Research Article

Untapped Ornamental Vines of Convolvulaceae-Potential Source of Antioxidants.

Ridhi Joshi*, Preeti Mishra, Vidya Patni
Plant Pathology, Tissue Culture and Biochemistry Laboratory, Department of Botany, University of Rajasthan, Jaipur, India.
***Corresponding author's E-mail:** ridhi.joshi316@gmail.com

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ABSTRACT

Secondary metabolites having antioxidative properties were screened in *in vitro* and *in vivo* plant parts of *Merremia aegyptia* and *Merremia dissecta*. The callus induction and establishment was achieved at NAA 1mg/L and BAP 0.5mg/L for *M. aegyptia* and NAA 2mg/L and BAP 0.5mg/L for *M. dissecta*. This is the first report of callus induction in these species. The free radical scavenging activity of the methanolic sample extracts was evaluated using established DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay. The highest phenol content was measured in *M. aegyptia* leaf (104 ± 0.8 mg Catechol equivalent/ g dry weight of sample) whereas highest flavonoid content was measured in *M. aegyptia* stem (38± 0.2 mg Quercetin equivalent/ g dry weight of sample). The maximum antioxidant activity was reported in *M. dissecta* stem having lowest IC_{50} value (60.5). Total phenolics, flavonoid contents and antioxidant activity showed that these species could practically be used in pharmaceutical industries.

Keywords: Antioxidants, 2,2-Diphenyl-1-Picrylhydrazyl, NAA, BAP, flavonoids

INTRODUCTION

Free radicals have significant role in the causation of several diseases such as diabetes, cirrhosis, cancer, and cardiovascular diseases[1]. Antioxidants are compounds that protect cells against the damaging effect of reactive oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrile which results in oxidative stress leading to cellular damage. Thus, compounds and antioxidants that can scavenge free radicals have vital role in the improvement of these diseased conditions[2]. Human body has an inherent antioxidative mechanism[3] and many of the biological functions such as the antimutagenic, anti-carcinogenic, and anti-aging responses originate from this property. Studies on herbal plants, vegetables, and fruits have indicated the presence of natural antioxidants, the best known are tocopherols, carotenoids, vitamin C, flavonoids, and different other phenolic compounds[4]. Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals[5]. Recently, among natural antioxidants, flavonoids have received increasing attention as compared with vitamin C and E, dietary. Flavonoids are known to be highly effective antioxidants as they scavenge free oxygen radicals, and have anti-cancer, hypolipidemic, anti-ageing, and anti inflammatory activities. The search for novel natural antioxidants of plant origin has ever since increased and antioxidants appear to play a major role in the protective effect of plant medicine.

The present study was designed to evaluate the total phenolic content, total flavonoid content and antioxidant activity in various sample extracts of *Merremia aegyptia* and *Merremia dissecta.*

MATERIALS AND METHODS

Callus induction and establishment

Various explants such as nodal explant, shoot tip, leaf disc and stem from mature plants were surface-sterilized with 0.1% (w/v) mercuric chloride for 2-3 min, then washed 3-4 times with sterile double-distilled water and inoculated on MS medium supplemented with various combinations and concentrations of auxins 2,4 D, NAA and cytokines like kinetin, or BAP with 3% (w/v) sucrose. The pH of the medium was adjusted to 5.8 before sterilization. Cultures were maintained at 26± 2 °C under 16 hours photoperiod illuminated by fluorescent light of 2000-3000 lux intensity and 55±5% relative humidity[6].

Determination of total phenolic content

Total Phenolic Content was determined by using Folin-Ciocalteu method with catechol as standard[7]. 1 ml of each sample extract was diluted and 1.0ml of Folin-Ciocalteu reagent was added. After 3min, 2 ml of 20% sodium carbonate was added and the contents were mixed thoroughly. The total volume was made upto 20 ml. The colour was developed and absorbance measured at 650nm in spectrophotometer (Shimadzu UV-1800)[8]. Different concentrations of catechol solution (0.1 mg to 1mg/1 ml) were used to plot the calibration curve. Results were expressed as mg catechol/g of dry weight material.

Determination of total flavonoid content

Aluminium chloride colorimetric method was used for flavonoids determination[9]. Aliquots of extract solutions (0.5 ml) were taken and made up to volume 2ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken and allowed to stand for 30 minutes of incubation.

