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Antioxidant and Antimicrobial Activities of *Wedelia trilobata* and *Morinda pubescens*

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The methanolic extracts of two Indian medicinal plants *Wedelia trilobata* (Linn.) and *Morinda pubescens* (Smith) belonging to the families of *Astraceae* and *Rubiaceae* were investigated for the antioxidant and antimicrobial activities. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used as an *in vitro* model to evaluate the antioxidant activities of different parts of the plants like root, stem, leaves, flower and fruit and it was compared with standard antioxidant L-ascorbic acid. Agar diffusion method was used to study the antibacterial activity against seven bacterial strains in Nutrient-Agar media. In Wedelia trilobata, flower had showed the highest antioxidant activity (99.12 %) at 800 µg when compared to other parts of the plant and exhibited reasonable good antibacterial activity and the inhibition was maximum for *Salmonella paratyphi* and *Bacillus cereus*. The antioxidant activities of all the parts of *Morinda pubescens* were minimum and the root had exhibited highest antibacterial activity against *Shigella sonnei* with the zone of growth inhibition 26 mm at 25 mg.

Key Words: *Wedelia trilobata*, *Morinda pubescens*, 2,2-Diphenyl-1-picrylhydrazyl, Antioxidant, Antimicrobial.

INTRODUCTION

Human cells are constantly exposed to reactive oxygen radicals generated by a number of biotic and abiotic factors such as irradiation, environmental factors, pollutants, stress or by products of metabolic processes. When the exposure overwhelms endogenous preventive systems, cells are exposed to potentially harmful load of oxidants, leading to various free radicals induced noxious effects. These include oxidation of nucleic acids, proteins, lipids and carbohydrates which may subsequently determine mutagenesis and diseases related to DNA damage[1]. The balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system. Hyper physiological burden of free radical causes imbalance between oxidants and antioxidants in the body. This imbalance leads to oxidative stress involving aging and various diseases like stroke, diabetes, cancer and neuro generative diseases such as Alzheimer's and Parkinsonism[2,3].

World Health Organisation (WHO) estimated that 70-80 % of the population of the developing countries depends on medicinal plants for their basic pharmaceutical care[4] and the plants constitute an important source of active natural products which differ widely in terms of structure and biological properties. They have a remarkable role in the traditional medicine in different countries. The protective effects of plant products are due to the presence of several compounds like amino acids, sugars, vitamins, flavonoids, polyphenols, phenolic acids, alkaloids, terpenoids etc which have distinct mechanisms of action[5].

In recent years, there has been a great interest in finding natural antioxidants and antimicrobials from plant materials and numerous crude extracts and pure natural compounds from plants were reported to have antioxidant and antimicrobial activities[6-8]. Antioxidant properties elicited by plant species have a full range of perspective applications in human healthcare. It is investigated that the prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruits, vegetables or tea which are rich in natural antioxidants[9]. Higher intake of such compounds should lower the risk of mortality from the degenerative diseases[10]. In search of some sources of novel antioxidants, in the last few years some medicinal plants have been extensively studied for their antioxidant activities[11-14].

Morinda pubescens and *Wedelia trilobata* are traditionally used as laxative and also as wound healing, antioxidant and antidepressant. Extensive literature survey shows that limited work has been carried out in *Wedelia trilobata* and *Morinda pubescens*. Thus an attempt has been made in the assessment of antioxidant and antibacterial efficiency of the above mentioned Indian medicinal plants.

EXPERIMENTAL

All chemicals and solvents were of analytical grade and supplied from Sigma-Aldrich and the bacterial strains were

purchased from the Department of Molecular Biology, Christian Medical College and Hospital, Vellore, Tamil Nadu, India.

Wedelia trilobata and *Morinda pubescens* were identified by Ms. Isabella Roseline, Head, Department of Botany in Auxilium College campus and authenticated by a Taxonomist Mr. Babu, Cholayil, Chennai and the Vouchers of the plant specimen were deposited in the Department of Botany, Auxilium College with the code DRC_wt1 and DRC_mp1.

Different parts of the plant materials like root, stem, leaves, flower and fruits were shade dried, finely ground and were percolated in 100 % methanol and filtered. The filtrate was evaporated at 40 ºC under reduced pressure by a rotary evaporator to give a semisolid residue of approximately 200 g each.

