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RESEARCH ARTICLE

IN VITRO ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT FROM THE LEAVES OF *WEDILIA TRILOBATA* AND *EMILIA SONCHIFOLIA* AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Now a day, a search is going on for new antimicrobial agents due to the negative effects of existing antibiotics and the constant development of bacterial resistance. This study assessed the phyto chemical screening and antibacterial activities of methanol extract from the leaves of *Wedilia trilobata* and *Emilia sonchifolia*. Methanol extract from the leaves of *Wedilia trilobata* and *Emilia sonchifoliahas* an antimicrobial activities against 5 bacteria *K. pneumoniae, E. coli, E. faecalis, S. aureus* and *P. Aeruginosa* and it was investigated using a disc diffusion and minimum inhibitory concentration assay (MIC). Both disc diffusion and (MIC) of the extracts ranged from 5 to 20 µg/m. Results of phyto chemical screening of methanol extracts of *W. trilobata* and *E. sonchifolia* showed the presence of tannins, flavonoids, terpenoids, alkaloid, poly phenols, saponins and cardiac glycosides absent. The total content of flavonoids and poly phenols in the methanol extracts of the studied species positively correlated with their antioxidant properties .The study findings indicate that bioactive natural products from these plants may be isolated for further testing as leads in the development of new pharmaceuticals in food preservation as well as natural plant-based medicine.

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INTRODUCTION

From olden days, an imposing number of modern drugs have been isolated from natural sources. Most of the medicinal agents were obtained from the nature for thousands of years; most of them were based on the uses of the agents in the traditional medicines. 80% of the world's inhabitants depending predominantly on traditional system of medicines for their primary health care (Raids *et al.,* 2010). Hence, the traditional system of medicines based on natural plants continues to play a major role in the health care. According to the World Health Organization, naturally occurring medicinal plants would be the best source to obtain a large number of drugs. Therefore, those plants should be taken under research to gain knowledge about their properties, safety and efficacy (Hassan *et al.,* 2009). In developing countries, there is a major cause of morbidity and reduced mortality due to the infectious diseases. Today, a vast range of synthetic and semi-synthetic antibacterial agents is available for the control of microorganisms; however bacterial resistance is rapidly growing to the antibacterial agents. The available antibiotics have beneficial effects of bacterial control and also have an adverse reactions such as hypersensitivity and immune suppression. Due to these negative effects and the constant development of bacterial resistance, there is a necessity to develop newer antimicrobial agents which is more effective against microorganisms and less. Hence the pharmaceutical industry is to be motivated to progress alternative antimicrobial drugs. Some of the essential-oil containing aromatic plants is significant natural sources of antimicrobial agents, which are used in traditional medicine primarily to combat infectious diseases. Plants containing natural products are a source of potential and powerful drugs have been used worldwide in traditional medicine. Among the estimated 250,000-500000 plant species, only a small proportion has been submitted to biological or pharmacological screening, since the capacity of higher plants as a source for new drugs is largely unexplored (Azzi *et al.,* 2012). The therapeutic action of plants is allocated mainly to their antimicrobial activities. Usage of antimicrobial agents derived from the natural plants might be effective in reducing the dependence on antibiotics and minimizing the chances of antibiotic resistance in food borne pathogenic microorganisms (Qi, 2015). *Wedilia trilobata* is a perennial herb with a creeping or climbing habit. The leaves are attractive, bright shiny green, somewhat fleshy oppositely arranged and simple, the blade obovate to elliptic or ovate and are stalk less. The single attractive bright yellow flower heads are daisy-like in appearance. The fruit is a 2 to 4-angled achene, with short, narrow pappus scales on the top. The aerial parts of

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this plant are used in traditional medicine in the Caribbean and Central America against bronchitis, colds, abdominal pains, dysmenorrheal, and even as a fertility enhancer. Also other folk medicine, it is employed to treat backache, muscle cramps, rheumatism, stubborn wounds, sores and swellings, and arthritic painful joints. The leaves used for treatment of kidney functioning, cold, stingray wounds, snakebite, purge and amenorrhea.

