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Antimicrobiological Studies on Different Essential Oils of *Wedelia* Species (*W. chinensis, W. trilobata* and *W. biflora*) and *Eclipta alba* (Asteraceae)

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The essential oil of *Wedelia chinensis, Wedelia biflora, Wedelia trilobata* and *Eclipta alba* (1 µg/mL) were evaluated for its anti microbiological study against (gram positive and gram negative) bacteria and also with fungi by paper disc diffusion technique. The oil of *Wedelia trilobata* found to be effective against gram positive and active against all the tested fungi. The other essential oils of *Wedelia chinensis, Wedelia biflora* and *Eclipta alba* were exhibit significant antibacterial and antifungal activity when it was compared to that of standard ciprofloxine (10 µg/disc).

Key Words: Antimicrobial activity, Essential oils, *Wedelia* and *Eclipta alba*.

INTRODUCTION

Natural products contribute to a great extent to fight against pathogenic micro organism. Many plants or their parts are used in food as spices and are thought to provide a natural preservation by inhibiting the microbial growth. Varieties of herbs and spices have been used traditionally in food preservation to extend shelf life[1]. All the four plants were belonging to Asteraceae, are commonly growing as a weed.

***Wedelia chinensis* (Osbeck) Merr:** This herb is said to possess properties similar to those of *Eclipta alba*[2]. This plant finds its place in indigenous Ayurvedic medicinal system for its hepatoprotective efficiency[3] and the herbal extract was effective in curing induced liver injury *in vivo*[4]. The leaves are regarded as tonic and alternative, useful in cough, cephalagia and diseases of the skin. A decoction of the herb is used in uterine haemorrhage and menorrhagia. An ethanol extract of the plant inhibits the growth of Ehrlich's ascites carcinoma and also found to affect the central nervous system[5].

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***Wedelia trilobata* (L.):** The flowers and leaf part of the plant were used by the women for the purpose of amenorrhea. It is also used in the treatment of toothache[6]. The fruits were used for bites and stings. The decoction of the plant also used for childbirth too[7]. It is also reported that the fresh entire plant were used as molluscicidal activity[8]. Flower and its essential oil screened for its antibacterial and antimycobacterial activity *in vitro* studies[9].

***Wedelia biflora* (DC.):** The dried and fresh leaves were used to relive of headache, diuretic and laxatives[10,11].

The fresh leaf were effectively used in the treatment of malarial fever, which is taken orally along with lime and sea water[12]. Fresh leaf juice used to treat tropical sores, wounds, scabies and cuts. The fresh leaves were effectively used in the treatment of diarrhoea and dysentery[13,14].

The young leaves are cooked and eaten in small quantities as flavouring with food. The pounded leaves are used for preparing a poultice for cuts, ulcers, sores and varicose veins. A decoction of the roots and leaves is prescribed for stomach ache. The leaves are also credited with diuretic properties. The flowers are violently purgative. The leaves contain a fair amount of protein, but have a high content of fiber. They also contain alkaloids[15]. The aqueous extract of leaves and stems is toxic to American cockroaches[15].

***Eclipta alba* (Linn.):** This is one of the ten auspicious herbs that constitute the group dasapuspam [= Ten flowers] which is considered to destroy the causative factors of all unhealthy and unpleasant features and bestow good health and prosperity. The members of this group cure wounds and ulcers as well as fever caused by derangement of the three dosas vata, pitta and kapha[16].

The juice of the leaves is given in one teaspoonful doses in jaundice and fevers. It is principally used as a tonic and deobstruent in hepatic and spleenic enlargements and in chronic skin diseases, pain in the liver, fevers, headache, antipyretic, stomachic, stomatitis and toothache (Unani). The plant has a bitter hot sharp dry taste; fattening, alternative, anthelmintic, good for the complexion, the hair, the eyes, the teeth, cures inflammations hernias, eye diseases, leucoderma, disesses of the heart and the skin, itching and for uterine pains after delivery used in formulation of Ayurveda[2].

EXPERIMENTAL

Collection and authentification: The plants *Wedelia chinensis, Wedelia trilobata* and *Eclipta alba* (Asteraceae) are found abundantly in the various parts of Tamilnadu, India and *Wedelia biflora* is found in Andaman Nicobar Island. For the present study, the entire plants of *Wedelia chinensis, Wedelia trilobata* and *Eclipta alba* were collected from the sur-

roundings of Salem, India and *Wedelia biflora* was collected from Andaman Nicobar Islands in the month of August 2002 and were authenticated by Dr. S. Jayaraman, Director, Medicinal Plant Research Unit and Plant Anatomy Research Centre, Chennai, India.

Extraction of oil: The essential oil was isolated from the fresh leaves by hydrodistilation in a Clevenger-type apparatus[17]. The distillation yielded *Wedelia chinensis*, (1 %), *Wedelia trilobata*, (4 %) *Wedelia biflora*, (3 %) and *Eclipta alba* (1.5 %) v/w oil on fresh weight basis. Finally, it was preserved under refrigeration.