The principle of the method used for the determination of the flavonoid content consists in the fact that aluminum chloride forms stable acid complexes with the carbonyl group at C_4 and hydroxyls at C_3 (flavonols) and C_5 in flavonols and flavones, besides forming labile acid complexes with hydroxyls in the ortho position in A or B rings of flavonoids[10]. A standard calibration plot was generated at 415 nm using known concentrations of quercetin (0.1mg to 1.0mg/ml). The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

Determination of antioxidant activity

The methanolic crude extracts of *Merremia aegyptia* and *Merremia dissecta* at four different concentrations (50, 100, 250 and 500 µg/ml) were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant[11]. Free radical scavenging activity[12] was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical assay. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in colour (from deep violet to light yellow) was measured at 517 nm on a UV visible light spectrophotometer. The more rapidly the absorbance decreases, the more potent is the antioxidant activity of the extract. 3 ml of 0.004% ethanolic DPPH free radical solution was added to 1ml of all four concentrations of each extracts. After 30 minutes, the absorbance of the preparations was taken at 517 nm by a UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentrations (1-500 µg/ml).

RESULT AND DISCUSSION

Callus initiation and establishment: Callus from leaf discs and stem explant were best induced and established on MS media with NAA 2.0 mg/L and BAP 0.5 mg/L for *M.aegyptia* and NAA 1.0 mg/L and BAP 0.5 mg/L for *M.dissecta*

Total phenolic content

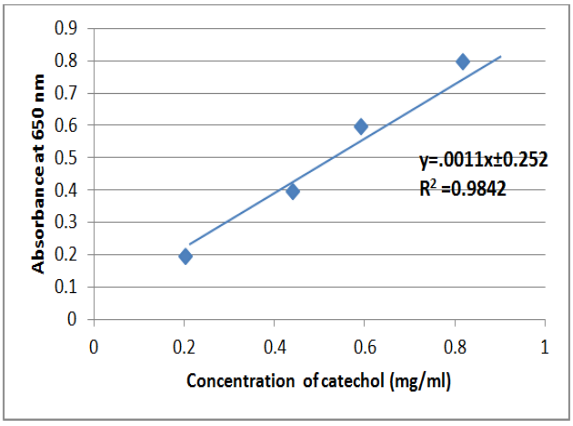

Figure 1: Standard calibration curve for phenol obtained using catechol as standard

The present study revealed the phenol content present in the *in vitro* and *in vivo* plant parts of *Merremia aegyptia* and *Merremia dissecta* in terms of mg catechol equivalent per gram dry sample. The values ranged in between 104 mg and 46 mg per gram for plant samples (leaf, stem, seed and callus) of *M. aegyptia* and 91 mg to 28mg per gram for *M.dissecta*. **(Table 1)** which were quantitatively determined comparing with the obtained standard calibration curve **(Fig.1).** Highest phenol content was found to be present in leaves of both the species as compared to other samples.

Table 1: Total Phenol content in various sample extracts of *M.aegyptia* and *M. dissecta*

Plant parts	Phenol content (mg catechol equivalent/g dry material)	
	M.aegyptia	*M.dissecta*
Leaf	104.80	91.30
Stem	46.48	88.49
Seed	54.34	48.36
Callus	62.04	28.03

Total flavonoid content

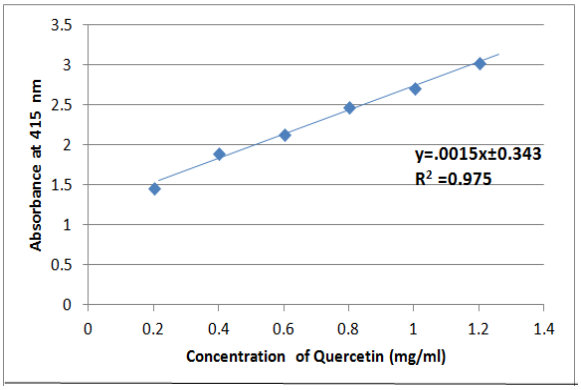

Figure 2: Standard calibration curve for flavonoid using quercetin as standard.

Using the standard plot **(Fig. 2**) of quercetin for flavonoid estimation, the *in vivo* and *in vitro* plant parts of both species found to contain mg quercetin equivalent/g dry weight of sample.

