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Free-Radical–Scavenging Activity in the Inflorescence of European Nettle/Sisnu (*Urtica dioica* L.)

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ABSTRACT

The inflorescence of *Urtica dioica* L. is used by ethnic people of Darjeeling Hills as therapeutics and green vegetables. As it is ethnomedicinally very important for curing several critical and emerging oxidative stress–induced diseases, the inflorescence of this plant may be considered as a potential source of antioxidants. In the present study, *U. dioica* inflorescences were successively extracted and purified with nine different solvents by using silica gel column chromatography to study their antioxidant properties and polyphenol contents. Ethyl acetate:acetone::1:3 fraction was found (91.50% inhibition) to be the best scavenger of DPPH at a dose of 100 µg/ml among 33 extracts, which was comparable with standard quercetin (44.77% inhibition). An NBT-NADH-PMS–based highest superoxide–scavenging activity (53.30% inhibition) was observed in acetone:ethanol::1:1 fraction, which was also comparable with the same standard (72.30%). Optimum anti-lipid peroxidation of 55.27% inhibition was detected in diethyl ether:ethyl acetate::1:1 fraction. TLC fingerprint of ethyl acetate:acetone::1:3 extract after derivatization with DPPH showed maximum number of separated components with the highest diameter at R_f = 0.907. Phenols and flavonoids were mostly concentrated in the same fraction with a concentration of 24.22 ± 0.26 and 43.34 ± 1.02 mg/g of dry mass, respectively. Significant correlations were found among phenolic phytochemicals and radical–scavenging activity through which it may be assumed that the antioxidants present in *U. dioica* inflorescence are polyphenolic in nature. The observations suggest the importance of ethnomedicinal use of *U. dioica*, which could be commercially exploited by the pharmaceutical industry as a natural antioxidant.

Key words: Antioxidant, flavonoids, phenols, radical scavenger, *Urtica dioica* L

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INTRODUCTION

It is widely accepted that many diseases and aging are closely related to the oxidation reactions occurring in living organisms. Recent studies show that free-radical scavenger, both exogenous and endogenous, whether synthetic or natural compounds, can be effective for the prevention of ailments attributed to free-radical damage. There is growing interest toward antioxidants from herbal resources such as tea, fruits and vegetables. In ancient times and even today, Indian Ayurvedic Pharmacists have used this type of holistic natural products as therapeutics. Traditional uses of less toxic phytopharmaceuticals in different disease conditions by the ethnic groups worldwide are the witness of life-saving medicinal approaches of natural products.

European Nettle/Stinging Nettle, locally called Sisnu (*Urtica dioica* L., Family: Urticaceae) is an erect perennial

herb widely distributed in the forest of Darjeeling hills and has been domesticated in the Indo-China belt at altitudes of 1000-2500 m. It is known as "Stinging Nettle or European Nettle" because the 4-sided stems are armed with stinging hairs, which characteristically possess an elongate stinging cell with multicellular pedestal. Leaves are oppositely arranged; leaf blades narrowly lanceolated with pointed apex and broadly ovate to cordate at base, and margin coarsely sharp toothed. Inflorescence bears inconspicuous, greenish to tinged pinkish, unisexual flowers with four tiny sepals. Fruits are sessile achenes, laterally compressed and loosely enclosed by inner sepals. All parts of the plant are slightly aromatic in odor.

The therapeutic properties of *U. dioica* have been known for decades in the Indian Ayurvedic medicines and traditionally used as adjuvant in rheumatoid arthritis by ethnic people of Darjeeling. Ethnomedicinally, it is used in various ailments such as fever, diarrhea, cough, pulmonary infection, hemorrhoids, asthma, backache and as a poultice to boils. The inflorescence of this plant has a high reputation in indigenous system of medicine and mostly applied in chronic granulomatous diseases and urinary trouble for dissolving stones in the kidney.[1] The components extracted from *U. dioica* have an antiproliferative effect on human prostate cancer.[2] Aerial parts of the *U. dioica* contain quercetin-3-*O*-glucoside, kaempherol-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside.[1]

Though ethnomedicinally it is considered very important, only few preliminary reports are available on the pharmacological properties of crude aqueous extracts of *U. dioica*.[3] In particular, the inflorescence of *U dioica* is very important because it is used as green vegetables by the people inhabiting the hills of Darjeeling. However, adequate information on the antioxidant properties of different fractions of *U. dioica* inflorescence in polar and nonpolar solvents is still lacking. Therefore, a study was undertaken to investigate the *in vitro* free-radical–scavenging activity and anti-lipid peroxidation of successive extracts of *U. dioica* inflorescences and their possible correlation with phenols and flavonoids.

