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In vitro antioxidant activity of *Hedyotis corymbosa* (L.) Lam. aerial parts

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The methanolic extract of the aerial part of *Hedyotis corymbosa* (L.) Lam. (Rubiaceae) was screened for antioxidant activity using 1,1-diphenyl-2-picryl hydroxyl (DPPH) quenching assay, 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) cation decolorization test, ferric reducing power (FRP), scavenging capacity towards hydroxyl ion ($OH^{\cdot}$) radicals and nitric oxide (NO) radical inhibition activity using established assay procedures. Total phenolics and total flavonoid contents were also determined. The plant yielded 210 mg gallic acid equivalent/100 g phenolic content and 55 mg quercetin equivalent/100 g flavonoid content. The extract exhibited high antiradical activity against DPPH, ABTS, nitric oxide and hydroxyl radicals with EC_{50} value of 82, 150, 130, and 170 µg/ml, respectively. The FRP increased with increasing concentration of the sample. The antioxidant activity of the extract was comparable with that of the standard butylated hydroxyl toluene (BHT). High correlation between total phenolic/flavonoid contents and scavenging potential of different reactive oxygen species ($R^2 = 0.785$-0.998) indicated the polyphenols as the main antioxidants.

Keywords: *Hedyotis corymbosa*, Antioxidant activity, DPPH, Ferric reducing power, ABTS, Nitric oxide scavenging activity.

Free radicals including reactive oxygen species (ROS) induce oxidative damage to biomolecules and have been implicated with variety of chronic diseases including cancer, diabetes, atherosclerosis, neurodegenerative disorders and arthritis[1,2]. Natural antioxidant mechanisms can be inefficient, hence dietary intake of antioxidant compounds becomes important[3-5]. Epidemiological studies have indicated the relationship between the plant antioxidants and reduction of chronic diseases[6-8]. These benefits are thought to result from the antioxidant components of plant origin, vitamins, flavonoids, and carotenoids[9-11]. The studies in recent years have shown that polyphenols in plants scavenge active oxygen species and effectively prevent oxidative cell damage[12].

Hedyotis corymbosa (L.) Lam. syn. *Oldenlandia corymbosa* (L.) Lam. (Rubiaceae) is a weedy herb, widely distributed throughout India. It is commonly known as 'Parppatakapullu' in traditional medicine of Kerala. It is extensively used in modern Chinese practice for treatment of viral infections, cancer, syndromes involving "toxic heart", acne, boils, skin aliments, appendicitis, hepatitis, eye disease and bleeding. The plant is used for treating venomous bites. It is bitter, acrid, cooling, febrifugal, pectoral, anthelmintic, diuretic, depurative, diaphoretic, expectorant, digestive and has stomachic properties[13]. It is given in jaundice, and other diseases of liver, heat eruption, vitiated conditions of pitta, hyperdypsia, giddiness, dyspepsia, flatulence, colic, constipation, helminthiasis, leprosy, skin diseases, cough, bronchitis, necrosis, nervous depression caused by deranged and hepatopathy[14]. Earlier, hepatoprotective effect of *H. corymbosa* against carbon tetrachloride (CCl_4)-induced liver damage in rats has been investigated[15] and three new iridoid glycosides[16], nine iridoid and lignan glucosides and rutin have been isolated from the whole plant[17]. In the present study, antioxidant activity of methanolic extract of aerial parts of *H. corymbosa* has been evaluated, employing a range of indices of antioxidant assays.

Materials and Methods

Chemicals

All chemicals used including the solvents were of analytical grade. 1,1-Diphenyl-2-picryl hydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS), quercetin, butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) were purchased from Merck (Mumbai). Potassium ferricyanide, folin ciocalteu reagent, methanol, gallic acid, trichloroacetic acid (TCA), ferric chloride, sodium carbonate, hydrogen peroxide, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, aluminium chloride, potassium persulfate,

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sodium nitroprusside, napthyl ethylene diamine dihydrochloride, sulfanilic acid, ferrous ammonium sulfate, EDTA, acetyl acetone, glacial acetic acid, DMSO, phosphoric acid and ammonium acetate were purchased from SD Fine Chemicals, Mumbai.

Plant material and sample preparation

The plant *Hedyotis corymbosa* L. was collected in September, 2008 from Coimbatore district, Tamil Nadu state, India and identified by Dr. R Gopalan, Karpagam University, and authenticated in the Botanical Survey of India, Coimbatore. Voucher specimens were deposited in the herbarium of Karpagam University, Coimbatore. The aerial parts (leaf and stem) were dried at room temperature for 7 days, finely powdered and the powder was exhaustively extracted with methanol (MeOH). The extract was centrifuged (3000 × *g*) thrice and the clear supernatants were filtered over Whatman No.1 filter paper and the extract was evaporated to dryness by rotary flash evaporator (Buchi type Rotavapor). Different concentrations of extract were prepared from the resultant crude methanolic extract to determine *in vitro* antioxidant assays.

