

Phytochemical analysis and biological evaluation of leaf extracts of *Wedelia Chinensis*

Abstract

Wedelia chinensis is a reputed herbal medicine in ayurvedic, siddha and Unani system of medicine. In the present study leaf of *Wedelia chinensis* was explored for phytochemical, cytotoxic and antioxidant potential. Leaf crude extract was positive for the presence of tannins and saponins. Methanol extract showed 100% activity at the higher dose of 400 microgram/ml in brine shrimp mortality assay where as acetone and methanol extract both exhibited more than 80% radical scavenging activity in DPPH radical scavenging assay.

Keywords: tannins, saponins, brine shrimp assay, DPPH, antioxidant

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Introduction

Wedelia chinensis is a small much branched annual herb, commonly known as “pilabhamgara” or “bhringraj” in Hindi. *Wedelia* in Chinese, “pitabhrnga” in Sanskrit.¹ The fresh juice from the leaves of *Wedelia chinensis* has been used by ayurvedic physicians in India for external use to treat skin problems, dermatitis, eczema and acne.² The leaves are regarded as tonic and alternative, useful in cough, cephalgia and diseases of the skin, especially alopecia. A decoction of the herb is used in uterine haemorrhage and menorrhagia.³ As can be seen that leaves are endowed with a number of medicinal properties hence in this study same were explored for their phytochemical, cytotoxic and antioxidant nature.

Materials and methods

Collection and processing of plant material

Leaves of *Wedelia chinensis* were collected from medicinal germplasm garden of Regional Plant Resource Centre (RPRC), Bhubaneswar. Weight of fresh leaves were taken and washed thoroughly and left for drying. Later after complete drying moisture content of the sample was evaluated. Dried samples were completely dried in incubator at 60°C for 1hr and then the dried leaves were made into fine powder in mechanical grinder of Lexus make. 15gms of powdered leaf sample of plant (*Wedelia chinensis*) was taken in a thimble and was subjected to extraction with increasing order of polarity of different solvents like Hexane, Chloroform, Acetone and Methanol. 250ml of solvent was taken in round bottom flask and extraction was carried out Soxhelt. Reflux was continued till solvent became colourless. Extract obtained was concentrated in Buchi (R-200) Rota vapour. Concentrated extract was stored in screw cap vials until further use.

Phytochemical tests

All the tests were conducted as per the standard protocols⁴

i. Tannin: Extracts were dissolved in 50ml distilled water and heated for 10 minutes on hot plate. Solution was cooled and 1% ferric chloride was added drop by drop and change in the color of solution was observed.

ii. Anthraquinone: Small amount of extracts were added to 5ml of 5% KOH, and color change was observed.

iii. Saponins: To the extracts 1% sodium bicarbonate was added and stirred vigorously and solution was observed for the presence of honey comb like structure.

iv. Flavonoids: 5ml of leaf extract was taken in flask to which 10% NaOH was added. From the side of the flask few drops of HCL were added. Sample was observed for change in color.

v. Terpenoid: 1ml of extract was mixed with 400µl chloroform. Then the mixture was added by drop of sulphuric acid. A reddish brown interface indicates the presence of terpenoids.

vi. Phlobatannin: Few amount of extracts was boiled with 1% aqueous HCL. Sample was observed for red precipitate.

vii. Protein: 1ml of extract was dissolved in 5ml distilled water. Then the mixture was boiled in hot water bath for few minutes. Sample was observed for the presence of coagulation.

viii. Alkaloids: In 1ml extract 5ml of 1% aq. HCL was added and then it was heated for few mins. 2 drops of dragendroff reagent was added to it. Sample was observed for reddish brown precipitate.

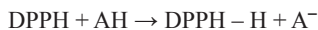
Brine shrimp lethality test

Brine shrimp (*Artemia salina*) eggs were incubated for 48hrs (3.6gm of black salt in 200ml distil water) to get the desired growth of the larvae for biological evaluation. Stock solutions of different extracts were prepared at a concentration of 10µg/µl. Cytotoxic assay was carried out at five doses 25, 50, 100, 200 and 400µg/ml, for each dose level 3 replicates were used. Motility, readings were taken every hour up to 4hrs. Motility was graded. After 24hrs the final reading was taken and percentage of inhibition was calculated by comparing the treated samples with the controls. Standard deviation was also calculated.

Antioxidant activity (Qualitative screening of antioxidants)

DPPH assay: To detect antioxidant activity, qualitative 2, 2 - Di-

phenyl-1-picrylhydrazyl (DPPH) assay was carried out. The plates were first air dried and then the chromatograms were sprayed with 0.2% DPPH in methanol as an indicator.⁵ The presences of antioxidant (AH) compounds were detected by yellow spots against a purple background on the TLC plates sprayed with 0.2% DPPH in methanol.