The concentration ranged in between 38.2mg to 9.9 mg for *M. aegyptia* and 37.7 mg to 13.6 mg for *M. dissecta*. **(Table 2**) Highest flavonoid content was found in stem extract of *Merremia aegyptia.*

Table 2: Total Flavonoid content in various sample extracts of *M.aegyptia* and *M. dissecta*

Plant parts	Flavonoid content (mg quercetin equivalent/g dry material)	
	M.aegyptia	***M.dissecta***
Leaf	34.4	13.6
Stem	38.2	23.2
Seed	18.3	37.7
Callus	9.9	15.4

Antioxidant activity

For measuring **radical scavenging activity** (RSA) against the stable radical N, N diphenyl- N'-picryl hydrazyl (DPPH) in percent the following formula was used:

RSA = (Acontrol - Asample / Acontrol) × 100

The methanolic extracts of *in vitro* and *in vivo* plant parts of *Merremia aegyptia* and *Merremia dissecta* showed free radical scavenging property at all the four concentrations studied.(**Table 3)**

It was observed that the scavenging effect on DPPH radical increased with the increasing concentration of plant extracts. **(Table 3)** The results obtained showed that among the different sample analyzed for DPPH scavenging activity, **stem, leaf and seed** extracts of *M. dissecta* and **seed** of *Merremia aegyptia* showed higher radical inhibition activity **(Fig. 3 and Table 3**) which is comparable and even better with that of standard ascorbic acid.

Table 3: Percent (%) radical scavenging activity of plant extracts of *M. aegyptia and M. dissecta.*

Conc. µg/ml	Std.	*M. aegyptia*				*M. dissecta*			
	Asc.acid	Leaf	Stem	Seed	Callus	Leaf	Stem	seed	Callus
50	24.103	14.347	6.456	23.242	6.599	43.041	46.054	24.964	9.899
100	47.345	25.538	33.715	59.397	31.133	60.688	82.639	62.410	32.855
250	76.901	75.750	74.461	77.761	64.418	72.453	86.226	87.804	78.909
500	91.965	78.900	82.352	90.961	86.226	74.748	92.826	90.100	88.665
IC_{50} Values		165.016	167.873	84.179	194.045	82.388	60.504	80.115	158.410

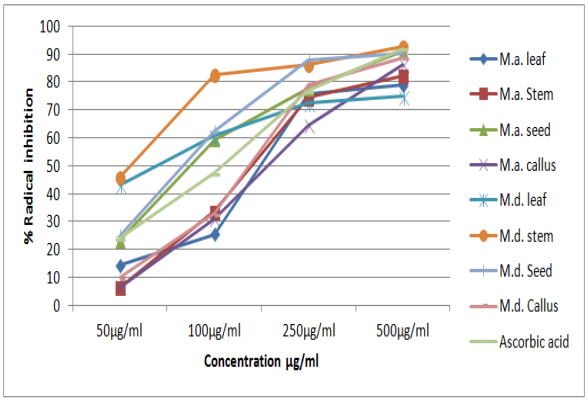

Figure 3: Antioxidant activity analysis in plant parts of *M. aegyptia* and *M. dissecta*