MATERIALS AND METHODS

Plant materials

Urtica dioica L. inflorescences were collected in July 2007 from Kurseong, Darjeeling. Taxonomic position was authenticated by the Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal. The material has been deposited in the "NBU Herbarium," compared with authenticated original sample and recorded [accession number 9486 dated January 27, 2009].

Animal materials

Goat liver were collected from the abattoir immediately after slaying and used for the anti-lipid peroxidation assay.

Chemicals

Solvents used for column chromatography are hexane (H), benzene (B), chloroform (C), diethyl ether (D), ethyl acetate (EA), ethanol (E) and methanol (M). TLC plates were purchased from Merck (Germany). Silica gel G_{60}, 2,2-diphenyl-1-picryl hydrazyl (DPPH), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide phosphate sodium salt monohydrate (NADPH), phenazine methosulfate (PMS), trichloroacetic acid (TCA), thiobarbituric acid (TBA), ferrous sulfate heptahydrate ($FeSO_4$,$7H_2O$), potassium hydroxide (KOH), potassium dihydrogen phosphate ($KH_2PO_{4)}$, formic acid, glacial acetic acid, gallic acid and quercetin were either obtained from Sigma Chemicals (USA) or were of Himedia grade.

Extraction, purification of *U. dioica* inflorescence

U. dioica inflorescences (20 g) were crushed within 24 h of collection and extracted with 100 ml of methanol water (4:1) by using Soxlet extractor for 8 h. The flask containing extracts were kept overnight for cold percolations. The extracts were filtered through double layer of muslin cloth and volume was reduced by evaporation under reduced pressure in a rotary evaporator. The residual part was treated with chloroform and filtered through the same and the solvent was removed *in vacuo*. Finally condensed extracts were used for purification of components through silica gel column chromatography (volume of the column, 47.1 cm^3) by using the mobile phase of hydrophobic to hydrophilic solvents with their increasing dielectric constant. All solvent fractions were evaporated to dryness under reduced pressure. Extracts were then reconstituted in methanol, used for evaluation of DPPH and superoxide-radical–scavenging, anti-lipid peroxidation assay and evaluation of phenolic components.

The design of successive extractions were chosen in such a way that the information related to the concentration and separation of antioxidants in a specific solvent system has been elucidated, which will assist the pharmaceutical industry to purify the same efficiently.

1. H (50 ml) → H:B (25 ml, 3:1) → H:B (25 ml, 1:1) → H:B (25 ml, 1:3)
2. B (50 ml) → H:B (25 ml, 3:1) → H:B (25 ml, 1:1) →H:B (25 ml, 1:3)
3. C (50 ml) → B:C (25 ml, 3:1) → B:C (25 ml, 1:1) → B:C (25 ml, 1:3)
4. D (50 ml) → D:EA (25 ml, 3:1) → D:EA (25 ml, 1:1) → D:EA (25 ml, 1:3)
5. EA (50 ml) → EA:A (25 ml, 3:1)→ EA:A (25 ml, 1:1) → EA:A (25 ml, 1:3)
6. A (50 ml) → A:E (25 ml, 3:1) → A:E (25 ml, 1:1) → A:E (25 ml, 1:3)
7. E (50 ml) → E:M (25 ml, 3:1) → E:M (25 ml, 1:1) → E:M (25 ml, 1:3)
8. M (50 ml) → M:W (25 ml, 3:1) → M:W (25 ml, 1:1) → M:W (25 ml, 1:3)
9. W (50 ml)

