A. J. A. Petrus et al /J. Pharm. Sci. & Res. Vol.4(7), 2012, 1856 - 1861

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Isolation and Characterisation of the Antioxidant Phenolic Metabolites of *Boerhaavia erecta* L. Leaves

A. J. A. Petrus*, S.Siva Hemalatha , G.Suguna

Department of Chemistry,
Kanchi Mamunivar Centre for Post-Graduate Studies
(Autonomous - College with Potential for Excellence-UGC),
Puducherry 605 008, India.

Abstract
Boerhaavia erecta L. (Nyctaginaceae) is a medicinal weed, indigenous to tropical America, but presently pantropical and appear to constitute a functional leafy vegetable of certain communities. The taxon is reported to possess antiparasitic, antifungal, antibacterial, antiviral, antihistaminic, antioxidant and hepatoprotective properties. The residue of the ethanolic leaf-extract was fractionated into diethyl ether, acetone and methanol solubles. All the three fractions of the leaf extract scavenged reactive oxygen and nitrogen species effectively. The most polar methanol fraction was determined to possess Vitamin C equivalent antioxidant capacity (VCEAC) of 64.57±1.07%, which is about 78.4% of the dietary flavonol, rutin. The acetone and ether fractions were determined to possess respectively, 51.5 and 47.1% of the rutin VCEAC. The percent nitric oxide inhibitions of the fractions were respectively found to be 84.0, 56.4 and 51.9% of the rutin capacity for the methanol, acetone and ether fractions. The per cent Fe^{II} chelating capacities of the leaf-extract were found to be in the order methanol > acetone > ether (*viz.*, 73.4, 63.1 and 59.4% of the chelating capacities of the standard trihydroxamic siderophoric drug, deferoxamine). Chemical analysis of the leafy matter has led to the isolation and characterisation of two potentially antioxidant flavan-3-ols, [(+)-catechin] (**1**) and [(-)-epicatechin] (**2**) and two dietary flavonols, quercetin (**3**) and isorhamnetin (**4**) together with their glycosides, rutin (**5**), narcissin (**6**) isoquercitrin (**7**) and isorhamnetin 3-O-β-D-glucopyranoside (**8**). The structures of all the eight antioxidant phenolic phytometabolites isolated were characterised on the basis of their physical, chemical and spectral features.
Keywords: *Boerhaavia erecta*, Nyctaginaceae, Antioxidant capacity, Polyphenolic metabolites, Flavonoid profile.

INTRODUCTION

Boerhaavia erecta L., syn. *B. punarnava* Saha & Krishn. *Spec. nov.* (Family: Nyctaginaceae; Common names: Tar vine, erect spiderling, spindlepod, erect boerhavia, and upright spiderling) is an erect, puberulous annual herb (Fig. 1), indigenous to tropical America, but presently a pantropical taxon [1-3]. The species is reported to occur throughout the regions of tropical Africa. In Asia, it is distributed in Guangdong of China, Taiwan, the Ryukyu Islands, Singapore, Malaysia, Philippines, Thailand, South Sumatra, Java, Lesser Sunda Islands, New Guinea and the Pacific as well as the Gangetic plains, Maharasthra, Tamil Nadu and Kerala of the Indian subcontinent [1,4]. This exotic weed has probably found its way to India with food grains imports [5] and subsequently got naturalized in open bush land, waste ground, gardens, in agricultural land and along roadsides, up to 1500–2500 m altitude, particularly on sandy or rocky soils, in various parts of the country [6].

Fig. 1 *B. erecta* L. in its natural habitat

The leaves are consumed traditionally as vegetable herbs in Senegal and are either cooked and eaten as spinach or used for the preparation of sauces in certain parts of the American tropics [6,7]. *B. erecta* is one among the 24 medicinal plants used, as decoction, in the traditional medicine of Niger to treat skin diseases and is also among the 189 native drugs of the Bolivian lowland ethnic group, Izoceño-Guaraní [8]. It has been assessed to possess antiparasitic, antifungal, antibacterial, antiviral, antihistaminic, antioxidant and hepatoprotective activities [9-12]. The amino acid content of the aerial portions, the phenolic, flavonoid and enzymic antioxidant contents of the leaf extract, the acute, short-term, and subchronic oral toxicity in Wistar rats have been reported from the earlier investigations of the leaf extract [6,12-14]. The only record of the potentially antioxidant phenolic metabolites of the edible medicinal leaves available in literature is the isolation of quercetin and isoquercitrin from its aerial parts [15]. Though there appears to exist in literature indications [16] of other chemical examinations, personal correspondence with the concerned author and the affiliated institution has clarified the absence of such recorded investigations. The present paper, therefore, reports the polyphenolic profile of the leaves that may potentially contribute to the reported antioxidant and the related biological activities of this medicinal herb.

