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Full Length Research Paper

Antioxidant potential of the extracts of *Putranjiva roxburghii*, *Conyza bonariensis*, *Woodfordia fruiticosa* and *Senecio chrysanthemoids*

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***Putranjiva roxburghii*, *Conyza bonariensis*, *Woodfordia fruiticosa* and *Senecio chrysanthemoids* were collected from different areas of Pakistan and extracted in methanol. The crude methanolic extract was dissolved in water and further partitioned with n-hexane, chloroform and n-butanol successively. Total phenols of all extracts were calculated using Folin-Ciocalteu (FC) reagent, while antioxidant activities were determined using standard protocols. All extracts contained reasonable amount of phenolic contents ranging from 36.9 ± 0.3 to 911.7 ± 1.4 mg GAE/g of extract, and maximum total phenols were present in the ethyl acetate extract of *S. chrysanthemoids* (911.7 ± 1.4 mg GAE/g of extract). Antiradical activity was measured as decrease in absorbance at 517 nm using diphenylpicrylhydrazyl radical (DPPH). The ethyl acetate extract of *W. fruiticosa* exhibited the highest activity (92.1 ± 1.6% with IC_{50} = 4 ± 0 µg). The reducing potential of the extracts was determined with ferric reducing antioxidant power (FRAP) and total antioxidant capacity (TAC) assays. The ethyl acetate extract of *C. bonariensis* showed the highest activity in FRAP (671.9 ± 1.6 µM) and the extract of *W. fruiticosa* (ethyl acetate) was the most active (1.882 ± 0.041) in TAC among the other extracts of the selected medicinal plants.**

Key words: DPPH, FRAP, TAC, medicinal plants.

INTRODUCTION

Cellular damage caused by the free radicals or reactive oxygen species (ROS) now appears to be the fundamental mechanism involved in a number of human neurodegenerative disorder, inflammation, diabetes, viral infections, digestive system disorders and autoimmune pathologies (Atawodi, 2005). In living systems, free radicals are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals, especially stress hormones (adrenalin and noradrenalin) (Sini et al., 2010). ROS can be scavenged through chemoprevention utilizing natural antioxidant compounds present in medicinal plants. Natural antioxidants such as phenolic compounds, are gaining importance due to their benefits for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation (Silva et al., 2004; Pulido et al., 2000; Tseng et al., 1997).

Putranjiva roxburghii Wall, also known as *Drypetes roxburghii* (Wall),is a member of the Euphorbiaceae family. It is widely grown in Thailand, Nepal, Bangladesh, India, Indochina, Myanmar and Sri Lanka (Phuphathanaphong and Chayamarit, 2006). In folklore medicine, its leaves and fruits have been traditionally used for the treatment of fever, muscle twisting, arthralgia and rheumatism (Boonyaprapat and Chokechaicharoenporn, 1999). It is also used as antinociceptive, antipyretic, anti-inflammatory and the whole plant of *P. roxburghii* has been used for the

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treatment of fever and hemorrhoids. Two triterpenoids, namely putranjivanonol and putranjic acid, were isolated from the trunk bark of *P. roxburghii* (Garg and Mitra, 1968). The isolation of four other triterpenoids, friedelin, putranjivadione, friedelanol and roxburgholone, from the bark of *P. roxburghii* has also been described (Sengupta and Mukherjee, 1968). Roxburghonic acid, a triterpene acid, and putraflavone, a biflavonoid, were isolated from the alcoholic extract of *P. roxburghii* leaves (Garg and Mitra, 1971).