DPPH-based free-radical–scavenging activity

The free-radical–scavenging activities of each fraction were assayed using a stable DPPH, following standard method.[4] The reaction mixture contained 2 ml of 0.1 mM DPPH and 0.1 ml of each methanol fraction. Simultaneously, a control was prepared without sample fraction. The reaction mixture was allowed to incubate for 5 min at room temperature in the dark and scavenging activity of each fraction were quantified by decolorization at 515 nm. Percentage of free-radical–scavenging activity was expressed as percent inhibition from the given formula:

$$\text{Percent inhibition} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

Superoxide-radical–scavenging assay

The superoxide-radical–scavenging assay was performed using standard method[5] with slight modification. The reaction mixture contained 1 ml of NBT solution (156 µM prepared in phosphate buffer, pH 7.4), 1 ml of NADH solution (468 µM prepared in phosphate buffer, pH 7.4) and standardized 50 times methanol-diluted different fractions of the sample were added. Finally, reaction were accelerated by adding 100 µl PMS solution (60 µM prepared in phosphate buffer, pH 7.4) to the mixture. The reaction mixture were shaken and incubated at 25° C for 5 min and absorbance at 560 nm was measured against blank sample. Decreasing value of absorbance of reaction mixture indicates increasing superoxide-anion–scavenging activity. Percentage inhibition was calculated using the formula given above.

Anti-lipid peroxidation assay

The anti-lipid peroxidation (ALP) assay in the goat liver homogenate was measured by standard method.[6] Briefly, concentration of the extract 100 $\mu g \cdot mL^{-1}$ was added to the liver homogenate (10% w/v prepared in normal saline phosphate buffer, pH 7.40). Lipid peroxidation was initiated by adding 100 µL of 15 mmol $FeSO_4$ solution to 3 ml of liver homogenate. After 30 min, 100 µl of this reaction mixture was placed in tube containing 1.5 ml of 10% trichloroacetic acid (TCA) and centrifuged after 10 min. The supernatant was separated and mixed with 1.5 ml of 0.67% thiobarbituric acid (TBA) in 50% acetic acid. The mixture was heated in a 100° C water bath at 85° C for 30 min to complete the reaction. The intensity of the pink colored complex was measured at 535 nm in a UV-VIS double-beam spectrophotometer 2201 (Systronics). ALP % was calculated using the following formula.

$$\text{ALP percent} = \frac{\text{Abs of } Fe^{2+} \text{ induced peroxidation} - \text{Abs of sample}}{\text{Abs of } Fe^{2+} \text{ induced peroxidation} - \text{Abs of control}} \times 100$$

TLC purification of components, assay of antioxidants and fingerprinting

Fractions (1) EA:A::1:1, (2) A, (3) A:E::1:1, (4) A:E::1:3, (5) D:EA::3:1, (6) D:EA::1:1, (7) EA:A::1:3 and (8) A:E::3:1 were run for TLC purification of components.[7] Extracts were solubilized in 5 ml of methanol and spotted on precoated TLC plate by using capillary tube and marked as 1, 2, 3 and 4. A parallel replica plate was made on the other half of the same plate also marked as 5, 6, 7 and 8 and was dried. The plate was developed in the solvent system ethyl acetate:formic acid:glacial acetic acid:double distilled water (50:7:5.5:13). The plate was dried at room temperature. Plate was sprayed with 0.2% DPPH methanol solution, left for 30 minutes and scanned. R_f values and diameter of each spot was recorded. R_f was calculated using the given formula

$$R_f = \frac{\text{Distance moved by the analytes from origin}}{\text{Distance moved by the solvent font from origin}}$$

Total phenols determination

Total phenols in the 33 fractions were determined as per standard method.[8] The standard curve was prepared using different aliquots of gallic acid in methanol:water (50:50, v/v). Total phenol values were expressed in terms of gallic acid equivalent.

Total flavonoids determination

Total flavonoids in 33 fractions were determined using standard method.[9] The calibration curve was prepared by preparing quercetin solution at different aliquots of 12.5 to 100 $\mu g \cdot ml^{-1}$ in methanol.

Statistical analysis

All experiments were performed in triplicates and data were represented as mean ± S.D. and analyzed by SPSS (version 11.0 for window XP, SPSS, Inc.).