MATERIALS AND METHODS

SOURCE OF MATERIALS

Plant material

Leaves of *B. erecta* (1.2 Kg) were collected from the Lawspet Nursery Garden premises, Puducherry (Fig. 1), during mid-January and the identity of the voucher

specimen was authenticated by the Botanical survey of India, Southern Circle, Coimbatore. The herbarium specimen is preserved in our laboratory.

Chemicals and instruments

2,2'-Azino*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) diammonium salt, 3-(2-pyridyl)-5,6-di(4-phenylsulphonic acid)-1,2,4-triazine (ferrozine) sodium salt, ethanol (EtOH), ferrous chloride, rutin and deferoxamine methanesulfonate were obtained from Sigma-Aldrich Inc. and Sephadex LH-20 from Pharmacia. Silica (SiO_2) gel (60-120 mesh for column chromatography), microcrystalline cellulose for thin layer chromatography (TLC) and methanol for spectroscopy were procured from Merck Specialities Private Limited. All other chemicals/reagents were of analytical/ laboratory grades from Himedia/Merck/Loba Chemie. Melting points have been determined in open capillaries and are uncorrected. Shimadzu UV-160 Spectrophotometer was used for electronic spectral measurements and the NMR spectral recordings were performed on Bruker DRX-500/AMX-400 spectrometers using tetramethylsilane (TMS) as internal standard.

METHODS

Solvent extraction and fractionation

The shade dried and coarsely powdered leaf-material was exhaustively extracted with boiling 90% aq. EtOH (4×10.0L, 6h), filtered through Whatman No. 3 filter paper and concentrated under reduced pressure. The crude aqueous concentrate was fractionated into C_6H_6 (benzene, 3×1.5L), Et_2O (diethyl ether, 5×1.0L) and EtCOMe (ethyl methyl ketone, 4×1.5L) solubles. Leaving aside the deeply coloured non phenolic C_6H_6 fraction, each one of the other two fractions was washed successively with Et_2O, acetone (Me_2CO) and methanol (MeOH). The fractions were concentrated under reduced pressure and stored in a refrigerator for a week.

Determination of *in vitro* antioxidant capacity

Aliquots of the Et_2O, Me_2CO and MeOH solubles were quantitatively diluted with appropriate amounts of EtOH/MeOH and subjected to *in vitro* antioxidant capacity measurements. Determination of reactive species-scavenging capacities was carried out by (i) measuring the capacity to scavenge ABTS radical cation ($ABTS^{\bullet+}$) and expressing the results as relative percentage of vitamin C equivalent antioxidant capacities (VCEAC) and (ii) measuring the percent inhibition of nitric oxide (NO), according to the protocols described previously with respect to the dietary flavonol, rutin as standard [17,18]. Metal ion chelating capacities were evaluated using ferrous ion and the percentage inhibition of the ferrous-ferrozine complex formation was calculated, with respect to deferoxamine standard, according to the protocol described previously [18,19].