Determination of total phenolic content and total flavonoid content

The total phenolics content was determined using Folin and Ciocalteu's phenol reagent[18]. The absorbance was measured at 650 nm using spectrophotometer. Total soluble phenolic content in the methanolic extract was measured and expressed as mg of gallic acid equivalents. The total flavonoid content was determined by spectrophotometric method measuring the flavonoids in $AlCl_3$-complex from the extract at 420 nm[19] and expressed as mg of quercetin equivalents.

Antioxidant activity determination

The antioxidant activity of methanolic extract was determined using DPPH radical quenching assay, ferric reducing power (FRP) capacity, scavenging of ABTS radical cation, nitric oxide and hydroxyl radical scavenging activities.

DPPH radical scavenging activity

DPPH stable free radical scavenging activity was determined based on the previously described method[20]. The absorbance of the various concentrations the extract was measured at 517 nm. The inhibition percentage was calculated as radical scavenging activity (%) = (Abs $_{control}$ − Abs $_{sample}$)/ Abs $_{control}$) × 100.

FRP capacity

The reducing power of the extract was quantified according to the method described previously[21]. The absorbance of the various concentrations of extract (1000, 500, 250 and 125 µg/ml) was read at 700 nm. The reducing power of the extract was linearly proportional to the concentration of the sample. Increased absorbance of the reaction mixture indicated increased reducing power.

Scavenging activity against ABTS$^{\cdot+}$, NO and OH$^{\cdot}$ radicals

Radical scavenging activity of the extract was assessed spectrometrically by $ABTS^{\cdot+}$ cation decolorization assay[22]. The test was based on the relative activity of antioxidants to quench the radical cation $ABTS^{\cdot+}$ and absorbance was taken at 734 nm. Percentage inhibition was calculated as $ABTS^{\cdot+}$ radical scavenging activity (%) = ($Abs_{control}$ − Abs_{sample})/($Abs_{control}$) × 100.

Sodium nitroprusside in aqueous solution at physiological *p*H spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions, which can be estimated using Griess Illosvoy reaction[23]. Scavengers of NO compete with oxygen, leading to reduced production of NO and a pink coloured chromophore is formed. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Percentage inhibition was calculated as NO scavenging activity (%) = (Abs $_{control}$ − Abs $_{sample}$)/(Abs $_{control}$) × 100.

The OH$^{\cdot}$ scavenging activity of the plant extract (250, 500 and 1000 µg) was measured according to the previously described method[24]. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The percentage of OH$^{\cdot}$ scavenging activity (HRSA) was calculated by the following formula: % HRSA = 1-(difference in absorbance of sample/difference in absorbance of blank) × 100.

Statistical analysis

The experimental results were mean ± S.D of three parallel measurements. Effective concentration (EC_{50}) value was calculated by regression analysis. Linear regression analysis was performed quoting the correlation coefficient. One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were carried out to determine significant differences

($P< 0.05$) between the means of assays by SPSS (version 10 for Windows 98, SPSS Inc.).

Results and Discussion

The total phenolic content in the methanolic extract of aerial parts was found to be 210 mg of gallic acid equivalents (GAE) in 100 g fresh weight basis whereas the total flavonoid content was 55 mg of quercetin equivalents (QE) in 100 g of fresh plant material. Polyphenols are present in a variety of plants utilized as important components of both human and animal diets[25-27]. The health benefits associated with the consumption of fruits and vegetables have been partly attributed to the flavonoid content[28]. Antioxidant quality is measure of the effectiveness of the antioxidant(s) present as a pure compound or a mixture[29].

The methanolic extract aerial parts of *H. corymbosa* was tested for their antioxidant properties in a range of *in vitro* assays to determine their potency to scavenge ROS. The extract exhibited a concentration-dependent antiradical activity by quenching DPPH radical (Fig. 1a) and the DPPH scavenging activity was comparable to that of BHT. The extract exhibited a concentration-dependent increase in reducing power (Table 1). It caused significant elevation of reducing power potential with OD value of 0.810± 0.031 at 1000 µg/ml and displayed better FRP efficiency, as compared with BHT. The reducing power of a compound may serve as a significant indicator of its potent antioxidant activity[30].

