



Antibacterial and antioxidant studies of individual and formulation of Indian medicinal plants

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ABSTRACT

Plants are indispensable sources of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from the natural sources. *Calotropis gigantea*, *Leucas aspera* and *Euphorbia hirta* are abundantly available weed plants. The present study states that evaluation of “antibacterial effect” on comparison to that of individual extracts and formulated extracts. The effect of cytotoxicity against the bacteria was analysed for individual plant extracts such as *Calotropis gigantea* (E1), *Leucas aspera* (E2), *Euphorbia hirta* (E3) and formulated plant extract in the ratio of 1:1:1. The leaf extract of three weed plants were performed by solvent extraction method. The antibacterial activity was done by disc diffusion method using different concentration (10, 20, 30, 40, 50µL). The DPPH assay was done to analyze the antioxidant effect. It was concluded that the combinational study has given the highest cytotoxic effect against bacterial infections than the individual extracts.

Key words: *Calotropis gigantea*, *Leucas aspera*, *Euphorbia hirta*, Formulated extract, antibacterial activity, DPPH assay.

INTRODUCTION

Plants are exploited as medicinal source since ancient age. The traditional and folk medicinal system uses the plant products for the treatment of various infectious diseases. In recent times, plants are being extensively explored for harbouring medicinal properties. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development [1,7,16]. These are reported to have antimicrobial, anticancer, anti-inflammatory, antidiabetic, haemolytic, antioxidant, larvicidal properties etc. Plant drugs could be effective and at the same time have less or no side effect [9,10,19]. Now a days 80% people (WHO estimated) from all over world are interested towards traditional medicines [5]. Drug therapy has a profound influence on the health statistics all over the world. Herbal medicine is the most ancient form of health care known to humankind.

Calotropis gigantea belongs to the family of Asclepiadaceae, a weed plant commonly known as giant milkweed. Various ailments are treated by different parts of plants [13]. Leaves and areal parts of the plant have been reported for anti-bacterial activity [18]. The leaf contains ascorbic acid, o-pyrocatechic acid, β-amyrin, taxasterol, tarasterol and β-sitosterol. Two new cardenolides, 19-Nor and 18, 20-epoxy-cardenolides were isolated from the leaves of *C. gigantea* [2,6,8,14]. Plants of genus *Leucas* is a member of Lamiaceae family which is widely used in traditional medicine to cure many diseases such as cold, cough, diarrhoea and inflammatory skin disorder [17]. A variety of phytoconstituents has been isolated from the *Leucas* species including flavonoids, coumarins, steroids, lignans, terpenes, fatty acids and aliphatic long chain compounds. The extracts of these plants and their phytoconstituents have shown the activities of Anti-inflammatory, analgesic, antidiarrhoeal, antimicrobial, antioxidant and insecticidal. *Euphorbia hirta* is a member of Euphorbiaceae family is commonly called pill bearing spurge and asthma herb [15]. The plant leaves are used to treat colic troubles, dysentery, cough, asthma, worms and vomiting [4,21].

The present study was planned to evaluate the antibacterial, antioxidant activities and to compare the cytotoxic effect for individual and formulated leaf extracts of *Calotropis gigantea*, *Leucas aspera* and *Euphorbia hirta*.

EXPERIMENTAL SECTION

Collection of plant

The leaves of *Calotropis gigantea*, *Leucas aspera* and *Euphorbia hirta* were collected from Tamil Nadu, Chennai. The leaves were washed and shade dried for about three weeks.

Preparation of plant extract

The dried leaves of *Calotropis gigantea*, *Leucas aspera* and *Euphorbia hirta* were moderately coarse powdered and the extraction was carried out by cold percolation process using methanol for 24 hours. The extracts were filtered through muslin cloth and Whatman No 1 filter paper. The filtrate was stored for further analysis.

Preparation of samples

The test samples were prepared by concentrating the filtrate in evaporator at 50°C to obtain residue. 0.5mg sample was dissolved in 1ml of methanol.