Isolation of polyphenolic metabolites

The Et_2O and Me_2CO fractions that were placed in the ice-chest deposited a yellow solid, which were filtered to separate the residue and the supernatant mother liquors. The supernatant of the Et_2O fraction was subjected to open column chromatography over silica gel and gradiently eluted with mixtures of *n*-hexane-ethyl acetate (EtOAc). The fraction eluted using *n*-hexane-EtOAc (1:1), was further chromatographed on silica gel with EtOAc-MeOH (20:1) as eluent to obtain (2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol [(+)-Catechin] (**1**) and (2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol [(-)-epicatechin] (**2**). The fraction eluted with *n*-hexane-EtOAc (1:3), when purified by a silica gel column ($CHCl_3$-MeOH, 5:1) yielded 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy chromen-4-one [quercetin] (**3**) and 2-(4-hydroxy-3-methoxyphenyl)-3,5,7-trihydroxychromen-4-one [isorhamnetin] (**4**). MeOH fraction was further separated, using a column of Sephadex LH-20 and eluting with 90% aq. MeOH, into 20 fractions (frs). The eluates were analysed by paper chromatography (PC) (Whatman No.1, 15% aq. HOAc (acetic acid), ascending, 28°C) for their phenolic composition. Based on the PC features, frs 5-11 and 12-18 were combined and concentrated as before to get respectively frs F-1 and F-2. The former was again passed through the same column and eluted with 90% aq. MeOH to separate ten further fractions. Frs 4-8 were combined together, concentrated and separated by preparatory TLC (cellulose, 20×20 cm, 0.5 mm, 5% aq. HOAc, ascending, 28°C) to yield quercetin 3-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside [rutin] (**5**) [R_f 0.43] and isorhamnetin 3-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside [narcissin] (**6**) [R_f 0.52]. F-2 was similarly processed using preparatory TLC (cellulose, 20×20 cm, 0.5 mm, 15% aq. HOAc, ascending, 28°C) to yield quercetin 3-O-β-D-glucopyranoside [isoquercitrin] (**7**) [R_f 0.26] and isorhamnetin 3-O-β-D-glucopyranoside (**8**) [R_f 0.19]. The yellow residual deposits from the Et_2O and Me_2CO solutions were dissolved in MeOH and purified through a column of sephadex LH-20 to isolate the homogeneous product, rutin (**5**). All the isolates were purified by passing their methanolic solutions through Sephadex LH-20 column followed by recrystallisation to get the homogeneous products. A similar systematic chromatographic analysis of the Me_2CO fraction yielded homogeneous compounds **5**-**8**, in minor quantities.

Characterisation of the phenolic isolates

All the eight flavonoids isolated were characterised based on their characteristic physical, chemical and spectral features [20-23] as briefed below.

(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol [(+)-Catechin] (1)

$C_{15}H_{14}O_6$, mp. 174-176°C; $[\alpha]_D^{26}$ +17 [*c* 2.8, 50% acetone]. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm) 6.84 (d, *J*=2.0 Hz, H-2'); 6.76 (d, *J*=8.1 Hz, H-5'); 6.72 (dd, *J*=8.1 and 2.0 Hz, H-6'); 5.93 (d, *J*=2.3 Hz, H-6); 5.86 (d, *J*=2.3 Hz, H-8);4.57 (d, *J*=7.5 Hz, H-2); 3.97 (m, H-3); 2.85 (dd, *J*=16.1 and 5.4 Hz, H-4eq); 2.51 (dd, *J*=16.1 and 8.1 Hz, H-4ax). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm) 82.0 (C-2); 67.9 (C-3); 28.5 (C-4); 157.1 (C-5); 96.0 (C-6); 156.9 (C-7); 95.1 (C-8); 156.1 (C-9); 100.1 (C-10); 131.4 (C-1'); 115.4 (C-2'); 145.4 (C-3'); 145.4 (C-4'); 115.7 (C-5'); 119.5 (C-6').

(2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol [(-)-epicatechin] (2)

$C_{15}H_{14}O_6$, mp. 242°C, $[\alpha]_D^{26}$ = -29.6 (*c* 0.23, CH_3OH). ^1H NMR (400 MHz, CD_3OD, δ, ppm) 6.98 (d, *J*=1.8 Hz, H-2'); 6.80 (dd, *J*=8.2 and 1.8 Hz, H-6'); 6.76 (d, *J*=8.2, H-5') 5.95 (d, *J*=2.3 Hz, H-6); 5.92 (d, *J*=2.3 Hz, H-8); 4.80 (br. s, H-2); 4.19 (br. s, H-3); 2.86 (dd, *J*=16.8 and 4.3 Hz, H-4eq); 2.74 (dd, *J*=16.8 and 2.9 Hz, H-4ax). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm) 80.0 (C-2); 67.5 (C-3); 29.1 (C-4); 158.2 (C-5); 96.9 (C-6); 158.0* (C-7); 96.8 (C-8); 157.5* (C-9); 100.7 (C-10); 132.5 (C-1'); 115.9 (C-2'); 145.8** (C-3'); 145.3** (C-4'); 116.4 (C-5'); 119.8 (C-6').* *and* ***values are interchangeable.*

2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one [quercetin] (3)