The marked antioxidant activity of plant extract seemed to be due to presence of polyphenols. Fig. 1b demonstrates scavenging activity of the extract against ABTS radical. It mopped up more than 60% ABTS radicals *in vitro*. The ABTS scavenging activity of the extract was comparable to that of BHT and might be due to variation in the types of phenolic compounds that differ significantly in their reactivity towards ABTS[31]. The extract scavenged OH radicals (Fig. 1c) and percentage inhibition was proportional to the concentration of the extract and was comparable with that of BHT at the concentration of 0.1 mg/ml. Extent of nitric oxide radical scavenged was determined by the decrease in intensity of pink coloured chromophore at 540 nm. The extract exerted compatible inhibitory (47.53%) potential at the concentration of 1000 µg/ml against nitric oxide generation (Fig. 1d). The antioxidant activity was compared with BHT as standard. The study demonstrated the potent nitric oxide scavenging activity of the extract of *H. corymbosa*.

The EC_{50} values of *H. corymbosa* in DPPH, ABTS, nitric oxide and hydroxyl ion scavenging test were 82, 150, 130 and 170 µg/ml, respectively (EC_{50} values for standard BHT were 79, 40, 68, and 91µg/ml respectively). Results showed that the possessed strong DPPH and moderate hydroxyl, ABTS and nitric oxide radical scavenging activities. These results were in accordance with the studies on antioxidant activity of methanolic extract of other *Hedyotis* spp[32,33].

Results obtained in the antioxidant assays were well correlated with total phenol and total flavonoid contents (Table 2). The total phenolic and total flavonoid contents were significantly correlated to the DPPH activity (R^2 = 0.971 and R^2 = 0.985, respectively). ABTS scavenging activity was highly correlated with total phenols (R^2 = 0.993), total

Table 1—Ferric reducing power (FRP) activity of *H. corymbosa* methanolic extract

[Values represent average of three experiments (n = 3) ± S D]

	Methanolic extract conc. (µg/ml)	OD Value at 700 nm	
		Control	Sample
H. corymbosa	1000	0.084 ± 0.024	0.810 ± 0.031*
	500		0.557 ± 0.015*
	250		0.281 ± 0.019*
	125		0.185 ± 0.023*
BHT	1000		0.709 ± 0.015*

Values represent average of three experiments (n = 3) ± S D. *Significantly different at P<0.05.

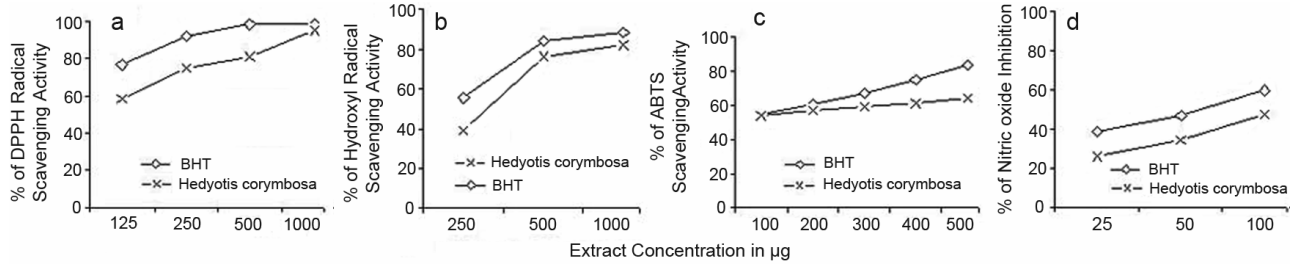

Fig. 1 — *In vitro* Antioxidant activity of *H. corymbosa* methanolic extract

Table 2—Linear correlation coefficients of *H. corymbosa* methanolic extract

TP	TF	DPPH	ABTS	NO	HR	FRP
TP	1					
TF	0.998	1				
DPPH	0.971	0.985	1			
ABTS	0.993	0.994	0.980	1		
NO	0.994	0. 994	0.927	0.998	1	
HR	0.851	0.922	0.992	0.920	0.873	0.785
FRP	0.983	0.995	0.945	0.962	0.987	1

TP, Total phenol content; TF, total flavonoid content; DPPH, diphenyl picryl hydrazyl
ABTS, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); NO, nitric oxide scavenging
HR, hydroxyl radical scavenging; FRP, ferric reducing power assay.

flavonoid (R^2 = 0.994) and DPPH (R^2 = 0.980). A linear relationship existed between nitric oxide values and total phenolic content, total flavonoid content, DPPH, and ABTS (R^2 = 0.994, R^2 = 0. 994, R^2 = 0.927 and R^2 = 0.998, respectively). The hydroxyl radical scavenging and FRP were also well correlated with total phenol (R^2 = 0.851 and 0.983) and flavonoid content (R^2 = 0.995 and 0.922), respectively. A positive correlation between antioxidant activity and polyphenol content suggested that antioxidant capacity of the extract was due to a great extent to their polyphenols[34,35].