RESULTS AND DISCUSSION

The tribal apothecaries prescribed *U. dioica* inflorescence and formulated active medicaments for the relief of patients' sufferings from many diseases at hills of Darjeeling. It has been observed that herbal antioxidants can prevent various stress-induced diseases in human beings. The other plants show antioxidant activities mainly due to the presence of phenyl propanoid derivatives, namely, phenols and flavonoids, besides other secondary metabolites widely distributed in plant kingdom.

Significant DPPH free-radical–scavenging activity of *U. dioica* inflorescence extracts was observed using 33 solvents at different ratios [Figure 1]. DPPH accept an electron or hydrogen radical to become a diamagnetic molecule. DPPH reacts with specific antioxidant molecule, which involves reduction of DPPH, as a result of the decrease in its optical density. Noticeable effects of scavenging free radicals were observed in the eight fractions, which were further established by TLC. The R_f values of the yellow spots with purple background on the TLC plate developed after the application of DPPH (0.2% in methanol) is the recognition of antioxidant molecules present in that fraction.[10] DPPH free-radical–scavenging activity of eight fractions of TLC-based antioxidant fingerprint [Table 1] interestingly proved the antioxidant property of *U. dioica* inflorescence.

Superoxide-anion–scavenging activities in the fractions were determined by NBT-NADH-PMS system. Decrease of optical density values against control is the indication of the presence of bioactive compounds possessing superoxide-radical–scavenging activity. Superoxide radical requires a small input of energy that is often provided by NADPH in biological systems.[11] It can be formed

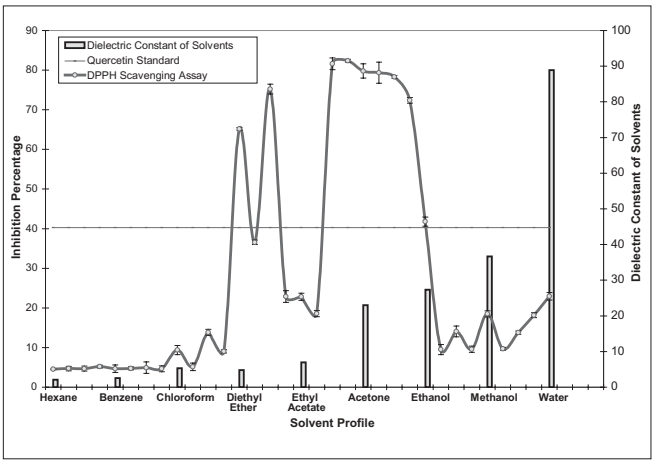

Figure 1: Percentage inhibition of DPPH-radical–scavenging activity in the different solvent fraction

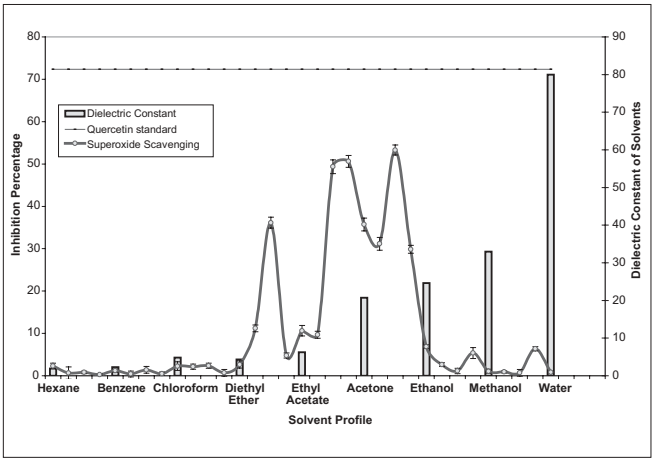

Figure 2: Percentage inhibition of superoxide-radical–scavenging activity in the different solvent fraction

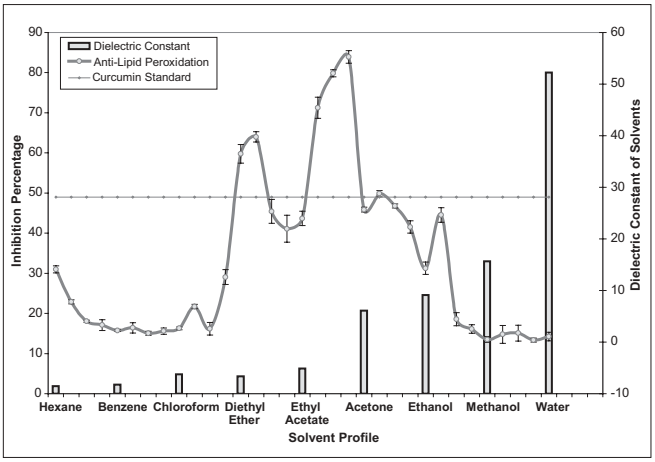

Figure 3: Percentage of anti-lipid peroxidation activity in the different solvent fraction