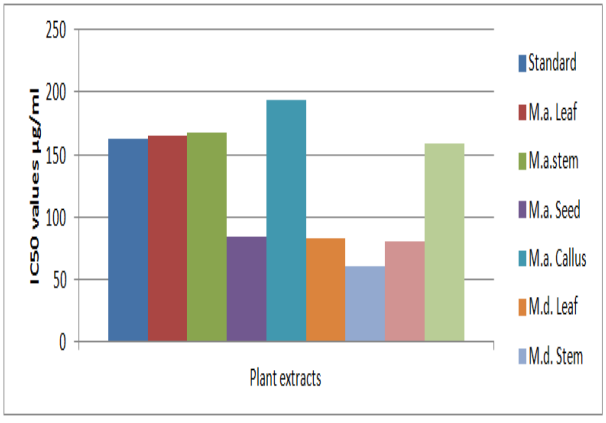

Figure 4: IC_{50} values of *in vivo* and *in vitro* parts of *Merremia* species

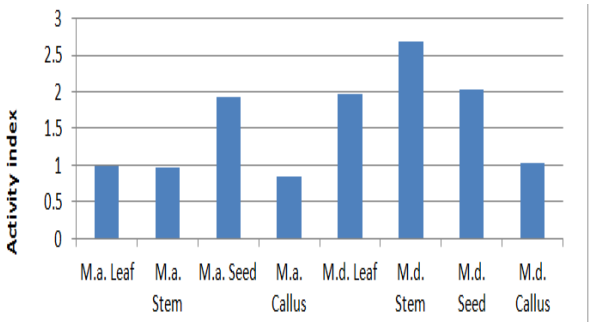

Figure 5: Antioxidant activity index of *in vivo* and *in vitro* parts of *Merremia* species

CONCLUSION

Both the plant species belonging to genus *Merremia* showed higher antioxidant potential. Total phenolics and flavonoid content measured were also satisfactory but did not show any correlation with the observed antioxidant activity of that particular sample. And hence, our study is in agreement with the authors[13], who reported no correlation between total phenolic content and antioxidant capacities of a number of medicinal plant extracts as antioxidant capacity may not solely depend upon the phenolic contents of the plants, but is attributable to the presence of various other phytochemicals such as ascorbic acid, tocopherol , pigments and flavonoid content of the plant etc. Also, phenolic content determined according to the Folin-Ciocalteu method is not an absolute measurement of the amount of phenolic materials. Different types of phenolic compounds have different antioxidant activities, which is dependent on their structure. The extracts possibly contained different type of phenolic compounds, which

had different antioxidant capacities. Further research in the area of isolation and identification of the active principles resulting in the medicinal properties of these plants could prove useful for the pharmaceutical industry.

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REFERENCES

1. Hertog MGL, Feskens EJM, Hollman PC, Katan MB, and Kromhout D, Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly study, The Lancet, 342, 1993, 1007-1011.
2. Wilson RL, Free radicals and tissue damage, mechanistic evidence from radiation studies. In: *Biochemical Mechanisms of Liver Injury*, Academic Press, New York, 1988, 123.
3. Devasagayam TPA, Tilak JC, Boloor KK, Review: Free radical and antioxidants in human health, Curr Stat Fut Pros JAPI, 53, 2004, 794-804.
4. Cai YZ, Sun M, Antioxidant activity of betalins from plants of the *Amaranthacea,* J. Agric, Food Chem, 51(8), 2003, 2288-2294.
5. Roya K, Fatemeh G, Screening of total phenol and flavonoid content, antioxidant and antibacterial activities of the methanolic extracts of three *Silene* species from Iran, Intl J Agri Crop Sci, 5(3), 2013, 305-312.
6. Sharma N, Patni V, Comparative analysis of total flavonoids, quercetin content and antioxidant activity of *in vivo* and *in vitro* plant parts of *Grewia asiatica* mast, International journal of pharmacy and pharmaceutical sciences, 5(2), 2013, 465-469.
7. Kaur S, Mondal P, Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants, J Microbiol Exp, 1(1), 2014, 01-00005.
8. Singleton VL, Rossi JA, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am J Enol Vitic, 16(3), 1965, 144-158.
9. Khatiwora E, Vaishali B, Manik MKA, Deshpande NR and Kashalkar RV, Spectroscopic determination of total phenol and flavonoid contents of *Ipomoea carnea,* International Journal of Chem Tech Research, 2(3), 2010, 1698-1701.
10. Chang CC., Yang MH., Wen HM And Chern JC, Estimation of total flavonoid content in *propolis* by two complementary colorimetric methods, J Food Drug Anal, 10, 2002, 178-182.
11. Bhuiyan MAR., Hoque MZ and Hossain SJ., Free Radical Scavenging Activities of *Zizyphus mauritiana*, World Journal of Agricultural Sciences, 5(3), 2009, 318-322.
12. Mensor LL, Menezes FS, Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, Phytother. Res, 15, 2001, 127–130.
13. Markham KR., Bloor S J, Analysis and identification of flavonoids in practice. In, flavanoids in health and disease, *Marcel Dekker Inc.,* New York, 1998, 1-33.
14. Sengul M, Yildiz H, Gungo N, Cetin B, Eser Z, Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants, Pak J Pharm Sci, 22(1), 2009, 102-106.

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