Antibacterial test

The individual and mixed extracts at different concentrations were tested for antibacterial activity using agar disc diffusion technique following the Kirby-Bauer method [3]. 250 µl of each bacterial subculture *E.coli* and *E.aerogenes* were micropipetted onto the solidified agar plates and using a sterile L-rod, the cultures in each plate were evenly spread over the agar plates. The individual and combined plant extracts in the ratio of 1:1:1 were added in different concentration (10-50 µl). Streptomycin used as an antibiotic disc. The plates were kept at room temperature for few minutes to allow diffusion and incubated 37°C for 24 hours in an incubator. The diameter of the zone of inhibition was calculated in millimetres (mm).

DPPH free radical scavenging assay:

The free radical-scavenging assay of individual and formulated extracts were measured in terms of hydrogen donating or radical scavenging ability using stable radical DPPH followed by Blois method [21]. Stock solution (0.5 mg/ml) was prepared in DMSO. Different concentrations (10, 20, 30, 40, 50 µl) of test solutions were prepared from stock. 1ml solution of DPPH (0.1mM) in methanol was added to each of the above test solutions. The mixture was shaken vigorously and incubated for 30 min and absorbance for each test solution was measured at 517 nm. Butylated hydroxyl anisole was used as a reference for standard or positive control and DMSO was used as negative control. The scavenging activity of the DPPH free radical was calculated using the following equation.

$$\bullet \text{ DPPH scavenging effect(\%)} = [(A_c) - A_b / A_c] * 100$$

Where, A_c is absorbance of negative control, A_b is the absorbance of test solution.

RESULTS AND DISCUSSION

The extracts were screened for the analysis of antibacterial and antioxidant activity. The analysis revealed the best activity in formulated extract. Table 1 shows the comparison of antibacterial effect of individual and mixed extract activity.

The antibacterial activity of methanol extract of *Calotropis gigantea* (E1), *Leucas aspera* (E2) and *Euphorbia hirta* (E3) were analyzed against two pathogenic gram negative bacteria followed by disc diffusion method. The methanolic extract of herbal plants have shown the better antimicrobial effect against the pathogenic organism [11, 12], so the three different herbal medicinal leaves were extracted using methanol to analyze the antibacterial and antioxidant activity. Table 1 shows the Inhibition zone formed by mixture and individual extracts E1, E2 and E3. From the inhibition levels of methanol extract against *E.coli* and *E.aerogenes*, formulated leaves extract showed the maximum zone of inhibition of 11 mm diameter for *E.coli* and 10.16 mm diameter for *E.aerogenes* was considered as highly susceptible at the concentration of 50 µL. whereas the antibacterial effect of individual extracts E1, E2 and E3 had not given a better activity when compared to the mixture. The combination of extract 1, 2 and 3 in the ratio of 1:1:1 was effective against *E.coli* and *E.aerogenes*. Thus the mixture of extracts showed the highest cytotoxic effect when compared to individual extracts of E1, E2 and E3.

Table 1: Inhibition zone formed by mixture of three plant extracts and individual extracts (*Calotropis gigantea*(E1), *Leucas aspera*(E2) and *Euphorbia hirta*(E3))