Cultures of seven human pathogenic bacteria made up of two gram positive and five gram negative bacteria were used for the *in vitro* antibacterial assay. The bacterial strains are *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 10031), *Salmonella typhi* (ATCC 10749), *Shigella sonnei* (ATCC25931), *Bacillus cereus* (ATCC 10987), *Salmonella paratyphi* (ATCC 11511) and *Staphylococcus auereus* (ATCC 25923).

General procedure

Radical scavenging activity: Experiments were carried out in triplicate according to the method of Blois (1958) with the slight modification[15]. Briefly, 25 mg/L solution of DPPH radical (Aldrich) in methanol was prepared and then 2 mL of this solution was mixed with different concentration (400, 600 and 800 µg) of sample solution to achieve the final volume of 3 mL. After 0.5 h the absorbance was measured at 517 nm. Decrease in the absorbance of the DPPH solution indicates an increase of the DPPH antioxidant activity. The antioxidant activity (AOA) was calculated using the equation

$$AOA = \frac{(A_o - A_s)}{A_o} \times 100$$

A_o = DPPH solution without the sample, A_s = DPPH solution with the sample.

Antimicrobial activity: Sterile nutrient broth was inoculated with freshly isolated bacterial culture and incubated for 24 h at 37 ºC. The bacterial suspension was found to be approximately 10^7-10^8 cells/mL. After the incubation period they were used as inoculum. About 0.1 mL of the suspension containing 10^8 colony forming unit (CFU/mL) of bacterial strains were taken which was studied by agar diffusion method[16]. About 500 mg/mL of methanolic extracts of the plant materials at different concentrations like 5.0, 10.0, 12.5 and 25.0 mg of sample were used and their zones of inhibitions were monitored after 24 h and the inhibition zone was compared with methanol which was a negative control.

Statistical analysis: The experiments were carried out in triplicate and the statistical software package (SPSS.12) was used for the statistical analysis and the results are given as a mean ± standard deviation (SD). Regression analysis was carried out for the comparison of concentration dependency and one-way analysis of variance (ANOVA) was used for comparison of more than two means. A difference was considered statistically significant when $p \leq 0.05$[17].

RESULTS AND DISCUSSION

Radical scavenging activity of *Morinda pubescens* and *Wedelia trilobata*: The results of the antioxidant activity of *Morinda pubescens* and *Wedelia trilobata* determined by DPPH assays at different concentrations are given in Tables 1 and 2, respectively. From Table-1 it was evident that all parts of *Morinda pubescens* showed moderate antioxidant activity when compared with standard antioxidant L-ascorbic acid whose radical scavenging activity at different concentrations like 400, 600 and 800 µg were 84, 88 and 94 % and the antioxidant activity of L-ascorbic acid at 800 µL (positive control) were in good agreement with the results of earlier workers[18,19]. The antioxidant activities followed the trend: leaves 65.89 % > stem 56.99 % > fruit 55.38 % > root 44.14 %. The radical scavenging activities of root were same at different concentrations. This clearly indicates that the antioxidant activity was highest at the 600 µg and the increase in concentration showed least effect on the antioxidant activity. It was concentration independent as proved by regression analysis.

TABLE-1
ANTIOXIDANT ACTIVITY OF *Morinda pubescens*

Part of plant	Conc. (µg)	Mean ± SD	R^2	Scavenging activity (%)
Leaves	400	0.2427 ± 0.0065	0.937	31.4
	600	0.2092 ± 0.0069		40.9
	800	0.1207 ± 0.0065		65.9
Stem	400	0.2622 ± 0.0057	1.000	33.9
	600	0.2155 ± 0.0071		45.7
	800	0.1707 ± 0.0065		57.0
Root	400	0.2504 ± 0.0053	0.768	36.9
	600	0.2224 ± 0.0074		44.0
	800	0.2217 ± 0.0062		44.1
Fruit	400	0.2045 ± 0.0058	0.999	42.7
	600	0.1757 ± 0.0075		45.4
	800	0.1437 ± 0.0085		55.4

TABLE-2
ANTIOXIDANT ACTIVITY OF *Wedelia trilobata*

Part of plant	Conc. (µg)	Mean ± SD	R^2	Scavenging activity (%)
Leaves	400	0.1626 ± 0.0073	0.998	54.4
	600	0.2183 ± 0.0141		38.8
	800	0.2657 ± 0.0092		25.6
Stem	400	0.1110 ± 0.0059	0.964	72.0
	600	0.0569 ± 0.0260		85.7
	800	0.0300 ± 0.0073		92.4
Flower*	400	0.1182 ± 0.0065	0.870	98.0
	600	0.0640 ± 0.0057		98.9
	800	0.0532 ± 0.0053		99.1

*Absorbance at different concentration were significant at confidence interval of 95 % by Tukey test.

