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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF DIFFERENT CRUDE EXTRACTS OF *Wedelia biflora*

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Abstract:- A Study was under taken to investigate the qualitative phytochemicals and antimicrobial properties of different solvent crude extracts of *Wedelia biflora* against various bacterial and fungal pathogens. The phytochemical analysis carried out revealed the presence of alkaloids, tannins, saponins, flavanoids, carbohydrates, terpenoids and glycosides were tested. The antimicrobial activity of crude extracts (hexane , chloroform , ethyl acetate, methanol and aqueous) of medicinal plant were evaluated using agar well diffusion method against *Bacillus Stubtilis, Pseudomonas aeruginosa, Staphylococcus aureus , Aspergillus niger, Penicillium chrysogenum* and *Candida albicans* .The plant extracts showed different diameters zone of inhibition ranging from 10 to 35mm. Chloroform and methanol extracts of *Staphylococcus aureus* and *Pseudomonas aeruginosa* displaying strong antimicrobial activity against *Bacillus subtilis.* The results provide justification for the use of the medicinal plant to treat various oral infections.

Keywords: *Wedelia Biflora*, phytochemicals Screening, Antimicrobial activity.

INTRODUCTION

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine, and food supplements, pharmaceuticals industries and chemical entities for synthetic drugs (Ncube NS et al; 2008). India is the birth place of renewed system of indigenous medicine such as siddha, ayurvedha and unani. Traditional systems of medicines are prepared from a single plant. The efficacy depends on the use of proper plant part and its biological activities which in turn depends up on the presence of required quantity and nature of secondary metabolite in a raw drug (Vinoth S *et al.*, 2011; Savithramma N. *et al;* 2010). Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude extracts. The objective of this paper study was to evaluate the qualitative analysis of phytochemicals and antibacterial efficacy of selected medicinal plants .

Wedelia biflora (Linn.) D.C. belongs to a family compositae, is an ancient weed found in Eastern and Western sea coasts in India and other Southern Asia. Ethno medically, the leaves of the plant is used by traditional healers and local peoples of Tamil Nadu, India for dressing of wounds, treatment of ulcers, sore throat, varicose of veins, skin diseases, headache and fever (Council of Scientific and Industrial Research ; 1986).Roots are used to check vaginal discharge and stomachache. The flowers are said to be violent urgative (Yoganarasimhan S.N, 2000).The pounded leaves are used for preparing poultice for treating cuts, ulcer, sore, and varicose veins. A decoction of the roots and leaves are prescribed for stomach ache. The leaves are also credited with diuretic properties (Yoganandam GP *et al*., 2009).

Various reports reveals that this plant contains useful antifungal *phytoconstituents* such as 3'-formyl-2',4',6'-trihydroxydihydrochalconeveratrylidenehydrazide, 3,3'-di-O-methylquercetin, 2,7-dihydroxy-3 (3t'-methoxy-4'-hydroxy)-5-methoxyisoflavone and 3',7-di-O-methylquercetin (Miles DH *et al*.,1993) With this

background, the present study was undertaken to emphasise the effect of leaves of *W. biflora* against experimentally and antimicrobial property against a wide range of microorganisms.

2. MATERIAL AND METHODS

2.1 Plant materials

Leaves of *Wedelia biflora* were collected in the coast of east India (costal region of Danushkodi). The collected plant materials were washed thoroughly with distilled water to remove the dirt and other contaminations. The plant material was dried at room temperature to retain their fresh green colour and also prevent the decomposition of active compounds. The dried plant material were milled to coarse powder and stored in air tight container.

2.2 Extraction of *Wedelia biflora*

The leaf coarse powder was extracted with various sequential solvents of hexane, chloroform, ethyl acetate, methanol, and aqueous extract using Soxhlet apparatus. The extracts were filtered and concentrated under vacuum evaporator. The dried residue was stored in air tight container and subjected to phytochemicals analysis and further studies.

2.3 Preliminary phytochemical analysis

The preliminary phytochemical screening were carried out to determine the presence of alkaloids (Draggen dorff's test) , flavonoids (Shinoda's reaction) , saponins (Frothing test), tannins (5% alcoholic ferric chloride) , terepenoids (2,4 – nitro phenyl hydrazine), glycosides (Fehling's test) , steroids (Libermann's Burchard test) , anthraquinons (Borntrager's test) , phylobalanins (Iyengar 1995).

2.4 Antimicrobial activity assay

To determine the biomedical potential of the leaf extract, the antimicrobial activity assay was carried out.