$C_{15}H_{10}O_7$, mp 305-306°C, UV (λmax, nm) (MeOH): 256, 267sh, 301sh, 370; +$AlCl_3$ 272, 304sh, 332, 457; +$AlCl_3$/HCl 265, 301sh, 328, 427; +NaOAc 257sh, 272, 300, 389 (dec); +NaOAc/H_3BO_3 260, 302sh, 388, 361; +NaOMe 245sh, 321, 412 (dec). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm) 7.68 (d, *J*=2.0 Hz, H-2'); 7.55 (dd, *J*=2.0 and 8.5 Hz, H-6'); 6.89 (d, *J*=8,5 Hz, H-5'); 6.42 (d, *J*=1.8 Hz, H-8); 6.20 (d, *J*=1.8 Hz, H-6). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) 146.8 (C-2); 135.8 (C-3); 175.9 (C-4); 160.8 (C-5); 98.2 (C-6); 163.9 (C-7); 93.4 (C-8); 156.2 (C-9); 103.0 (C-10); 122.0 (C-1'); 115.1 (C-2'); 145.1 (C-3'); 147.7 (C-4'); 115.6 (C-5'); 120.0 (C-6').

2-(4-hydroxy-3-methoxyphenyl)-3,5,7-trihydroxychromen-4-one** [isorhamnetin] **(4)

$C_{16}H_{12}O_7$, mp 304-306°C, UV (λmax, nm) (MeOH): 254, 267sh, 306sh, 326sh, 370; +$AlCl_3$ 264, 304sh, 361sh, 431; +$AlCl_3$/HCl 263, 271sh, 303sh, 357, 428; +NaOAc 260sh, 270, 320, 393; +NaOAc/H_3BO_3 255, 270sh, 308sh, 328sh, 377; +NaOMe 242sh, 273, 330, 440. ^1H NMR (500 MHz, DMSO-d_6, δ, ppm) 7.73 (d, *J*=2.0 Hz, H-2'), 7.56 (dd, *J*=8.5 and 2.0 Hz, H-6'), 6.84 (d, *J*=8.5 Hz, H-5'), 6.55 (d, *J*=2.9 Hz, H-8), 6.39 (d, *J*=2.9, H-6), 3.87 (s, OCH_3-3').^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) 147.5 (C-2); 136.4 (C-3); 176.8 (C-4); 161.6 (C-5); 98.9 (C-6); 164.9 (C-7); 94.2 (C-8); 157.1 (C-9); 103.9 (C-10); 122.9 (C-1'); 113.1 (C-2'); 149.8 (C-3'); 148.0 (C-4'); 116.4 (C-5'); 121.8 (C-6'); 54.7 (OCH_3).

Quercetin 3-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside [rutin] (5)

$C_{27}H_{30}O_{16}$, mp 188-189°C, UV (λmax, nm) (MeOH): 259, 266sh, 300sh, 359; +$AlCl_3$ 275, 303sh, 434; +$AlCl_3$/HCl 275, 300sh, 412; +NaOAc 270, 325, 394; +NaOAc/H_3BO_3 262, 298, 387; +NaOMe 272, 326, 410. ^1H NMR (500 MHz, DMSO-d_6, δ, ppm) 7.58 (d, *J*=2.2 Hz, H-2'), 7.53 (dd, *J*=8.6 and 2.2 Hz, H-6'), 6.90 (d, *J*=8.6 Hz, H-5'), 6.42 (d, *J*=2.0 Hz, H-8), 6.22 (d, *J*=2.0 Hz, H-6), 5.16 (d, *J*=8.7 Hz, H-1"), 4.40 (d, *J*=2 Hz, H'"), 3.2 to 3.7 (m, other sugar protons) and 1.1 (s, rhamnosyl CH_3). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) 156.3 (C-2); 133.2 (C-3); 177.2 (C-4); 161.1 (C-5); 98.6 (C-6); 163.9 (C-7); 93.5 (C-8); 156.5 (C-9); 103.9 (C-10); 121.5 (C-1'); 116.2 (C-2'); 144.6 (C-3'); 148.3 (C-4'); 115.1 (C-5'); 121.3 (C-6'); 101.6 (C-1″); 74.5 (C-2″); 76.5 (C-3″); 70.3 (C-4″); 75.8 (C-5″); 66.2 (C-6″); 100.6 (C-1'"); 70.8 (C-2'"); 70.5 (C-3'"); 71.6 (C-4'"); 68.6 (C-5'"); 17.6 (C-6'"). Hydrolytic products : 2N HCl , quercetin/D-glucose/L-rhamnose ; 1% H_2SO_4, quercetin-3-O-β-D-glucopyranoside/quercetin/ D-glucose/L-rhamnose ; takadiastase enzyme, quercetin-3-O-β-D-glucopyranoside/L-rhamnose ; H_2O_2 oxidation, rutinose.