The genus Conyza composed of 50 species which are found on the tropical Himalaya from Nepal to Sikhim, extending to Assam, Khasia hills, Chittagong and Burma. The species *C. bonariensis* (Asteraceae) is distributed in many parts of the Punjab province along the edges of roads, gardens and maize lands. The genus Conyza is found to be very rich in terpenoids such as celarodanes (Zdero et al., 1990), sesquiterpenes (Bohlmann and Wagner, 1982) and diterpenes (Ahmad et al., 1992; Mata et al., 1997). Woodfordia fruticosa Kurz (Woodfordia floribunda Salisb.) belonging to the Lythraceae family, is abundantly present throughout India, and also in a majority of the countries of South East and Far East Asia like Malaysia, Indonesia, Sri Lanka, China, Japan and Pakistan, as well as Tropical Africa (Kirtikar and Basu, 1935). It is a much-used medicinal plant in Ayurvedic and Unani systems of medicines (Chopra et al., 1956). Although all parts of this plant possess valuable medicinal properties (Chougale et al., 2009), there is a heavy demand for the flowers, both in domestic and international markets specialized in the preparation of herbal medicines (Oudhia, 2003). According to the Indian Systems of Medicine, this flower is pungent, acrid, cooling, toxic, alexiteric, uterine sedative and antihelminthic, and is useful in dysentery, leprosy, erysipelas, blood diseases, leucorrhoea, menorrhagia and toothache. The compounds identified are predominantly phenolics (phenolic acids, flavonoids and hydrolysable tannins) (Desai et al., 1971). The other non-phenolic constituents reported include the triterpenoids lupeol, betulin, betulinic acid, oleanolic acid and ursolic acid from the leaves (Dan and Dan, 1984).

The genus Senecio (family: Asteraceae) consists of more than 1,500 species of aromatic herbs and shrubby plants native to Southern Europe, but now spread all over the world (Joffe, 2001). It is herbaceous, common in Pakistani's flora, along roadsides, waste grounds, forests and crops and the plains to the mountain zone. Senecio species contain a large variety of pyrrolizidine alkaloids, flavonoids, volatile oils, sterols, triterpenes, tannins, fatty acids, sugars and vitamins (Bohlman et al., 1986; Bohlmann and Ziesche, 1981). The aerial parts of Senecio species are used as traditional remedies for treatment of asthma, coughs, bronchitis, enemas in chest complaints, eczema and wound healing (Yang et al., 2011).

The aim of the present study was to determine a link between the antioxidant activity and traditional uses of the selected medicinal plants against various diseases such as ulcer, diabetes and neurodegenerative diseases. To the best of our knowledge, antioxidant activity of the selected plants has not been reported on stems.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent (FC), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, ascorbic acid and quercetin were purchased from Sigma-Aldrich (USA). All other solvents, chemicals and reagents of analytical grade were from Merck (Germany).

Collection of plant materials

C. bonariensis, W. fruticosa and *S. chrysanthemoids* were collected from Azad Kashmir, while *P. roxburghii* was collected from the Botanical Garden, GC University, Lahore. The plants were identified at Botany Department and voucher specimens of all plants were submitted at Sultan Herbarium.

Extraction

The stem of *S. chrysanthemoids*, W. fruticosa, *P. roxburghii* and *C. bonariensis* were shade dried, pulverized and extracted with methanol separately (300 g each). The crude extracts were filtered and concentrated on rotary evaporator at reduced pressure. Methanolic extracts of all plants were dissolved in water, and fractionated successively with n-hexane, ethyl acetate, chloroform and n-butanol

Determination of total phenolic contents

The total phenolic content was estimated through spectroscopic method (Shahwar et al., 2010a) using FC reagent. For this assay, 0.2 ml of each extract (1 mg/ml) was mixed with 0.05 ml of FC reagent and 0.2 ml of sodium carbonate (10%). The mixtures were shaken thoroughly and made up to 3 ml with distilled water. The absorbance at 760 nm was determined after incubation at room temperature for 30 min. Total phenolic contents were estimated as milligrams of gallic acid equivalent (GAE) per gram of extract by computing with standard calibration curve obtained at different concentrations of gallic acid. Tests were carried out in triplicate.

DPPH radical scavenging assay

The radical scavenging ability of different extracts was measured using DPPH (Brand-Williams et al., 1995). Methanol solution (1.0 ml) of all the extract/fraction at various concentrations (0.01 to 1.5 mg/ml) was added to 1.0 ml (0.2 mg/ml) methanol solution of DPPH and kept in dark. The decrease in absorbance at 517 nm was noted after 30 min. Tests were carried out in triplicate. The % scavenging of radical was determined by the following formula:

$$\% \text{ inhibition of DPPH} = \frac{A - B}{A} \times 100$$

Where A is the absorbance of blank and B is the absorbance of sample.