2.4.1 Preparation of antibiotic disc

Sterile empty antibiotic discs (6mm diameter) were purchased from Hi-Media Company, Mumbai. 20mg of dried crude extract was dissolved in l ml of 20% DMSO (dim ethyl Sulphoxide). From this stock solution 10µl of respective solvent extracts was added to the disc (0.2mg/disc) individually and aseptically. Each disc contained 0.2mg of extract. Then the discs were allowed for drying at room temperature. After drying they were used for screening the antibacterial and antifungal activity.

Three reference bacteria strains (*Bacillus Stubtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96)), fungal strains *Aspergillus niger* (MTCC 1344) *Penicillium chrysogenum* (MTCC 5108) and *Candida albicans* (MTCC 227)) were used in this study.

2.4.2 Antibacterial activity

Antibacterial assay was carried out by agar diffusion method (Bayer's et al., 1966). The sterile Muller-Hinton agar plates were prepared. The reference strains were spread over the Muller - Hinton agar plates using separate sterile cotton swabs. The prepared sterile disc was placed on the surface of the medium at equidistance. Then the plates were incubated at 37°C for 24 hrs to determine the bacterial activity of the respective solvent extraction. Ciprofloxacin antibiotic discs (30mg/disc) were used as positive control and disc with respective solvents (10µl) were used as negative control. Each extract was treated in triplicate to calculate the mean value.

2.4.3 Antifungal activity

Antifungal assay was carried out using the agar diffusion method (Bayer's et al., 1966). The sterile Muller-Hinton agar plates were prepared and a small amount of tryptophan is added to avoid bacterial growth. The reference strains were spread over the Muller - Hinton agar plates by using separate sterile cotton swabs. The prepared sterile disc was placed on the surface of the medium at equidistance. Then the plates were incubated at 37°C for 24 hrs to determine the bacterial activity of the respective solvent extraction. Amphotericin antibiotic discs (30mg/disc) were used as positive control and disc with respective solvents (10µl) were used as negative control. Each extract was treated in triplicate to calculate the mean value.

2.4.5 Statistical analysis

.Experimental results concerning this study were mean ± S.D of three parallel measurements. Analysis of variance was performed using ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests. P values, 0.05 were regarded as significant and p value 0.01 is very significant.

RESULT AND DISCUSSION

Preliminary phytochemical characterization

The qualitative phytochemical screening of *Wedelia biflora* leaf extract using various solvent showed that most of the biologically active phytochemicals are present in chloroform extract of *Wedelia biflora* leaves (Table 1). It confirms the result that the therapeutic potent compounds are found in leaf extract of *Wedelia biflora*. It also shows that tannins and alkaloids predominantly found in all five extracts, terpenoids were also found in extracts chloroform, ethyl acetate, methanol and aqueous extracts, saponin and carbohydrates were found in chloroform, methanol and aqueous extracts, flavonoids were found in the extracts of hexane and methanol, and glycosides were found in ethyl acetate and aqueous), whereas amino acids and proteins were found in one extract (ethyl acetate). Phenol, phylobalanins, and volatile oil were not found in all the five extracts (chloroform, hexane, ethyl acetate, methanol, and aqueous).

In a comparative study of *Wedelia biflora* and *W. chinensis*, (Meena A K *et al.,* 2010) reported the presence of alkaloids, coumarin, flavones, steroid, tannin, glycoside, terpenoids, and saponin in the whole plant extracts corroborating with our present results. Moreover, (Yoganandam G P *et al*., 2009) reported that alkaloids were present in the leaf extract of *Wedelia biflora* using different solvents, such as benzene, ethanol, hexane and chloroform, whereas anthraquinone, glycosides, and cardiac glycosides were not found in all the four extracts. Sugars, flavonoids, tannins, terpenoids, proteins, carbohydrates, and coumarin were present only in ethanol and absent in all other three extracts (benzene, hexane, and chloroform). The qualitative phytochemical analysis of Wedelia species (*W.chinensis, W.tribolata, W.biflora*) in ethanol extract, showed the presence of alkaloids and flavones in *w.chinensis* and *w.biflora* . *Flavonoids, phenolic compounds,saponin, steroids* and terpenoids were present in all three species((W.chinensis, W.tribolata,and W.biflora), Proteins were present in *W.biflora*, which was found to be absent in *W.chinensis* and *W.tribolata*. Carbohydrates were present in *W.chinensis* and was found to be absent in *W.tribolata* and *W.biflora* where as anthraquinone and glycosides were absent in all three species(*W.chinensis, W.tribolata, W.biflora*) (Shanmugam and Sureshkumar et al.,2011).