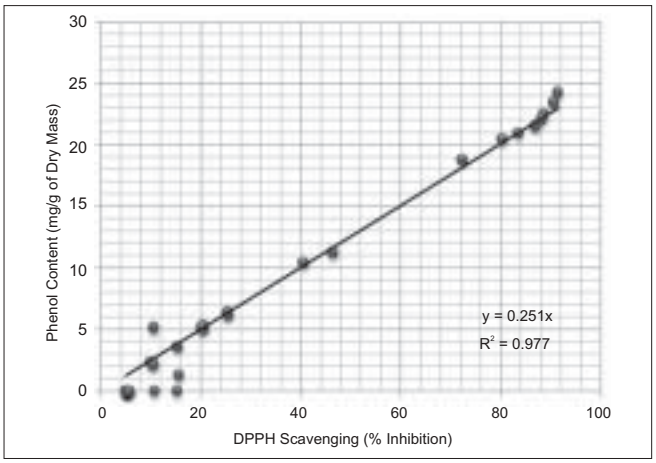

Figure 4: Percentage inhibition of DPPH-radical–scavenging activity vs phenol content (mg•g^{-1})

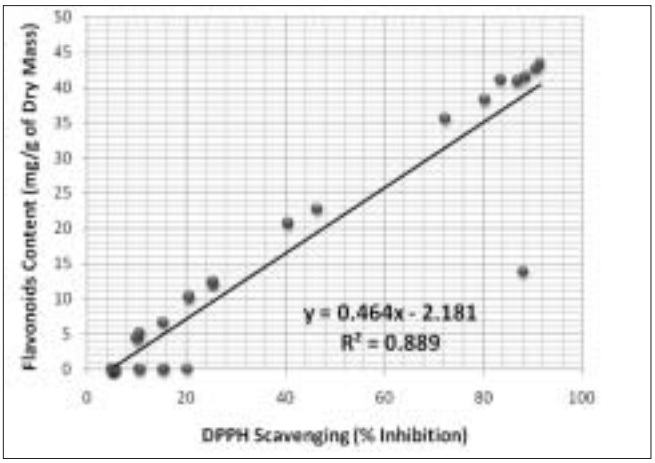

Figure 5: Percentage inhibition of DPPH-radical–scavenging activity vs flavonoids content (mg•g^{-1})

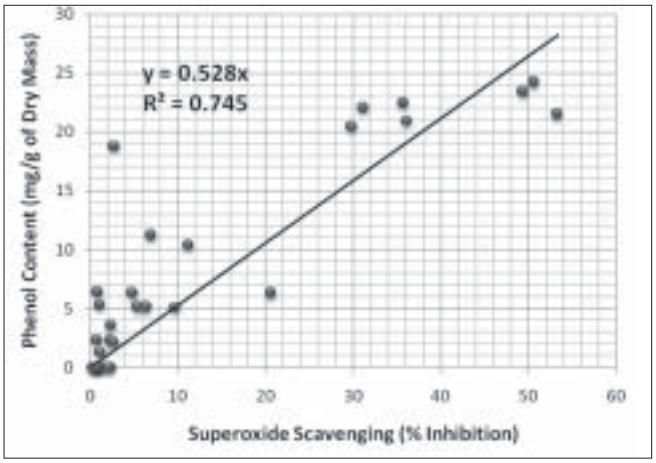

Figure 6: Percentage inhibition of superoxide-radical–scavenging activity vs phenol content (mg•g^{-1})