In conclusion, the results presented in this report indicated that *H. corymbosa* extract efficiently attenuated oxidative stress via its antioxidant properties. However, further studies are needed to isolate active principles responsible for the overall antioxidant activity of the extract.

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References

1 Aruoma O I (1998) *J Am Oil Chem Soc* 75, 199–212
2 Sawa T, Nakao M, Akaike T, Ono K & Maeda H (1999) *J Agric Food Chem* 47, 397-402
3 Halliwell B (1994) *Lancet* 344, 721-724
4 Duh P D (1998) *J Am Oil Chem Soc* 75, 455-461
5 Espin J C, Soler-Rivas C & Wichers H J (2000) *J Agric Food Chem* 48, 648-656
6 Frie B (1995) *Crit Rev Food Sci Nutr* 35, 83-98
7 Halliwell B (1997) *Nutr Rev* 52, 253-265
8 Lieu R H (2003) *Am J Clin Nutr* 78, 517-520
9 Pietta P G (2000) *J Nat Prod* 63, 1035-1042
10 Rice-Evans C (2001) *Curr Med Chem* 8, 797-807
11 Prior R L (2003) *Am J Clin Nutr* 78, 570S-578S
12 Yagi E, Ragavi E, Hadolin M, Peredi V K, Balazs A, lazovics A & Simadi B (2005) *J Agric Food Chem* 53, 17-21
13 Kirthikar K R & Basu B D (1994) *Indian Medicinal Plants*, Vol. 2, p 1263, Bishen Singh Mahendrapal Singh Publisher, Dehradun
14 Warrier R K, Nambiar V P K & Ramankutty C (1995) *Indian Medicinal Plants: A compendium of 500 species;* Vol. 3, pp 120-123, Orient Longman Ltd, Madras
15 Chiu H F, Lin Yang C C & Yang F (1998) *Am J Chin Med* 16, 127-137
16 Jiang W, Kuang L S, Hou A J, Qian M & Li J Z (2007) *Helvet Chim Acta* 90, 1296–1301
17 Noiarsa P, Ruchirawat S, Otsuka H & Kanchanapoom T (2008) *J Nat Med* 62, 1340-3443
18 Sadasivam S & Manickam A (1996) *Biochemical Methods,* Vol. 8, pp 193-194, New Age International (P) Ltd. Publishers, New Delhi
19 Ordon Ez A A L, Gomez J D, Vattuone M A & Isla M I (2006) *Food Chem* 97, 452-458
20 Blois M S (1958) *Nature* 181, 1199-1150
21 Oyaizu M (1986) *Jpn J Nutr* 44, 307–315
22 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M & Rice-Evans C (1999) Free *Radic Biol Med* 26, 1231-1237
23 Garrat D C (1964) *The Quantitative Analysis of Drugs,* Vol, 3, pp 456-458, Chapman and Hall Ltd., Japan
24 Klein S M, Cohen G & Cederbaum A I (1991) *Biochem* 20, 6006-6012
25 Bravo L (1998) *Nutr Rev* 56, 317-333
26 Chung K T, Wong T Y, Wei C I, Huang Y W & Lin Y (1998) *Crit Rev* Food *Sci Nutr* 38, 421-464
27 Crozier A, Lean M E J, McDonald M S & Black C (1997) *J Agric Food Chem* 45, 590-595
28 Lampila P, Lieshout M V, Gremmen B & Lähteenmäki L (2009) *Food Res Int* 42, 122-129
29 Vinson J A & Zhang J (2005) *J Agric Food Chem* 53, 3710-3713
30 Meir S, Kanner J, Akiri B & Hodas S P (1995) *J Agric Food Chem* 43, 1813-1817
31 Katalinic V, Milos M, Kulisic T & Jukic M (2006) *Food Chem* 94, 550-557
32 Lin C C, Ng L T & Yang J J (2004) *Am J Chin Med* 32, 339-349
33 Ahmad R, Ali A M, Israf D A, Ismail H N, Shaari K & Lajis N H (2005) *Life Sci* 17, 1953-1964
34 Ng T B, Liu F & Wang Z T (2000) *Life Sci* 66, 709-723
35 Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B & Yankova T (2006) *Phytother Res* 11, 961-965