The phytochemical compounds identified in the leaf extract of *Wedelia biflora* has been reported to be biologically active with different bioactivities, hence we strongly speculated that the leaf extracts Wedelia biflora, especially chloroform and methanol extracts could be adopted for pharmacological studies.

Table:1 Preliminary phytochemicals studies on various extract of *Wedelia biflora* leaf powder

S.No.	Phytochemical test	Aqueous	Chloroform	Ethyl acetate	Hexane	Methanol
1	Saponin	++	++	--	--	++
2	Tannins	++	++	++	++	++
3	Phenol	--	--	--	--	--
4	Terpenoids	++	++	++	--	++
5	Flavonoids	--	--	--	++	++
6	Amino acid and proteins	--	--	++	--	--
7	Carbohydrate	++	++	++	--	--
8	Phylobalannis	--	--	--	--	--
9	Volatile Oil	--	--	--	--	--
10	Glycosides	++	--	++	--	--
11	Alkaloids	++	++	++	++	++
12	Steriods	--	--	--	--	--

Antimicrobial Activity

Antimicrobial activity of the leaf extract of *Wedelia biflora* revealed that the chloroform and methanol extracts has greater potential to inhibit the growth of the pathogenic strains compared to that of the other three extracts (hexane, aqueous, and ethyl acetate). The chloroform and methanol extracts showed the diameter of inhibition zones ranging from 10 to 35 mm. The highest zone of inhibition was observed against *Staphylococcus aureus* with 32 and 34 mm, followed by *Pseudomonas aeruginosa* 22 and 23mm respectively for chloroform and methanol extract and the least inhibition zone was obtained against *Bacillus subtilis* of 11mm however the diameter observed was slightly similar to that of the standard antimicrobial agent, ciprofloxacin that is 13.50. Both the extracts were effective against the gram-positive and gram-negative bacteria.

Furthermore, the aqueous and organic extracts (chloroform, ethyl acetate, hexane, and methanol) of *Wedelia biflora* displayed notable antifungal activity against the selected fungal pathogens, Aspergillus niger, *Penicillium chrysogenum* and Candida albicans. As shown in Table 3, the hexane extract exhibited the highest zone of inhibition of 9 mm followed by ethyl acetate and methanol of 7 and 6 mm against A. niger. The chloroform, ethylacetate and methanol extracts showed merely 8, 9 and 10 mm zone of inhibition against *P. chrysogenum* which is lower than that observed with the standard, amphotericin of 13 mm. However, the chloroform extract showed makeable zone of inhibition of 10 mm against *C. albicans* when compared to the standard of 7 mm. In our study the potential antimicrobial activity of different extracts from the leaves of *Wedelia biflora* were found to be in the order of chloroform>methanol>ethyl acetate>hexane>aqueous with respect to the zone of inhibition. Hence, chloroform and methanol extracts showed the highest antimicrobial activity among the three extracts. In corroboration with our study, (Preethi et al.2010) had showed that the methanol extract of *Holarrhena antidysenterica* leaves showed high antimicrobial, anticancer, and antioxidant activity against food-borne pathogens.

Table 2. Antimicrobial activity of *Wedelia biflora* various leaf extract against disease causing pathogenic bacteria

S. No.	Name of the Bacteria	Zone of inhibition mm in diameter(M±SD) (n=6)						
		Standard S*	Control	Aqueous	Chloroform	Ethyl acetate	Hexane	Methanol
1.	*Bacillus subitils*	13.50±1.25 ^{a}	-	-	11.50 ±1.25 ^{a}	12.00 ±1.06 ^{a}	10.50±1.33 ^{a}	11.5 ±0.76^{a}
2	*Pseudomonass auerogeans*	18.00±1.06^{cd}	-	-	22.00±1.06^{ab}	16.00 ±1.06 ^{d}	20.00±1.06^{bc}	23.00 ±1.06^{a}
3	*Staphylococcus aureus*	20.00 ±1.06^{b}	-	-	32.00±1.06^{a}	14.00±1.06^{c}	22.00±1.06^{b}	34.00 ±1.06^{a}

S* - Ciprofloxacin (disc 30mg) Ref. Hi Media Standard value
Values are expressed in Mean ± SD and the values in vertically rows are significantly different at P>0.05% level

The antifungal and anti-inflammatory activity of methanolic extract of *Wedelia chinensis* leaves was determined. It revealed that higher concentration of methanolic extract reduces inflammation more as compared to low concentration of methanolic extract (Manjamalai A et al., 2011). The antimicrobial activity of *Wedelia chinensis* leaves was reported. It was tested against fifteen (gram+ve and gram-ve bacteria) and five fungal strains using disc diffusion method. Susceptibility of test microbes depends on the solvent used for extraction. It was found that methanolic extract showed strong antimicrobial activity. Most susceptible gram +ve bacteria is *streptococcus species*. The methanolic extract showed significant antifungal activity against *Candida albicans* (Banu R and Nagarajan N. 2012).