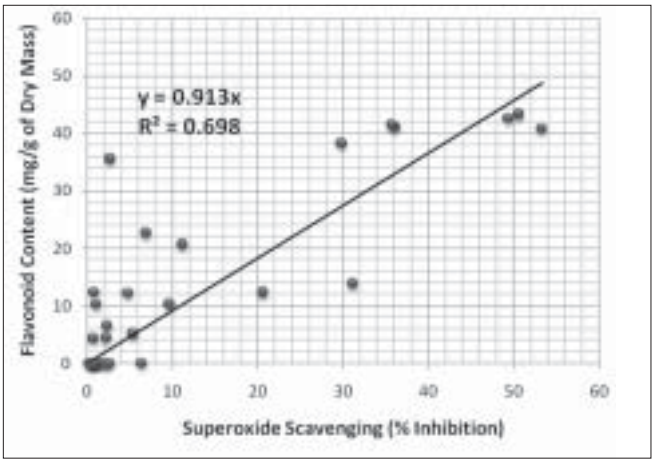

Figure 7: Percentage inhibition of DPPH-radical–scavenging activity vs flavonoids content (mg•g^{-1})

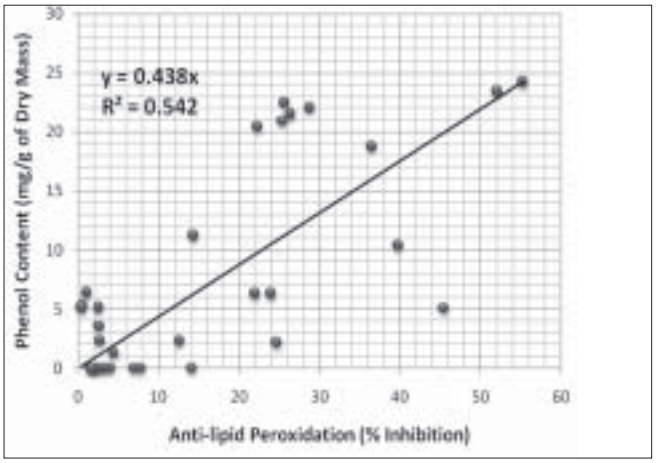

Figure 8: Percentage of anti-lipid peroxidation activity vs phenol content (mg•g^{-1})

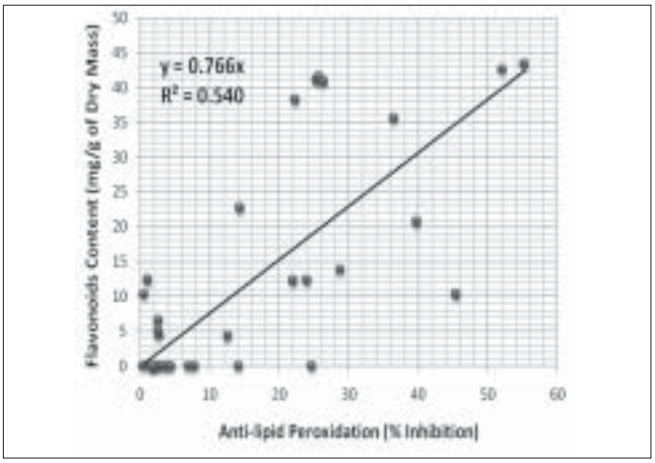

Figure 9: Percentage of anti-lipid peroxidation activity vs flavonoids content (mg•g^{-1})

enzymatically or nonenzymatically. Superoxide may decrease the activity of other antioxidant defense, an enzyme like catalases. In this assay, only 6 fractions have shown significant superoxide scavenging properties, which are comparable with reference standard [Figure 2]. Other plants that have proved their activity against superoxide anion are *Cissus quadrangularis*,[12] *Orthosiphon stamineus*,[13] *Rosa damascena* [14] and *Helichrysum italicum*.[15]

Table 1: DPPH antioxidant activity of *Urtica dioica* L. inflorescence in solvent fractions observed on TLC plate

Spot serial number	Solvent fraction	Ratio	Rf values	Diameter of the spot (cm)
1	EA	75%	0.656	1.51
			0.72	1.12
2	EA	25%	0.307	1.40
			0.421	0.91
			0.640	1.10
			0.720	0.60
			0.907	2.56
			0.993	1.05
3	A	50%	0.824	1.20
			0.751	1.01
4	D	100%	0.826	1.32
			0.950	1.00
5	D	75%	0.993	0.85
6	D	50%	0.988	0.67
7	EA	50%	0.513	0.80
			0.422	0.63
			0.805	0.60
			0.720	0.45
8	A	75%	0.540	0.45
			0.291	0.61
			0.527	0.95
			0.591	0.40
			0.820	0.65