Table-2 shows the antioxidant activities of different parts in *Wedelia trilobata* at various concentrations. From the Table-2 it is clear that the stem and flower exhibits the highest antioxidant activity of 92.44 and 99.12 %. Antioxidant activity of flower is higher than L-ascorbic acid at all concentrations. This may be due to the presence of phenolic compounds and flavonoids as detected by photochemical analysis were responsible for the antioxidant activity even at lower concentrations[20]. Thus the antioxidant activity of flower was independent of concen-

tration. The antioxidant activity of leaf decreased proportionately with increase in concentration. This discrepancy may be because of the interference of the phytochemicals at the wavelength of 517 nm. From the Tables 1 and 2 it was also evident that good precision is observed which was evident from the standard deviation values.

From the regression analysis it was clear that there was a good linearity between concentration and absorbance for all the parts of *Morinda pubescens* and *Wedelia trilobata* except the root of *Morinda pubescens* whose R^2 value was 0.768. Hence the root of *Morinda pubescens* was concentration independent at higher concentrations. In *Wedelia trilobata* good linearity with negative slope was observed in leaves which may be due to the interference of some of the phytochemicals. The poor linearity in flower ($R^2 = 0.870$) may be due to the presence of phenolic compounds and flavonoids which may be present in higher concentration.

One way analysis of variance (ANOVA) was used to test the level of significance between absorbance and concentration. The experiments were conducted in triplicate and the probability factor (p) was < 0.05 between different parts of *Morinda pubescens* and *Wedelia trilobata*. So it was considered as significant.

Antibacterial activity: In the present study, the methanolic extract of various parts of *Morinda pubescens* and *Wedelia trilobatao* were selected for antibacterial activity on seven different microorganisms like *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Shigella sonnei*, *Bacillus cereus*, *Salmonella paratyphi* and *Staphylococcus auereus*. Depending on the measured values of the complete inhibition diameter of the zone including the well in millimeter, the antibacterial activity can be classified into the following types, such as > 12 mm zone of inhibition - high sensitivity, 9-12 mm zone of inhibition - moderate sensitivity, 6-9 mm zone of inhibition - less sensitivity and < 6 mm zone of inhibition - resistant[21].

Antibacterial activity of *Morinda pubescens*: The antibacterial activities of various parts of *Morinda pubescens* for selected test bacterial strains at different concentrations were given in Table-3. From Table-3, it was clear that, all the samples at different concentrations (5.0, 10.0, 12.5 and 25.0 mg) gave different inhibition activities towards tested organisms when compared with the negative control methanol whose inhibition zone was 6 mm. Among the different plant extracts, root showed the highest antibacterial activity against *Shigella sonnei* with the net inhibition zone of 26 mm for 25 mg and the zone inhibition increased as the concentration of the sample increased for *Bacillus cereus* and *Shigella sonnei* and the sensitivity was very high at 25 mg. The zone of inhibition was moderate for the fruit of *Morinda pubescens* to the bacterial strains *Salmonella paratyphi*, whose zone of inhibition was 11 mm at concentration of 25 mg. The inhibitory effects of leaves, stem and fruit were less at higher concentrations (25 mg) for the bacterial strains *Bacillus cereus*, *Escherichia coli*, *Salmonella paratyphi* and *Salmonella typhi* and had exhibited no inhibitory action against *Klebsiella pneumoniae* and *Staphylococcus aureus*. The leaves and root showed no inhibition against *Salmonella paratyphi* and the fruit showed no inhibition against *Shigella sonnei*.