(Purple color) (Yellow color)

Qualitative screening of the constituents in each of the leaf extracts of *Wedelia chinensis* for antioxidant activity was done by TLC analysis. The process was carried out using TLC sheets. For about 5µl of each sample was loaded on the TLC sheet and the chromatograms were developed in following solvent systems:

- Ethyl acetate: methanol: water (40:5.4:4) [EMW] (polar neutral)
- Chloroform: ethyl acetate: formic acid (5:4:1) [CEF] (Intermediate polarity/acidic)
- Benzene: ethanol: ammonium hydroxide (90:10:1) [BEA] (Non polar/basic)

Quantitative antioxidant assay: DPPH radical scavenging assay

For DPPH free radical scavenging assay 1mM DPPH (2, 2-Diphenyl-1- picryl hydrazyl) (Mol. Wt. 394.33) solution was prepared. 4mg DPPH was weighed and dissolved in 10ml methanol. DPPH assay was done by serial dilution method starting from concentration 5000µg, 2500µg, 1250µg, 625µg, 312.5µg, 156.25µg, 78.13µg, 39.06µg, 19.53µg, 0.976µg. 500µl DPPH solution was added to each test-tube and stirred thoroughly before incubated for 30min. Then optical density (OD) was measured at $\lambda = 517\text{nm}$ in spectrophotometer. The percentage radical scavenging activity was calculated from the following formula:

$$\% \text{ Scavenging [DPPH]} = \left[\frac{(A_0 - A_1)}{A_0} \right] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of sample.

Results and discussion

Moisture content of *Wedelia chinensis* was slightly higher upto the tune of 82%. Amongst all extracts methanol extract showed highest yield as compared to other extracts.

Phytochemical analysis

As shown in Table 1. Tannins and saponins were present in the fresh samples of leaf. Tanins are well known for their astringent properties⁶ and they also promote formation of tissues on wounds and also used in case of varicose veins.⁷ Saponins are also well known for their association with cytotoxic, antitumor and anti inflammatory activity,⁸ thus the presence of both these class of compounds could be indicative of the biological activity of the leaves of *Wedelia chinensis*.

Cytotoxic activity/Brine shrimp assay

As can be seen from the Figure 1 methanol extract exhibited highest activity (100%) at a higher dose of 400microgram/ml, all the extracts showed dose dependent activity. Significant cytotoxic

activity could be due the presence of saponins which are well known to possess such cytotoxic potential.⁹

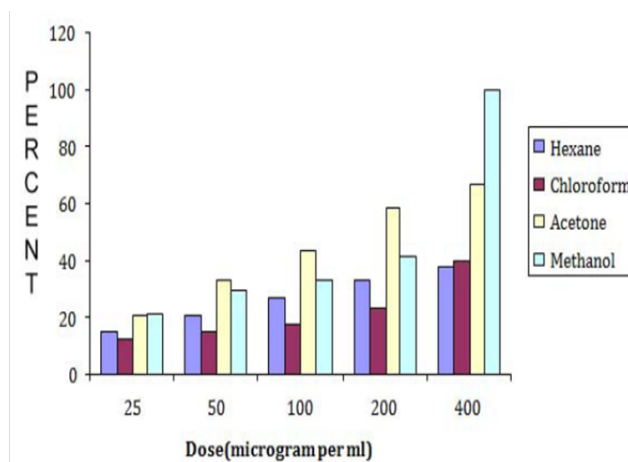


Figure 1 Cytotoxic assay of *Wedelia chinensis*.

Table 1 Phytochemical results of *Wedelia chinensis*

Class of compounds	Results
Tannin	+ve
Anthraquinone	-ve
Saponin	+ve
Flavonoids	-ve
Terpenoids	-ve
Phlobotanin	-ve
Protein	-ve
Alkaloids	-ve

Antioxidant activity of *Wedelia chinensis* leaf extracts

Qualitative antioxidant activity: Three solvents were used for qualitative antioxidant assay. As can be seen from the Table 2 Chloroform extracts showed highest number of antioxidant bands as compared to other extracts

Quantitative radical scavenging assay: At higher doses acetone and methanol extracts showed similar activity of more than 80%, qualitative and Quantitative results varied as number of antioxidant bands were more in chloroform extracts, it seems molecules in chloroform extracts had antagonistic effect where as number of antioxidant molecules were less in acetone and methanol extracts yet they showed more radical scavenging activity could be due to synergistic action of anti oxidant molecules present in the two extracts.¹⁰ Thus qualitative assays could give only the number of antioxidant bands corresponding to the number of antioxidant molecules but they cannot depict the overall antioxidant potential of the extracts.

Table 2 Qualitative antioxidant assay of *Wedelia chinensis* solvent extracts

Solvent extract	Ethyl acetate: methanol: water (40:5.4:4) [Emw]	Benzene: ethanol: ammonium hydroxide (90:10:1) [Bea]	Chloroform: ethyl acetate: formic acid (5:4:1) [Cef]
Hexane	2	3	2
Chloroform	5	6	5
Acetone	2	0	2
Methanol	2	0	2

Conclusion

Thus, amongst leaf extracts of *Wedelia chinensis*, methanol extract showed significant cytotoxic as well as antioxidant potential and further needs extensive exploration for the isolation of antioxidant as well as cytotoxic active principles.

Acknowledgements

None.

Conflict of interest

Author declares that there is no conflict of interest.

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