Isorhamnetin 3-O-α-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside [narcissin] (6)

$C_{28}H_{32}O_{16}$, mp 182°C, UV (λmax, nm) (MeOH): 254, 267sh, 356; +$AlCl_3$ 270, 301sh, 408; +$AlCl_3$/HCl 270, 300sh, 406; +NaOAc 272, 346, 382; +NaOAc/H_3BO_3 258, 354; +NaOMe 271, 300sh, 410. ^1H NMR (500 MHz, DMSO-d_6, δ, ppm) 7.88 (d, *J*=2.0 Hz, H-2'), 7.56 (dd, *J*=8.5 and 2.0 Hz, H-6'), 6.75 (d, *J*=8.5 Hz, H-5'), 6.47 (d, *J*=2.2 Hz, H-6), 6.18 (d, *J*=2.2 Hz, H-8), 5.68 (d, *J*=8.7 Hz, H-1"), 4.25 (d, *J*=2 Hz, H-1'"), 3.85 (s, OCH_3-3'), 3.2 to 3.7 (m, other sugar protons) and 1.1 (s, rhamnosyl CH_3). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) 156.3 (C-2); 133.2 (C-3); 177.2 (C-4); 161.1 (C-5); 98.6 (C-6); 163.9 (C-7); 93.5 (C-8); 156.5 (C-9); 103.9 (C-10); 121.5 (C-1'); 116.2 (C-2'); 144.6 (C-3'); 148.3 (C-4'); 115.1 (C-5'); 121.3 (C-6'); 55.1 (OCH_3-3') 101.6 (C-1″); 74.5 (C-2″); 76.5 (C-3″); 70.3 (C-4″); 75.8 (C-5″); 66.2 (C-6″); 100.6 (C-1'"); 70.8 (C-2'"); 71.5 (C-3'"); 71.6 (C-4'"); 68.6 (C-5'"); 17.6 (C-6'"). Hydrolytic products : 2N HCl, isorhamnetin/D-glucose/L-rhamnose ; 1% H_2SO_4, isorhamnetin -3-O-β-D-glucopyranoside/isorhamnetin/D-glucose/L-rhamnose ; takadiastase enzyme, isorhamnetin-3-O-β-D-glucopyranoside/L-rhamnose ; H_2O_2 oxidation, rutinose.

Quercetin 3-O-β-D-glucopyranoside [isoquercitrin] (7)

$C_{21}H_{20}O_{12}$, mp. 229–231°C, UV (λmax, nm) (MeOH): 257, 263sh, 352; +$AlCl_3$ 272, 301sh, 429; +$AlCl_3$/HCl 273, 300sh, 410; +NaOAc 271, 325sh, 398; +NaOAc/H_3BO_3 268, 298, 384; +NaOMe 272, 326, 410. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm) 7.59 (d, *J*=2.5 Hz, H-2′), 7.57 (dd, *J*=8.7, 2.5 Hz, H-6′), 6.84 (d, *J*=8.7 Hz, H-5′), 6.40 (d, *J*=2.3 Hz, H-8), 6.20 (d, *J*=2.3 Hz, H-6), 5.47 (d, *J*=6.9 Hz, H-1″), 4.4-3.0 (m, other sugar protons). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) 156.6 (C-2), 133.5 (C-3), 177.5 (C-4), 161.3 (C-5), 98.8 (C-6), 164.0 (C-7), 93.8 (C-8), 156.4 (C-9), 104.2 (C-10), 121.7 (C-1'), 113.5 (C-2'), 147.9 (C-3'), 148.4 (C-4'), 115.6 (C-5'), 122.3 (C-6'), 101.6 (C-1″), 74.2 (C-2″), 77.0 (C-3″), 70.3 (C-4″), 76.1 (C-5″), 61.2 (C-6″). Hydrolytic products : 2N HCl, quercetin/D-glucose; β-glucosidase enzyme, quercetin/D-glucose.