Extracts of *U. dioica* inflorescence have been found to reduce lipid peroxidation of microsomes in goat liver. Malonaldehyde formed during the lipid peroxidation is known as the cleavage products of adipose tissue, which is accumulated in the central nervous system and in cardiac muscle fibers.[16] *U. dioica*–derived phenols and flavonoids may serve as active antioxidant molecules in the different fractions by inhibiting this type of reaction [Figure 3]. The antioxidant properties are generally associated with the presence of reductones. Reductones are believed to break radical chain by donation of a hydrogen atom, indicating that antioxidative properties are concomitant with the development of reducing power. Therefore, the marked anti-lipid peroxidation properties in different fraction may be related with its higher reducing power.

It is already established that flavonoids are potent antioxidants and have considerable effects on human health and nutrition. The flavonoids with more than one hydroxyl group are enacted in biological system through scavenging or chelating processes.[17,18] Phenolic compounds are the special class of antioxidant agents, which act as free-radical–chain terminators.[19] The highest range of flavonoids content among 33 fractions were between 43.34 ± 1.02 mg quercetin equivalent per gram of dry mass of *U. dioica* in the EA:A::1:3 fraction. The phenol content of 33 fractions was also determined by Folin-Ciocalteu reagent

Table 2: Phenols (gallic acid equivalent) and flavonoids content (quercetin equivalent) in solvent fractions of *Urtica dioica* L. inflorescence*

Solvent Fraction (v/v)	100%		75%		50%		25%	
Active principles	Phenols	Flavonoids	Phenols	Flavonoids	Phenols	Flavonoids	Phenols	Flavonoids
Hexane (H) in benzene (B)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzene (B) in chloroform (C)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloroform (C) in diethyl ether (D)	2.31 ± 0.03	4.46 ± 0.18	0.0	0.0	3.56 ± 0.09	6.51 ± 0.38	2.31 ± 0.04	4.33 ± 0.18
Diethyl ether (D) in ethyl acetate (EA)	18.77 ± 0.21	35.53 ± 0.41	10.39 ± 0.15	20.66 ± 0.34	20.94 ± 0.27	41.07 ± 0.14	6.31 ± 0.04	12.20 ± 0.17
Ethyl acetate (EA) in acetone (A)	6.31 ± 0.04	12.30 ± 0.37	5.07 ± 0.37	10.27 ± 0.25	23.43 ± 0.24	42.61 ± 0.54	24.22 ± 0.26	43.34 ± 1.02
Acetone (A) in ethanol (E)	22.47 ± 0.16	41.50 ± 0.61	22.07 ± 0.08	13.84 ± 0.23	21.52 ± 0.47	40.85 ± 0.53	20.44 ± 0.13	38.25 ± 2.00
Ethanol (E) in methanol (M)	11.21 ± 0.11	22.66 ± 0.31	2.15 ± 0.04	0.0	1.27 ± 0.04	0.0	5.13 ± 0.10	5.08 ± 0.11
Methanol (M) in water (W)	5.32 ± 0.04	10.29 ± 0.32	0.0	0.0	0.0	0.0	5.13 ± 0.10	0.0
Aqueous fraction (W)	6.39 ± 0.16				12.35 ± 0.32			

*Mean values of active constituents ($mg \cdot g^{-1}$ of dry mass) over control ± standard deviations (n = 3)

and the highest concentration was measured as 24.22 ± 0.26 mg of gallic acid equivalent per gram of dry mass in the same fraction [Table 2]. According to our study, the high contents of these phytochemicals in EA:A::1:3 fraction of *U. dioica* can explain its high radical-scavenging activity. Linear regression analysis strongly proved significant correlation coefficient between phenolic phytochemicals and DPPH or superoxide-radical–scavenging activity [Figures 4 to 7, respectively], but comparatively lower correlations were established between polyphenols and anti-lipid peroxidation [Figure 8 to 9]. These relationships may serve as indices to directly select extracting lines through which maximum polyphenolic antioxidants can be obtained.