TABLE-3
ANTIBACTERIAL ACTIVITY OF *Morinda pubescens* AGAINST SELECTED BACTERIAL STRAINS

Part of plant	Conc. (mg)	BC (mm)	EC (mm)	KP (mm)	SA (mm)	SS (mm)	SP (mm)	ST (mm)
Leaves	05.0	–	–	–	–	–	–	–
	10.0	–	–	–	–	–	–	–
	12.5	–	–	–	–	–	–	–
	25.0	8	9	–	–	8	–	8
Stem	05.0	–	–	–	–	–	–	–
	10.0	–	–	–	–	–	6	–
	12.5	–	–	–	–	–	7	–
	25.0	10	6	–	8	8	9	7
Root	05.0	6	–	–	–	6	–	–
	10.0	10	–	–	–	8	–	–
	12.5	14	–	–	–	18	–	–
	25.0	18	10	–	–	26	–	13
Fruit	05.0	–	–	–	–	–	8	–
	10.0	–	–	–	–	–	8	–
	12.5	–	–	–	–	–	11	–
	25.0	8	8	6	6	–	11	8

BC: *Bacillus cereus*, EC: *Escherichia coli*, KP: *Klebsiella pneumonia*, SA: *Staphylococcus auereus*, SS: *Shigella sonnei*, SP: *Salmonella paratyphi*, ST: *Salmonella typhi*.

Antibacterial activity of *Wedelia trilobata*: Preliminary screening for antibacterial activities of different parts like leaves, stem and flower of *Wedelia trilobata* against the seven selected bacterial strains at various concentrations are given in Table-4. The zone of inhibition was the highest (18 mm) for the concentration of 25 mg against *Salmonella paratyphi* and flower showed good inhibition activity against *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella paratyphi* whose zone of inhibitions were 15, 15, 17 and 15 mm, respectively and the inhibition zone was high for *Salmonella paratyphi*. The stem showed moderate inhibition against *Bacillus cereus*, *Staphylococcus auereus* and *Salmonella paratyphi* at the maximum concentration of 25 mg. Flower was insensitive against *Shigella sonnei*, *Staphylococcus auereus* and *Salmonella typhi* and the stem was insensitive to *Escherichia coli*, *Klebsiella pneumonia* and *Shigella sonnei* at all concentrations. The zone of inhibition was moderate for *Bacillus cereus* and *Salmonella typhi* and the leaves were insensitive towards *Escherichia coli*, *Klebsiella pneumonia*

TABLE-4
ANTIBACTERIAL ACTIVITY OF *Wedelia trilobata* AGAINST SELECTED BACTERIAL STRAINS

Part of plant	Conc. (mg)	BC (mm)	EC (mm)	KP (mm)	SA (mm)	SS (mm)	SP (mm)	ST (mm)
Leaves	05.0	–	–	–	–	–	6	–
	10.0	6	–	–	–	–	7	–
	12.5	7	–	–	–	–	9	7
	25.0	11	6	6	6	8	18	9
Stem	05.0	–	–	–	–	–	–	–
	10.0	–	–	–	–	–	6	6
	12.5	6	–	–	8	–	8	7
	25.0	9	6	8	12	8	13	11
Flower	05.0	6	–	6	–	–	6	–
	10.0	8	–	6	–	–	8	–
	12.5	10	–	8	–	–	11	–
	25.0	15	15	16.7	6	–	15	–

BC: *Bacillus cereus*, EC: *Escherichia coli*, KP: *Klebsiella pneumonia*, SA: *Staphylococcus auereus*, SS: *Shigella sonnei*, SP: *Salmonella paratyphi*, ST: *Salmonella typhi*.

and *Staphylococcus auereus* and showed moderate sensitivity to *Bacillus cereus* and *Salmonella typhi*.

The good antioxidant and antimicrobial activity may be due to the presence of phytochemicals like phenolic compounds, flavonoids, coumarin, quinones, terpenoids, *etc*. as proved by the phytochemical analysis (Table-5).