Isorhamnetin 3-O-β-D-glucopyranoside (8)

$C_{22}H_{22}O_{12}$, mp. 267-269°C; UV (λmax, nm) (MeOH): 255, 269sh, 302sh, 357; +$AlCl_3$ 271, 300sh, 365sh 404; +$AlCl_3$/HCl 270, 300sh, 357, 402; +NaOAc 274, 316, 387; +NaOAc/H_3BO_3 257, 268sh, 303sh, 361; +NaOMe 273, 327, 413. ^1H-NMR (400 MHz, DMSOd_6) δ = 7.73 (d, *J*=2.0 Hz, H-2'), 7.69 (dd, *J*=2.0, 8.4 Hz, H-6'), 7.05 (d, *J*=8.4 Hz, H-5'), 6.39 (d, *J*=1.8 Hz, H-8), 6.20 (d, *J*=1.8 Hz, H-6), 5.40 (d, *J*=8.5 Hz, H-1″), 3.89 (s, OCH_3-

3'), 3.07-3.83 (m, other sugar protons). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm)156.4 (C-2), 133.5 (C-3), 177.8 (C-4), 161.7 (C-5), 98.9 (C-6), 164.5 (C-7), 93.7 (C-8), 156.8 (C-9), 104.6 (C-10), 121.9 (C-1'), 112.0 (C-2'), 149.6 (C-3'), 146.9 (C-4'), 115.8 (C-5'), 122.8 (C-6'), 55.9 (OCH_3-3'), 101.4 (C-1″), 74.8 (C-2″), 77.5 (C-3″), 70.3 (C-4″), 76.9 (C-5″), 61.3 (C-6″). Hydrolytic products: 2N HCl, isorhamnetin/D-glucose; β-glucosidase enzyme, isorhamnetin/D-glucose.

RESULTS AND DISCUSSIONS

Multiple lines of evidence accumulating to date from both laboratory and clinical studies support that oxidative stress imposed by reactive oxygen and nitrogen species (RONS) plays a crucial role in the pathophysiology associated with atherosclerosis, neurodegenerative disorders, immune functions, and all stages of carcinogenesis [24]. RONS are generated as a result of naturally occurring processes (such as mitochondrial electron transport and exercise), environmental stimuli (including ionizing radiation from the sun and pollutants) and lifestyle stressors. Oxidative stress is created when there is an imbalance between the generation of reactive species and their quenching. Oxidative stress due to high flux of oxidants has been implicated in the pathogenesis of several modern human ailments. Consequently, antioxidant protection against damages that could be caused by free radicals is vital for the integrity of cellular structures and macromolecules. A number of bioactive exogenous dietary antioxidants have been demonstrated to be effective in preventing this damage and the consequent chronic disease states [25]. Polyphenols, notably the ubiquitously distributed flavonoids, which contribute considerably to dietary antioxidants have been shown to exhibit substantial protective effects on human carcinogenesis [26,27], cardiovascular and renal disorders [28-34], memory and cognitive function [35], age-related neurological dysfunctions such as Alzheimer's disease [36,37], ulcers [38] and several other human ailments [39].

Food industries are also concerned with oxidative processes since lipids, the natural constituents of cellular membranes, are oxidised during peroxidation, producing partial or total changes in food sensorial properties and in nutritional values. Plant-based antioxidants and colourants are the order of the day to preserve food quality because of safety concerns. In the recent past, there has been growing interest in functional foods, which not only offer the basic nutrition and energy, but also added physiological benefits to the consumers. The functionality of a food usually has a close relationship with some of its ingredients and those ingredients that could be derived from food/natural sources are preferred over synthetic ones, whose applications are restricted due to suspected harmful health effects.

Characterisation of the dietary antioxidants and their capacities are also essential to validate the safety and traditional uses and to standardise preparations of these plants. Furthermore, this information may be used to establish flavonoid databases, such as the USDA flavonoid database (http:// www.nal. usda.gov/fnic/ foodcomp/Data/Flav/Flav02-1.pdf).