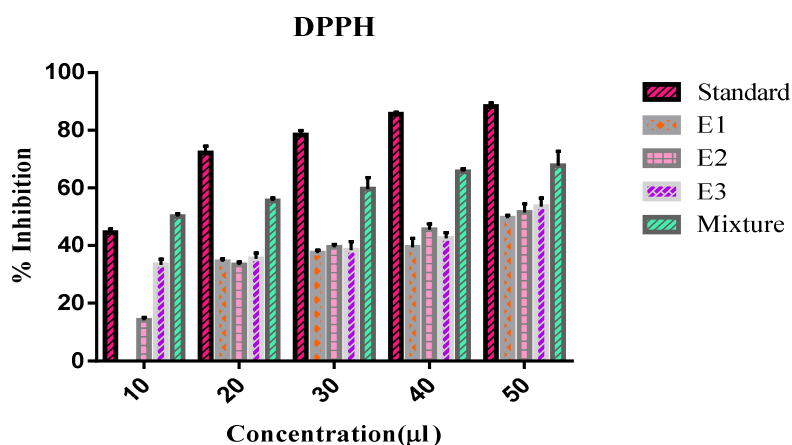
S.No	Microorganism	Diameter of inhibition zone(mm/30µL)				
		Concentration (µl)	Individual(ME)			Mixture (1:1:1) (ME)
			E1	E2	E3	
1	<i>E.Coli</i>	10	5.66±0.929	5.65±0.839	-	6.66±0.808
		20	6.16±0.351	5.83±0.723	6±0.800	8.86±0.808
		30	6.16±0.971	6±0.800	6.16±0.351	10.66±0.01
		40	6.33±0.451	6.16±0.971	6.33±0.961	11±0.900
		50	6.33±0.451	6.5±0.600	9.66±0.902	11±0.500
2	<i>E.aerogenesa</i>	10	-	-	-	6±0.800
		20	-	5.5±0.513	5.33±0.577	8±1.000
		30	6±0.854	5.66±0.839	6±0.854	9.33±0.74
		40	6.16±0.971	6.16±0.351	6.33±0.751	10.16±1.04
		50	6.33±0.751	7.75±1.050	6.33±0.961	10.16±0.76

Concentration: 30µL of 0.5 mg/mL of combined leaf extracts.

E1- Extract1- *Calotropis gigantea*, E2- Extract2- *Leucas aspera*, E3- Extract3- *Euphorbia hirta*.

Table 2: DPPH (2, 2 diphenyl-1-picryl hydrazyl) assay

Sample	10µl	20µl	30µl	40µl	50µl	IC ₅₀ (µl)
E1	0	34.34±0.121	37.37±0.119	39.39±0.782	49.49±0.189	50
E2	14.14±0.220	33.33±0.228	39.39±0.319	45.45±0.582	51.51±0.708	48.88
E3	33.33±0.502	35.35±0.620	38.38±0.712	42.42±0.609	53.54±0.754	46.82
Mixture(1:1:1)	50.00±0.198	55.56±2.005	59.6±0.846	65.66±0.193	67.68±0.919	10
BHA	44.46±0.250	72.16±0.450	78.41±0.330	85.51±0.065	88.27±0.550	14.4

**Fig 1: Graphical analysis of DPPH method**

DPPH radical scavenging activity was determined as previously described by Li [20], where Butylated Hydroxy Anisole (BHA) used as a standard. According to this study the antioxidant activity was carried out and the results were tabulated which were shown in Table 2. Methanol extract showed effective DPPH radical scavenging ability than other extracts like L-ascorbic acid, acetate and chloroform [19]. The individual extracts E1, E2, and E3 shows the 50% of inhibition (IC₅₀) at the concentration of 50 µl, 48.88 µl, 46.82 µl respectively, whereas the mixture showed 10 µl to be the inhibitory concentration (i.e.) lower IC₅₀ value that suggests highest anti-oxidant activity. Fig 1 shows the comparison of antioxidant activity of individual and mixture of methanolic leaf extracts. From the graph we can conclude that the concentration of 10 µl is enough to perform 50% inhibition against free radicals. Thus the formulation of E1, E2 and E3 in the ratio of 1:1:1 is effective to scavenge the free radicals.

CONCLUSION

Based upon the results obtained the present investigation revealed that the leaves of three extracts of *Calotropis gigantea*, *Leucas aspera* and *Euphorbia hirta* possessed highest activity against *E.coli* and *E.aerogenesa* and the formulated extract showed the highest free radical scavenging activity. (i.e.) combination of these three extracts possessed highest antibacterial and antioxidant effects than the individual extract. So this formulation studies might be useful for the study of bacterial diseases and to prevent the progress of various oxidative stresses. Future research is needed to isolate and purify the active components to design this formulated herbs as a drug.

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