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power assay of extracts and organic soluble fractions was carried out using 2,4,6-tri(2-pyridyl)-1,3,5-triazine (Shahwar et al., 2010b). One hundred and fifty microliters of FRAP reagent was mixed with sample and absorbance was read at 595 nm after 15 min. The results were expressed in µM equivalent to $FeSO_4.7H_2O$ by calculating from calibration curve. Tests were carried out in triplicate.

Evaluation of total antioxidant capacity by phosphomolybdate method

The total antioxidant capacity of the plant extract/fraction was evaluated by the method of Prieto et al. (1999). An aliquot of 0.2 ml (500 µg/ml) of the sample solution was mixed with 2.0 ml of the reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 60 min and absorbance was measured at 695 nm against a blank containing 2 ml of reagent solution. Tests were carried out in triplicate.

Statistical analysis

All the experiments were performed in triplicate and statistical analysis were made with one way ANOVA. Statistical calculation was performed using SPSS 15.0 software.

RESULTS

Determination of radical scavenging activity

In vitro assessment of antioxidant potency is commonly carried out using DPPH radical, which is a very stable organic free radical that shows absorption maxima at 517 nm. It loses chromophoric group upon receiving a proton and becomes yellow. As the radical, it is very sensitive to the presence of hydrogen donors (therefore the assay needs very low concentration) and a large number of samples can be evaluated in a short time. Table 1 reports the results of DPPH assay of all the extracts of the selected plants. Remarkable results were obtained with ethyl acetate extracts of all the tested plants in the decreasing order WFE > PRE > CBE, except *S. chrysanthemoids* (SCE) followed by the methanolic extracts (WFM > CBM > PRM). The butanolic extracts were also significantly active and the result of % inhibition was in the range of 58.8 ± 0.4 to 80.6 ± 0.9%. Moreover, CBC of *C. bonariensis* showed comparatively higher inhibition % than PRC of *P. roxburghii* (Figure 1). Water extracts of the selected plants showed moderate results, except CBW.

IC_{50} values were calculated in terms of 50% inhibition of the available radical and the results are presented in Table 1. A quantitative difference in the IC_{50} values of all the extracts was determined. Significant results of IC_{50} values were shown by WFE, WFM and PRE (IC_{50} = 4 ± 0, 4 ± 1 and 5± 1 µg, respectively) comparable with the standard (gallic acid, IC_{50} = 2 ± 0 µg).

Determination of reducing capacity

The reducing capacity of a compound is an indication of its antioxidant activity. FRAP assay is based upon the reduction of Fe^{3+} ions by the sample and estimation of the colored complex of Fe^{2+} with TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) at 595 nm. The data is expressed in terms of Fe^{2+} equivalents.

It was noticed that the same plant extracts showing substantial antioxidant activity against DPPH radical also showed reducing activity against Fe^{3+} ions in FRAP assay, although FRAP equivalent values were lower in the latter case for *P. roxburghii* and *W. fruiticosa*. Surprisingly, hexane extracts of *C. bonariensis* was found second in the order of FRAP results (CBE > CBH > CBC > CBM > CBB). Among all the extracts, polar extracts of *W. fruiticosa* except WFW showed significant results in the FRAP assay (Figure 2).

Total antioxidant capacity

Total antioxidant capacity of the extracts were calculated using phosphomolybdate assay which is based upon the color measurement of a green colored phosphomolybdenum (V) complex formed through reduction of Mo (VI) and shows a maximum absorbance at 700 nm. WFE, WFM and WFW of *W. fruiticosa* exhibited total antioxidant capacity higher than the standard (gallic acid). The results obtained for all other extracts were in the range of 0.692 ± 0.029 to 1.882 ± 0.041 (Table 1, Figure 3).