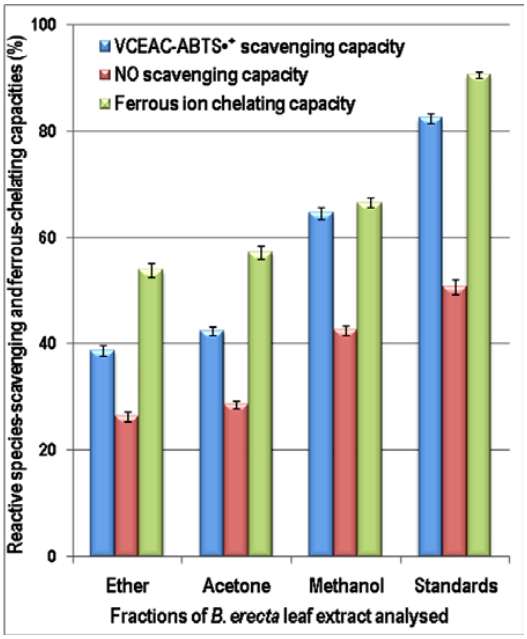
As a result, widespread screening of medicinal and food plants with antioxidant potentials are in progress. *B. erecta*, which grows wild in waste ground, open bush land and along roadsides as a weed, appear to be a functional food plant among certain cultures [6-8] and is recorded to possess potential antiparasitic, antifungal, antibacterial, antiviral, antihistaminic, antioxidant and hepatoprotective activities [9-12]. Earlier investigations have emphasised the significance and putative modes of action of specific flavonoids as bioactive components of the diet in both *in vivo* and *in vitro* models and hence, it is important to have a clear idea of the major phenolic families of the plants consumed and their levels contained therein [40]. We were, therefore, prompted to determine the polyphenolic antioxidant profile of this functional food plant.

Determination of *in vitro* antioxidant capacity

The antioxidant capacity of the leaf extract has been evaluated by measuring the capacities of the various fractions of the extract to scavenge reactive species ($ABTS^{\bullet+}$ and NO) and to chelate transition metal (Fe^{II}) ions. One of the most commonly employed and operationally simple assay of antioxidant capacity measurements involves the generation of the coloured radical cationic oxidant $ABTS^{\bullet+}$ and determining the ability of an extract/a metabolite to scavenge the same [17]. This decolourisation assay is widely recommended for plant extracts because of its several advantages, particularly (i) the λ_{max} employed for monitoring the stable blue-green chromogen eliminates colour interference in the extracts, (ii) the absorbance reduction tends to become a constant in <10 min, and (iii) $ABTS^{\bullet+}$ is soluble in both aqueous and organic phases and is not affected by ionic strength, so that it is capable of reacting with both lipophilic tocopherols, carotenes and flavonoids and hydrophilic polyphenolic glycosides and phenolic acids. All the three fractions of the analysed leaf extract scavenged $ABTS^{\bullet+}$ and the most polar MeOH fraction was found to possess the maximum VCEAC (64.57±1.07%), which is about 78.4% of the standard dietary flavonol, rutin (Fig. 2). The Me_2CO and Et_2O fractions were determined to possess respectively, 51.5% (42.43±0.74%) and 47.1% (38.77±1.03%) of the rutin VCEAC.

Large amounts of reactive nitrogen intermediates (RNS), such as NO, peroxynitrite and other reactive nitrogen oxide species, have been reported to play significant roles in inflammatory process and are considered to be potentially cytotoxic, causing injury to the surrounding cells. In addition, NO rapidly and spontaneously reacts with other reactive species, causing modification of proteins or nucleic acids. As a result, determination of the reactive nitrogen species-scavenging capacities has also gained significance [19]. In the present determination, MeOH fraction was again found to possess the maximum RNS-scavenging capacity, viz., 84% (42.57±0.87%) of the rutin capacity (Fig. 2). Me_2CO and Et_2O fractions were respectively found to be 56.4% (28.63±0.67%) and 51.9% (26.33±0.87%).

An increase in intracellular non protein-bound iron concentration occurs, for instance, as a result of (i) long-term transfusions to patients with anemia of genetic disorders such as β-thalassemia, sickle cell disease, and Diamond Blackfan syndrome, and (ii) bone-marrow failures such as aplastic anaemia and myelodysplastic syndromes [18]. As there exists no active mechanism to excrete iron from the body, a progressive accumulation of body iron easily occurs. Such redox-active iron can be taken up by liver, cardiac, and endocrinal cells through uptake mechanisms that are independent of transferrin receptor and the excess iron in parenchymal tissues can cause serious clinical sequelae, such as cardiac failure, liver disease, diabetes, and eventual death. Since iron-mediated damages have also been implicated in disease development, exploration of iron-chelating mechanism of polyphenol antioxidant behaviour has also become necessary. Iron-mediated oxidative damage is just not limited only to living organisms. Due to the presence of iron in the environment, iron-generated hydroxyl radical is also responsible for food spoilage and wood rotting. Hence, polyphenolics of natural origin that can be used as preservatives for food, cosmetics, and pharmaceuticals have been widely investigated. Per cent Fe^{II} chelating capacities of the fractions of the *B. erecta* leaf-extract were found to be in the order MeOH > Me_2CO > Et_2O (Fig. 2). MeOH fraction possessed 73.4 % of the chelating capacity of the standard drug studied, viz., deferoxamine. Deferoxamine is a trihydroxamic siderophore, which is widely employed for the treatment of iron over-load and it chelates Fe^{II} with high affinity under aerobic conditions, probably due to the formation of the more stable Fe^{III}-deferoxamine[18]. The other two fractions respectively exhibited 63.1 and 59.4% of the deferoxamine-chelating capacity (Fig. 2).