Emilia sonchifolia (L.) DC (Family: Asteraceae), is an annual herb with erect or prostrate at base and up to 10-150 cm tall. It often branches from the very base, usually purplish-green and deep rooting. The leaves (4-16 cm x 1-8 cm) are sessile, with alternate arrangement, dark green above and lighter green or tinged with purple beneath, and more or less irregularly coarsely dentate. The inflorescence is a terminal head and few together in slender corymbs or rarely solitary. The flowers are orange, pink, purple and white in colour. The fruit is one seeded (2.5-3.0 mm), linear oblongoid, soft and brown in colour. *Emilia sonchifolia* is an edible plant used in the ayurvedic system of medicine for the treatment of gastropathy, diarrhea, opthalmia, nyctolpia, cuts and wounds, intermittent fevers, pharyngodyma, and asthma (Nair and Chopra, 1996). The aqueous and the methanolic extracts of the leaves of *Emilia sonchifolia* progressively exhibit antitumour activities (Shylesh and Padikkala, 2000). The n-hexane extract of *Emilia sonchifolia* (250 mg/kg) has anticancer effect and rich in terpenoids (Shylesh *et al.,* 2005). However, the effect of methanol leaves extract of *Wedilia trilobata* and *Emilia sonchifoliaon* antibacterial activity have not been studied. The purpose of this study is to evaluate the antibacterial activity of methanol leaves extract of *Wedilia trilobata* and *Emilia sonchifolia.*

MATERIALS AND METHODS

Plant material: The leaves of *Wedilia trilobata* and *Emilia sonchifolia* were collected from Government siddha medical college, Arumbakkam, Chennai-600 106, Tamilnadu, India. The botanical identification of the plants was done by Dr. Sankaranarayanan, Head, Department of Medicinal Botany. A voucher specimen (GSMC-MB/215 and GSMC-MB/2016) was deposited in the herbarium of Department of Medicinal Botany. These plant materials were air-dried at room temperature and powdered. Then 500 g of each powder were macerated in methanol (2.5 l) at room temperature for 48 h. The filtrate was then concentrated under vacuum to give crude extracts from leaves of *Wedilia trilobata* and *Emilia sonchifolia*. These extracts were stored at room temperature till further use.

Phytochemical analysis: Phyto chemical analysis was carried out by using the standard procedures to identify the constituents qualitatively in plant extracts, fractions and quantitatively in dried whole plant as described by Edeoga *et al.* 2005.

Bacterial strains: Antibacterial activities were conducted by Disc Diffusion method and Minimal Inhibitory Concentration Test (MIC) for all the selected plants against pathogenic bacteria (Gram positive and Gram negative). Bacteria used for the determination of antibacterial activities were Gram positive viz; *Staphylococcus aureus* (MTCC 29213), *Klebsiella pneumoniae* (MTCC 1771) and *Enterococcus faecalis* (MTCC 439) and gram negative viz; *Pseudomonas aeruginosa* (MTCC 2488), and *Escherichia coli* (MTCC 25922).

Disc diffusion method: The agar disc diffusion method (NCCLS, 1997) was used for determination of diameters of inhibition zones made by methanol, leaves of *W.trilobata* and *E. sonchifolia* against various bacterial (*K. pneumoniae*, *E. coli, E. faecalis, S. aureus* and *P. aeruginosa*). Sterile nutrient agar was inoculated with 100 mL suspension of tested bacteria. The inoculated nutrient agar and potato dextrose agar were then poured into sterilized petri plates individually. Sterile filter discs impregnated with50 mL of sample solution were placed in inoculated petri plates using sterile forceps. Streptomycin was used as positive control in bacterial inoculated plates, respectively. The plates were incubated at 37 °C for 24 hours and at 27°C for 48 hours for maximum bacterial growth, respectively. Antibacterial activities were evaluated by measuring diameter (mm) of inhibition zones using a zone reader.

