders (Chopra *et al*., 1956; Karnick and Kulkarni, 1990; Karthikumar *et al*., 2007).

P. amarus leaves and whole plants are usually used for the treatment of gonorrhea, jaundice, rickets and asthma (Schlage *et al*., 1992) which is mostly used by traditional healers (Leaman *et al*., 1995). The *Phyllanthus* genus is a source of active chemicals. Extracts of *Phyllanthus* have secondary metabolites like alkaloid, flavonoid, lignin, phenol, tannin and terpene. Many of the active constituents like lignin, glycosides, flavonoids, alkaloids, ellagilannins, phenyl propanoids, sterols and flavonols are found in the leaf, stem and roots of the plant. Infectious diseases account for high proportion of health problems in the developing

countries (Sashi *et al*., 2003). Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents (Gomathi *et al*., 2011). Phytochemicals such as tannins and phenol from *P. amarus* have been reported to associate with antimicrobial activity (Cordell, 1995). From this study, an ingredient for the control of the test pathogenic microbes can be obtained for the benefit of mankind.

MATERIALS AND METHODS

Collection of plants

E. alba, *C. asiatica* and *P. amarus* (Figure 1) plants were collected from agricultural lands, Vaniyambadi, Vellore district. Fresh plant materials were first washed under running tap water and surface sterilized by 0.01% of $HgCl_2$ for 2 minutes subsequently soaked with double distilled water. After that the leaves were air dried under shade at room temperature for one week. The dried leaves are later ground with an electric grinder into fine powder which was stored in an air tight container at room temperature.

Extraction

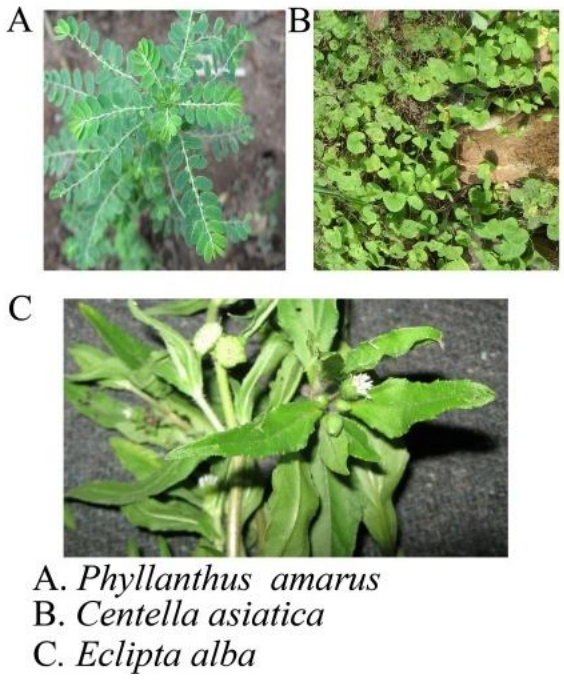
The 100g powder of individual plants and three plants powder in equal ratio was simultaneously extracted with methanol by keeping in a shaker (200 rpm) for 24 hrs at room temperature in separate flasks. The extracts were filtered using Whatman No. 1 filter paper and then concentrated in the vacuum rotary evaporator at 40°C. The concentrated crude extract (blackish-green) was stored at 4ºC for further use.

Drug preparation

The solvent free methanolic extract of *E. alba*, *C. asiatica and P. amarus* was used for further investigation. All the plant extracts were prepared at the concentration of 50 mg in 1 ml of 10% DMSO in a sterile vial.

Test organisms

Three human pathogenic bacterial strains like *Salmonella Typhi* (MTCC 733), *Escherchia coli* (MTCC 443) and *Bacillus subtilis* (MTCC 441), were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and were maintained on nutrient agar slants at refrigerated condition was used in the present study.



A. *Phyllanthus amarus*
B. *Centella asiatica*
C. *Eclipta alba*

Figure 1: Morphology of collected traditional plants

Culture media and inoculum preparation

Muller Hinton Agar (Himedia) is used to check the antibacterial activity and Nutrient broth (NB) is used to prepare inoculums. A loopful of gram positive and gram negative bacterial strains such as *Salmonella Typhi*, *Escherchia coli* and *Bacillus subtilis* were inoculated in 5 ml of nutrient broth in a test tube and incubated for 18 hrs to activate the strain.

Agar well diffusion method

The extracts obtained from three plants were studied for their antimicrobial activity. In agar well diffusion method, the media and the test bacterial cultures were inoculated into Petri dishes. The test strain 0.1 ml was inoculated into the media. Adequate care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (6mm). The methanolic extract at the concentration of (1, 1.5, 2, 2.5 and 3mg) was introduced into the well, the 10% DMSO served as control and the plates were incubated at 37 °C for 24 hrs. The antibacterial activity was determined by measuring the diameter of the zone of inhibition.

RESULTS

Three methanolic leaves extracts of *E. alba*, *C. asiatica* and *P. amarus* were examined for their

Table: 1. Antibacterial activity of methanolic extract of *E. alba*, *C. asiatica* and *P. amarus*.