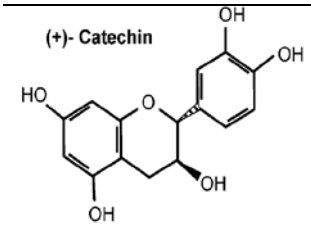
Data represents Mean±SD, n=3
Standard for VCEAC and NO scavenging = Rutin and for Fe^{II} chelation = Deferoxamine.

Fig. 2 Reactive species scavenging and Ferrous-chelating capacities of *B. erecta* leaf-extract fractions

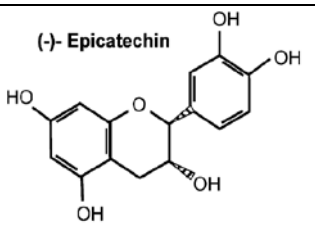
Isolation and characterisation of the phenolic metabolites

A systematic chemical analysis of the leafy matter has resulted in the isolation and characterisation of antioxidant phenolic metabolites belonging to two flavonoid sub-classes (Fig. 3). The two dietary flavonols, viz., quercetin (**3**) and isorhamnetin (**4**) and their glycosides, rutin (**5**), narcissin (**6**) isoquercitrin (**7**) and isorhamnetin 3-O-β-D-glucopyranoside (**8**), as well as the two flavan-3-ols, [(+)-catechin] (**1**) and [(-)-epicatechin] (**2**) are well known antioxidant phytometabolites. All the phenolic isolates were characterised on the basis of their physical properties, chemical reactions (including characteristically coloured products formation and rigorous hydrolytic studies) and spectral (UV, ^{1}H- and ^{13}C-nmr) features [20-23].

Flavan-3-ols

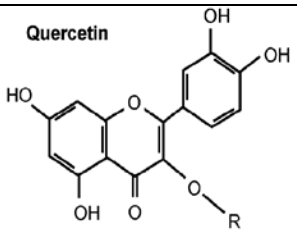


(**1**)



(**2**)

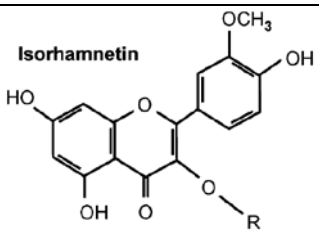
Flavonols



R= H; quercetin (**3**)

R= α-L-rhamnopyranosyl(1→6)-β-D- glucopyranoside; rutin (**5**)

R= β-D-glucopyranoside; isoquercitrin (**7**)



R= H; isorhamnetin (**4**)

R= α-L-rhamnopyranosyl(1→6)-β-D- glucopyranoside; narcissin (**6**)

R= β-D-glucopyranoside; isorhamnetin 3-O-β-D-glucopyranoside (**8**)

Fig. 3 Sub-classes of flavonoid metabolites isolated from *B. erecta* leaves

CONCLUSION

Countless number of studies have substantiated that Traditional Medicine is often the only accessible and the most affordable treatment available in developing countries and also offer an opportunity of improving the nutritional status and health care of the rural communities. Plant-based antioxidants and colourants are being used more commonly today to preserve food quality in the food industries. Characterisation of the dietary antioxidants and their capacities are necessary to validate the safety and traditional uses and also to standardise preparations involving these plants. As a result, widespread screening of medicinal and food plants possessing antioxidant capacities are in progress. The

common weed, *B. erecta*, which is determined to possess reactive oxygen and nitrogen scavenging capacity and transition metal ion chelating capability, can well be exploited as a natural and healthy source of antioxidants. The flavonoids elaborated in the taxon may potentially contribute, synergistically with other classes of phytometabolites, to the antioxidant and other biological activities observed for this functional leafy vegetable.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. A. G. R. Nair, Former Head, Department of Chemical Sciences, Pondicherry University, Puducherry for the generous donation of the authentic compounds, SIF, IISc., Bengaluru and SAIF, CDRI, Lucknow for the analytical facilities.

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