Minimum inhibitory concentrations (MIC): Minimum inhibitory concentrations (MIC) were calculated by a modification of the reported method of Sarker *et al.* (2007). For the evaluation of minimum inhibitory concentrations (MIC), different concentrations of plant extract, fractions and essential oil were prepared by serial dilution. The range of dilution was determined by keeping in mind the antimicrobial activity determined in the inhibition zone assay. For the samples showing better activity in the first assay the serial dilution for MIC determination was carried out at less concentration and for the samples showing less activity the higher concentration was used for serial dilution. Ciprofloxacin and fun gone at 10µg/mL were used as reference standards for the bacterial and fungal strains, respectively.

Statistical analysis: Minitab software version 16 (Statistical software, Minitab Inc., State College, PA, USA) was used to perform analysis of variance (ANOVA) and to determine significant differences ($p < 0.05$).

RESULTS AND DISCUSSION

Phytochemical analysis: In the present study, efforts were made to qualitatively assessment the various medicinally active constituents such as flavonoids, saponins, tannins, steroids, alkaloids and terpenoids present in leaves methanol extract of *Wedilia trilobata* and *Emilia sonchifolia* and absence of cardiac glycosides (Table 1).

Antimicrobial activity: The results of antimicrobial potential of methanol, extract of *W. trilobata*, and *E. sonchifol*ia against five pathogenic microorganisms *K. pneumoniae*, *E. coli,* E. faecalis, *S. aureus* and *P. aeruginosa* are shown in Table-2. The one investigated extracts exhibited considerable antimicrobial effects against all tested microorganisms. Maximum antibacterial activity was observed in *W. trilobata* against *K. pneumoniae*, *P. aeruginosa, K. pneumoniae,* while minimum activity was observed in *E. sonchifolia* against *E. coli* and *P. aeruginosa*. The highest activity of methanol extract of *W. trilobata*was observed against *P. aeruginosa, K. pneumoniae***,** *S. aureus* with inhibition zone of 17.6 ± 1.46, 16.2± 0.87, 15.6 ± 0.47mm respectively. A previous study Taddei and Rosas-Romero 1999) the ethyl acetate extract of *W. trilobata* was active only against *Salmonella* group C; and the aqueous extract was inactive against the tested bacteria. In the present study, antibacterial potential may be attributed to bioactive components present in the plant.

Table 1. Phytochemical screenings of methanol leaves extract of *W. trilobata* and *E. sonchifolia*

Sl. No.	Phytochemical Constituents	Observation	Methanol leaves extract of *W. trilobata*	Methanol leaves extract of *E. sonchifolia*
1	**Alkaloids**	Orange /	+	+
	Dragendorff's test	red precipitate		
	Mayers test	Cream pie ppt	+	+
2.	**Flavonoids**			
	Alkalai Reagent	Intense yellow colour	+	+
	Lead aceate test	Precipitate formed	+	+
3.	**Glycosides**			
	Keller-Killiani test	Pink colour (Ammonia layers)	-	-
4.	**Tannin**			
	$FeCl_3$ test	Blue-blackcolour	+	+
5.	**Saponins**			
	Frothing test	Foam	+	+
6.	**Terpenoids**	Reddish brown colour ring formed in interface		
	Salkowski test		+	+
7.	**Polyphenols**			
	Ferrozine test	Raddish blue	+	+
8.	**Anthocyanin**			
	Ammonia test	Pink color in ammonia layer	+	+

+ Positive result; - Negative result

Table 2. The antibacterial activity of methanol leaves extract of *W. trilobata* by disc diffusion method