Further studies have been undertaken for the characterization of individual components of bioactive fractions of *U. dioica* inflorescence to elucidate the structure-activity relationship.

CONCLUSION

The results of the present study showed that the EA:A::1:3 fraction of successive extract of *U. dioica* inflorescence contains the highest amount of phenols and flavonoids. Furthermore, significant correlations existed between phenols or flavonoids and the free-radical–scavenging activities. The extracting solvents significantly affected the purification of antioxidant molecules. Optimal antioxidants can only be obtained by extracting *U. dioica* inflorescence with medium polar solvents such as diethyl ether, ethyl acetate, and acetone. Highly non-polar or polar solvents are unsuitable for this extraction process. Thus, our results indicate the targeted extraction of natural polyphenolic materials by an appropriate solvent that have high radical-scavenging and anti-lipid peroxidation activities. The TLC fingerprint data can also help the apothecary to recognize the true *U. dioica* inflorescence drugs from its adulterants.

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REFERENCES

1. Akbay P, Basant AA, Undeger U, Basaran N. *In vitro* immunomodulator activity of flavonoid glycosides from *Urtica dioica* L. Phytother Res 2003;17:34-7.
2. Konrad L, Muller HH, Lenz C, Laubinger H, Aumuller G, Lichius JJ. Antiproliferative effect on human prostate cancer cells by a stinging nettle root (*Urtica dioica)* extract. Planta Med 2000;66:44-7.
3. Gulcin I, Kufrevioglu OI, Oktay M, Buyukokuroglu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J Ethnopharmacol 2004;90:205-15.
4. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958;181:1199-2000.
5. Nishikimi M, Rao NA, Yagi K. The occurrence of super oxide anion in the reaction of reduced Phenazine methosulphate and molecular oxygen. Biochem Biophys Res Commun 1972;46:849-53.
6. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
7. Souri E, Sarkhail P, Kaymahesh P, Amini M, Farsam H. Antioxidant activity and a new isolated Dioxaspiran derivative of Tripleurospermum disciforme. Pharma Biol 2005;43:620-3.
8. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic contents and antioxidant activity of olive extracts. Food Chemistry 2001;73:73-84.
9. Chang C, Yang M, Wen H, Chern J. estimation of total flavonoids content in propolis by two complementary colorimetric methods. J Food Drug Analysis 2002;10:178-82.
10. Conforti F, Statti GA, Tundis R, Menichini F, Houghton P. Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial plant. Fitoterapia 2002;73:479-83.
11. Dhalwal K, Deshpande YS, Prohit AP, Kadam SS. Evaluation of the antioxidant activity of *Sida cordifoli*. Pharma Biol 2005;43:754-61.
12. Jainu M, Shyamala Devi CS. In vitro and in vivo evaluation of free radical scavenging potential of *Cissus quadrangularis*. Pharma Biol 2005;43:773-9.
13. Akowuah GA, Zhari I, Norhayanti I, Sadikum A. Radical scavenging activity of methanol leaf extracts of *Orthosiphon stamineous*. Pharma Biol 2004;42:629-35.
14. Achuthan CR, Babu BH, Padikkala J. Antioxidant and hepatoprotective effects of *Rosa domascena*. Pharma Biol 2003;41:357-64.
15. Poli F, Muzzolim M, Sacchetti G, Tassinato G, Lazzarin R, Burni A. Antioxidant activity of super critical CO_2 extract of *Helichrysum italicum*. Pharma Biol 2003;41:379-83.
16. Nohl H. Involvement of free radicals in ageing: A consequence or cause of senescence. Br Med Bull 1993;49:653-7.
17. Kessler M, Ubeaud G, Jung L. Anti and pro-oxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol 2003;55:131-42.
18. Das NP, Pereira TA. Effects of flavonoids on the thermal autooxidation of palm oil: Structurally active relationship. J American Oil Chemists Society 1990;67:255-8.
19. Shahidi F, Wanasundara PK. Phenolic antioxidants. Crit Rev Food Sci Nutr 1992;32:67-103.

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