TABLE-5
PHYTOCHEMICAL ANALYSIS OF
Morinda pubescens AND *Wedelia trilobata*

Compounds	*Morinda pubescens*				*Wedelia trilobata*		
	Leaves	Stem	Root	Fruit	Leaves	Stem	Flower
Phenolic compounds	–	–	+	+	–	–	+
Steroids	+	–	+	–	+	+	+
Terpenoids	–	–	+	–	–	–	–
Flavonoids	+	–	+	+	–	–	+
Sugars	+	–	+	+	+	+	+
Coumarins	–	–	+	+	+	+	+
Quinone	+	–	+	+	–	+	+
Tannins	+	+	–	–	+	–	+
Saponin	+	+	+	+	+	+	+

Conclusion

The present study revealed that all the parts of *Wedelia trilobata* showed good radical scavenging activity when compared to *Morinda pubescens*. In *Wedelia trilobata* flower showed highest antioxidant activity when compared to stem and leaves. The results of this study also reveal that the zone of inhibition was different for different parts of *Morinda pubescens* and *Wedelia trilobata*. The root of *Morinda pubescens* was highly sensitive to *Shigella sonnei* and the flower and leaves of *Wedelia trilobata* were highly sensitive to the bacterial strains *Klebsiella pneumonia* and *Salmonella paratyphi*. Hence the present study supports the view, that these medicinal plants might be useful as antioxidant and antimicrobial agents. Further research could pay way for the development of novel drugs to control diseases and infections.

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REFERENCES

1. R. Bruni, D. Rossi, M. Muzzoli, C. Romagnoli, G. Paganetto, E. Besco, G. Choquechillo, K. Peralta, W.S. Lora and G. Sacchetti, *Fitoterapia*, **77**, 538 (2006).
2. B. Halliwell, *Lancet*, **344**, 721 (1994).
3. M.E. Letelier, A. Molina-Berrios, J.C. Troncoso, J.J. Sandoval, M. Holst, K. Palma, M. Montoya, D. Miranda and V. Gonzalez-Lira, *Toxicol. in Vitro*, **22**, 279 (2008).
4. A.A. Boligon, R.P. Pereira, A.C. Feltrin, M.M. Machado, V. Janovik, J.B.T. Rocha and M.L. Athayde, *Bioresour. Tech*., **100**, 6592 (2009).
5. A.C.C. Argolo, A.E.G. Sant Ana, M. Pletsch and L.C.B.B. Coelho, *Bioresour. Tech*., **95**, 229 (2004).
6. J.F. Lopez, N. Zhi, L.A Carbonell and V. Kuri, *Meat Sci*., **69**, 371 (2005).
7. R Torres, F Faini, B. Modak and J. Guerrero, *Phytochemistry*, **67**, 984 (2006).
8. V. Brezova, A. Slebodova and A. Stasko, *Food Chem*., **114**, 859 (2009).
9. L.P. Leong and G. Shui, *Food Chem*., **76**, 69 (2002).
10. D. Pratico and N. Delanty, *Am. J. Med*., **109**, 577 (2000).
11. Z. Xie, L. Duan, X. Tian, B. Wang and A. Egrinya, *J. Plant Physiol.*, **165**, 375 (2008).
12. K. Marxen, K.H. Vanselow, S. Lippemeier and R. Hintze, *Sensors*, **7**, 2080 (2007).
13. Y.S. Shyu and L.S. Hwang, *Food Res. Int*., **35**, 357 (2002).
14. N. Coruh, A.G. Sagdicglu Celep, F. Ozgokee and M. Iscan, *Food Chem.*, **100**, 249 (2007).
15. A. Cakir, A. Mavi, A. Yildirim, M.E. Duru, M. Harmandar and C. Kazaz, *J. Ethnopharmacol*., **87**, 73 (2003).
16. L. Estevinho, A.P. Pereira, L. Moreira and G. Luis, *Food Chem. Toxicol*., **49**, 3774 (2008).
17. S.M. Sabir and J.B.T. Rocha, *J. Ethnopharmacol*., **120**, 226 (2008).
18. T. Taechowisan, N. Chanaphat, A. Wanbenjob and Y. Shen, *Int. J. Biol. Chem*., **3**, 11 (2009).
19. S. Milardovic, *Bioelectrochemistry*, **68**, 175 (2006).
20. D. Villano, M.S. Fernandez Pachon, M.L. Moya, A.M. Troncoso and M.C.G. Parrilla, *Talanta*, **71**, 230 (2007).
21. P. Uma Devi, S. Murugan, S. Suja, S. Selvi, P. Chinnasamy and E. Vijayaanad, *Pak. J. Nutr*., **6**, 44 (2007).