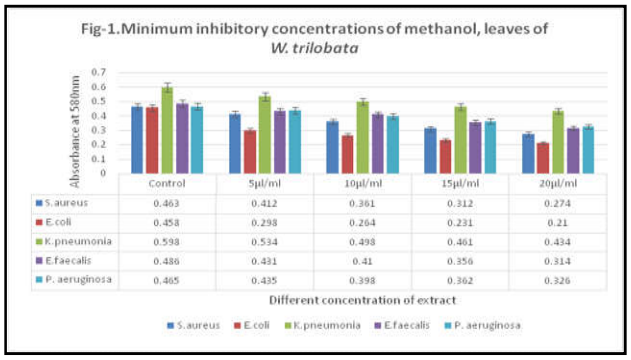
Pathogenic bacteria	Methanol leaves extract of *W. trilobata* Zone of inhibition (mm)[a]				
	Positive control 10 µl Ampicillin	Different concentrations of methanol leaves extract of *W. trilobata* (µl/ml)			
		5 µl	10 µl	15 µl	20 µl
Staphylococcus aureus	13.5±1.2	8.2±1.36	11.2±1.24	13.9±1.45	15.6±0.47
Escherichia coli	13.3±0.9	7.8±1.54	9.25±0.36	11.4±1.69	13.7±2.8
Enterococcus faecalis	14.4±1.4	8.5±0.68	10.9±0.24	13.5±0.98	15.2±0.64
Klebsiellapneumoniae	14.2±0.8	9.2±0.28	12.4±1.69	15.4±0.87	17.6±1.46
Pseudomonas aeruginosa	13.8±1.4	8.9±0.28	12.6±1.37	14.8±1.48	16.2±0.87

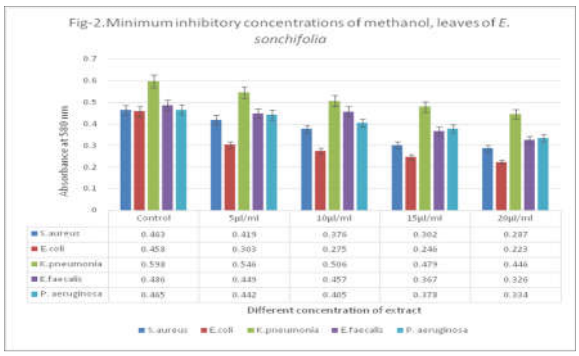
[a]The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.

Table 3. The antibacterial activity of methanol leaves extract of *E. sonchifolia* by disc diffusion method

Pathogenic bacteria	Methanol leaves extract of *E. sonchifolia* Zone of inhibition (mm)[a]				
	Positive control 10 µl Ampicillin	Different concentrations of methanol leaves extract of *E. sonchifolia* (µl/ml)			
		5 µl	10 µl	15 µl	20 µl
Staphylococcus aureus	13.5±1.2	7.5±0.24	10.5±0.36	12.5±0.21	14.2±1.27
Escherichia coli	13.3±0.9	6.5±1.68	8.1±1.69	10.8±0.29	12.4±1.68
Enterococcus faecalis	14.4±1.4	8.1±1.47	9.8±1.47	12.4±1.62	14.2±1.24
Klebsiellapneumoniae	14.2±0.8	8.6±1.26	11.3±1.52	14.2±1.6	16.2±1.64
Pseudomonas aeruginosa	13.8±1.4	7.2±1.89	11.9±0.49	13.2±0.24	14.8±1.89

[a]The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.





Govindappa *et al*. (2011) the antimicrobial activity of the whole plant *W. trilobata*ex tracts are low compared to antibiotics or fungicide, purification of the active natural compounds could derive a metabolite more active.MIC of methanol leaf extract of *W. trilobata* and *E. sonchifolia* against bacteria ranged from 5 to 20 µl/ml (Fig-1). The leaf extract was more active against all bacterial strains. The methanol leaf extract of *W. trilobata* highly potential for broadly active antimicrobial activity than *E. sonchifolia*.

Conclusion

In the present study results indicate that the methanol extract of *W. trilobata* and *E. sonchifolia* possess antimicrobial, properties. These activities may be due to the strong occurrence of some phyto molecules like polyphenol flavonoids, tannins, terpenoids and saponins. The methanol extract of these plants showed strong antibacterial activity compared standard antibiotic. These reports provide a basic scientific evidence to support its traditional medicinal uses. In this study might suggest a possible use of *W. trilobata* and *E. sonchifolia* as source of natural antibacterial, agent.

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