S. No	Test organisms	Zone of inhibition (mm)*														
		E. alba					*C. asiatica*					*P. amarus*				
		0.25 mg	0.5 mg	1.0 mg	1.5 mg	2.0 mg	0.25 mg	0.5 mg	1.0 mg	1.5 mg	2.0 mg	0.25 mg	0.5 mg	1.0 mg	1.5 mg	2.0 mg
1	***E. coli***	18±0.54	20±0.23	24±0.19	29±0.56	29±0.45	09±0.43	11±0.23	14±0.32	20±0.43	21±0.54	15±0.58	20±0.22	23±0.33	30±0.5	30±0.5
2	***S. Typhi***	08±0.45	09±0.35	11±0.33	17±0.45	18±0.46	10±0.64	13±0.27	15±0.39	20±0.39	21±0.27	13±0.42	16±0.33	20±0.48	23±0.31	24±0.16
3	***B. subtilis***	08±0.51	09±0.43	14±0.38	20±0.49	21±0.34	08±0.23	10±0.45	13±0.44	19±0.32	19±0.58	15±0.33	20±0.54	23±0.38	28±0.33	28±0.32

*Values are mean of three replicates.

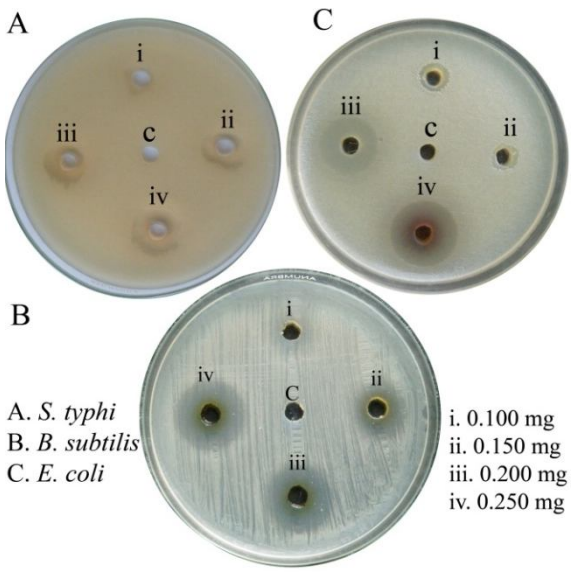


Figure 2: Antibacterial activity of methonolic extract of synergistic effect of kayan karpam

Table 2: Synergistic antibacterial activity of methanolic extract of *E. alba*, *C. asiatica* and *P. amarus*.

S. No	Test organisms	Zone of inhibition (mm)*			
		0.100 mg	0.150 mg	0.200 mg	0.250 mg
1	***E. coli***	10±0.55	11±0.21	25±0.32	28±0.53
2	***S. Typhi***	10±0.26	16±0.52	20±0.12	22±0.31
3	***B. subtilis***	9±0.33	11±43	15±0.48	21±0.29

*values are mean of three replicates.

antibacterial activity against certain Gram positive and Gram negative bacteria. Out of the three plant extracts tested at the concentration of (1, 1.5, 2, 2.5 and 3mg /well), *P. amarus* showed maximum activity at the range of 30 mm against *E. coli* followed by *B. subtilis* and *S. Typhi.* The zone of inhibition lies in the range of 08-30 mm (Table 1). Synergistic activity of these three plants altogether shows the maximum zone of 28 mm at the low concentration of 150 µg against *E. coli* followed by *S. Typhi* and *B. subtilis* (Figure 2). The zone of inhibition lies in the range of 09-28 mm (Table 2). The zone of inhibition for synergistic methanolic extract exhibited higher antibacterial activity even at low concentration.

DISCUSSION

There is a need to discover new drugs to treat pathogenic infections because of the resistant potential of the pathogens to the existing drugs. Plant plays a pivotal role in treating all kinds of disease. The plants used in this study are based on the prior information in treating diseases. Prabu *et al.*, 2011 reported that *E. alba* showed significant antibacterial activity in methanolic extract. This report supported our claim. Jagtap *et al*. (2009) reported that, the aqueous extract of *C. asiatica* did not show any antibacterial effects at lower concentrations and petroleum ether shows moderately activity. However, our methanolic extract was effective at the concentration below 1 mg/mL against *E. coli* followed by *S. Typhi* and *B. subtilis*. Flora Oluwafemi and Folasade Debiri, 2008 has reported *P. amarus* showed good inhibitory activity against *S. Typhi* for ethanol and hot water extract, but the methanolic extract showed effective activity against *E.coli* and *S. Typhi*, similar results was reported by Komuraiah *et al*., 2009. With reference to these reports, the plants *E. alba*, *C. asiatica* and *P. amarus* were chosen in the present investigation. This attempt has showed good antibacterial activity in single and synergistic effect. The synergistic effect exhibited pronounced antibacterial activity.

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