Further, leaves and stem extracts of *Mikania micrantha* H.B.K were prepared with different solvent (both polar and non –polar), which displayed a broad range of antimicrobial activity against fungi. Specifically, the leaf extracts showed higher activity against bacteria and fungi than the stem extracts, moreover the chloroform extract showed the highest bacterial activity (Yan Li *et al.*, 2013). In addition,(Manjamalai *et al*. 2011) had reported the leaf extract of *Wedelia chinense* demonstrated antimicrobial activity against fifteen bacterial strains tested and five fungal strains. Several studies reported the antioxidant activity of essential oil from *Wedelia chinensis* in vitro and in vivo by lung cancer bearing C57BL/6.It showed a significant co-relation that exists between the concentration of

essential oil and percentage of inhibition free radicals. It revealed that essential oil can be recommended for treating diseases related to free radicals and for the treatment of cancer (Manjamalai.A *et al.*,2012). The result showed that Wedlolactone from *Wedelia calandulaceae* was found to possess 5-lipoxygenase and caspase inhibitory properties. *Wedelia chinensis* is having potent analgesic and anti-inflamatory effects and therapeutic efficacy of *Wedelia chinensis* extract on animal models was comparable with those of standard drugs such as morphine, aspirin and indomethacine (Wagner H et al., 1986).

Extracts showed antifeedant activity which led to an investigation of antifungal properties in the stem of *Wedelia biflora* (D. Howard Miles.*et al.,* 1990). Different crude extracts (n-hexane, ethyl acetate, and aqueous) of *Wedelia trilobata* reported the biological screening of activity of gram positive and gram negative bacteria, yeast and fungi. Here n- hexane only showed the against gram positive and ,gram negative bacteria when compared to other two extracts . None of them were active against the yeast and fungi (Taddei A *et al* ., 1999).Antifungal activity of *Wedelia paludosa* (Sartori M.R.K *et al*.,2003). The aqueous extract showed highest antibacterial activity against S. epidermidis (14.3 mm) followed by S. aureus(13 mm) and K. pneumonia (10.6 mm). The antimicrobial properties of different solvent crude extracts of four Indian medicinal plants namely, Nerium oleander, Lippia nodiflora,Wattakaka volubilis and *Wrightia tinctoria* against various bacterial pathogens. The plant extracts showed different diameters of inhibition zone ranging between 4 to28 mm. Among themselves, methanol, ethyl acetate, hexane and petroleum ether extracts of N. oleander exhibited the highest inhibition zone against S. typhi than standard antibiotics(Chloramphenical) around 22 mm (Jeyachandran *et al* ., 2010) .

The ethanolic extract showed significant result than hexane extract against all the test samples(Merina Paul Das *et al.*, 2013).The studied *phytochemical constituents* and *antimicrobial activity* of some medicinal plants including ethanol extract of Wedelia chinensis. The antibacterial activity was determineed against different bacterial strains using minimum inhibitory concentration and zone of inhibition (.Manjamalai A *et al* ., 2010).

Table 3. Antimicrobial activity of *Wedelia biflora* various leaf extract against disease causing pathogenic fungi

S. No.	Name of the Bacteria	Zone of inhibition mm in diameter(M±SD) (n=6)						
		Standard S*	Control	Aqueous	Chloroform	Ethyl acetate	Hexane	Methanol
1.	*Aspergillus niger*	9.00 ±1.57^{a}	-	6.00±1.52^{ab}	2.00±0.76^{b}	7.00±1.52^{a}	9.00±1.52^{a}	6.00±1.52^{ab}
2	*Penicillium chrysogenum*	13.0±1.52^{a}	-		8.00±1.52^{b}	9.00±1.52^{b}	-	10.00±1.52^{ab}
3	*Candida albicans*	7.00±1.52^{b}	-	5.00±1.00^{bc}	10.00±1.06^{a}	5.00±1.06^{bc}	3.50±1.52^{d}	-

S* - Amphotericin (disc 15mg) Ref. Hi Media Standard value
Values are expressed in Mean ± SD and the values in vertically rows are significantly different at P>0.05% level

CONCLUSIONS

From the present study, it is concluded that most of the biologically active phytochemicals were present in the various extracts of *Wedelia biflora* leaves; the results confirmed the presence of therapeutically potent compound in leaf extract of *Wedelia biflora,* and further using of this plant extract in pharmaceutical industry.

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