Screening for antibacterial and antifungal activity: Assay of antibacterial activity was performed by reported methods[18,19].

Method: The assay of antibacterial activity of essential oil of four different plants like *Wedelia chinensis, Wedelia trilobata, Wedelia biflora* and *Eclipta alba* were evaluated by disc plate method for different dilutions like 1, 0.8, 0.6, 0.4 and 0.2 µL/mL in DMSO. 360 mL of Muller Hinton nutrient agar medium was prepared and distributed to 30 mL each in 12 sample test tubes and were sterilized by autoclaving. 0.5 mL Standard inoculums of test micro organism (18 h broath culture) were inoculated to the Muller Hinton agar medium (40-45ºC).The agar plates were prepared by pour plate technique[15]. The paper discs were gently pressed with sterile forceps to ensure proper contact with the agar medium. Then plates were kept in refrigeration for 0.5 h to allow the diffusion of the oil. The antibiotic disc of ciprofloxine (10 µg/disc) was used as standard, while the discs soaked in DMSO were used as blank control. The zone of inhibition formed against different concentration of oils, standard and blank were recorded after 24 h incubation at 37ºC. The observations were given in the Table-1.

TABLE-1
EFFECT OF ESSENTIAL OILS ON BACTERIAL GROWTH BY DISC PLATE METHOD

Bacterial strain	Zone of inhibition (mm)*				
	SC	WC	WT	WB	EA
Salmonella thyphi	24	14	17	13	12
Escherichia coli	22	13	15	11	10
Klebsiellapneumonia	22	12	16	11	10
Staphylococcus aureus	26	14	18	13	11
Bacillus substillus	28	14	18	12	11
Streptococcus mutans	28	13	19	12	11

SC = Standard Ciproflaxine; WC = *W. chinensis*; WT = *W. trilobata*; WB = *W. biflora*; EA = *E. alba*; *Diameter of cups = 8 mm.

Preparation of standard fungal culture: The fungal cultures were grown on SDA (Sabours Dextrose Agar-Himedia) at 28ºC for 7 d. Suspension of fungi was prepared in 0.85 % normal saline containing 0.1 % Tween 80 (Suspending agent) for use as inoculums, the turbidity of the suspension was adjusted to the McFarland no: 5 turbidity standards.

Method: The assay of antifungal activity of essential oil of four different plants like *Wedelia chinensis, Wedelia trilobata, Wedelia biflora* and *Eclipta alba* were tested by the method given by Wannisom *et al.*[16], cup plate method. The seeded SDA plates were prepared by pouring 20 mL of sterilized SDA medium in to each sterile plates. After solidification of medium, each plate was over laid with 5 mL of inoculum. The plates were slightly tilted to and fro once for uniform spreading of inoculums.

The essential oils were applied on the seeded SDA medium by cup plate technique. Four wells (cup-8 mm in diameter) were bored using sterile borrer in all the seeded Petri plates aseptically. The drugs were dissolved in DMSO, so as to get a concentration of 1 µg/L. Three cups out of four cups were filled (0.2 mL each) with the oils and the remaining well is filled with the standard drug. After charging the oils and standard drugs, the Petri plates were left in refrigerator (10-20ºC) for 0.5 h to facilitate the diffusion of charged essential oils into the SDA medium. Then the plates were incubated at 28ºC for 48 h. The zones of inhibition of the test were measured as the diameter of the clear zone around each disc and the potency of test were estimated with reference to standard drug. The tests were performed in triplicate to confirm the observations (Table-2).

TABLE-2
EFFECT OF ESSENTIAL OILS ON FUNGAL GROWTH BY CUP-PLATE METHOD

Name of organism	Zone of inhibition (mm)*				
	SC	WC	WT	WB	EA
Control	-	-	-	-	-
Trychophyton mentographytes	38	25	29	23	24
Trycophytonm rubrum	40	25	33	22	21
Aspargellus ruauatii	29	20	22	19	19
Rhizopus	28	19	22	19	20
Streptococcus mutans	28	13	19	12	11

SC = Standard Ciproflaxine; WC = *W. chinensis*; WT = *W. trilobata*; WB = *W. biflora*; EA = *E. alba*; *Diameter of cups = 8 mm.
Solvents control: Did not show any activity.

RESULTS AND DISCUSSION

The antibacterial and antifungal activity of the essential oil has been presented in Tables 1 and 2. The results of study indicated that the essential oil of *Wedelia trilobata* was found to be effective against gram positive and active against the all tested fungi. While the gram negative organisms shows a moderate activity (= 50 %) at the tested concentrations compared to standards. The oil of *Wedelia chinensis, Wedelia biflora* and *Eclipta alba* were found to be moderate active against the tested gram positive and fungus strains comparing with the Standard. The fungal activities of the oils may be the presence of terpinoids, steroids and flavonoids.

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