Determination of total phenols

The amount of total phenolic contents expressed as mg GAE/g of extract varied widely among plant extracts and ranged from 36.9 ± 0.3 to 373.6 ± 1.4 mg GAE/g of extract in *P. roxburghii*, 505.2 ± 1.0 to 891.3 ± 1.6 mg GAE/g of extract in *W. fruiticosa*, 108.6 ± 0.7 to 395.6 ± 1.1 mg GAE/g of extract in *C. bonariensis*, and 150.6 ± 0.6 to 911.7 ± 1.4 mg GAE/g of extract in *S. chrysanthemoids* (Table 1).

Correlation between total phenols and antioxidant activities

The antioxidant activities of the extracts of *C.*

Table 1. Total phenol and antioxidant activities of the extracts of *P. roxburghii*, *C. bonariensis*, *W. fruiticosa* and *S. chrysanthemoids*.

Sample/Standards	Total phenols[a]	DPPH activity		FRAP[c]	Total Antioxidant capacity[d]
		% inhibition[b]	IC_{50} (µg)		
Putranjiva roxburghii					
PRM	176.0 ± 1.3	76.1 ± 1.0	119 ± 4	576.2 ± 1.4	1.029 ± 0.056
PRH	-	29.5 ± 0.4	-	22.1 ± 0.5	1.016 ± 0.031
PRC	150.0 ± 0.8	69.4 ± 0.5	265 ± 5	490.1 ± 1.1	0.992 ± 0.026
PRE	373.6 ± 1.4	91.9 ± 2.3	5 ± 1	638.7 ± 1.3	1.393 ± 0.062
PRB	59.2 ± 0.6	58.8 ± 0.4	492 ± 4	260.3 ± 0.7	0.996 ± 0.043
PRW	36.9 ± 0.3	52.7 ± 0.2	444 ± 6	204.0 ± 0.5	0.863 ± 0.019
Conyza bonariensis					
CBM	241.3 ± 0.9	78.7 ± 0.3	230 ± 3	410.3 ± 1.0	0.728 ± 0.034
CBH	167.9 ± 1.1	62.84 ± 1.6	336 ± 4	449.5 ± 1.3	0.692 ± 0.029
CBC	304.7 ± 1.5	74.08 ± 1.3	223 ± 5	444.1 ± 0.9	0.905 ± 0.062
CBE	395.6 ± 1.1	90.3 ± 0.8	89 ± 3	671.9 ± 1.6	0.875 ± 0.051
CBB	108.6 ± 0.7	67.81 ± 1.4	251 ± 6	367.4 ± 1.1	0.698 ± 0.043
CBW	-	15.1 ± 0.3	-	26.4 ± 0.6	0.996 ± 0.068
Woodfordia fruiticosa					
WFM	771.7 ± 1.4	84.4 ± 1.5	4 ± 1	201.1 ± 1.0	1.520 ± 0.062
WFH	-	-	-	-	-
WFC	505.2 ± 1.0	58.9 ± 0.2	147 ± 2	144.2 ± 0.7	1.136 ± 0.069
WFE	891.3 ± 1.6	92.1 ± 1.6	4 ± 0	222.2 ± 0.9	1.882 ± 0.041
WFB	815.2 ± 1.2	80.6 ± 0.9	80 ± 3	190.3 ± 0.4	1.613 ± 0.050
WFW	660.1 ± 0.8	65.4 ± 0.8	190 ± 4	151.6 ± 0.6	1.455 ± 0.059
Senecio chrysanthemoids					
SCM	361.7 ± 0.4	37.2 ± 0.4	-	103.6 ± 0.9	0.852 ± 0.035
SCH	225.7 ± 0.8	19.5 ± 0.3	-	121.5 ± 1.0	0.893 ± 0.041
SCC	604.0 ± 1.1	39.9 ± 0.4	-	169.1 ± 0.8	1.015 ± 0.062
SCE	911.7 ± 1.4	57.4 ± 1.6	497 ± 9	193.7 ± 1.2	1.052 ± 0.037
SCB	879.3 ± 1.0	73.2 ± 1.1	460 ± 6	171.9 ± 1.5	1.041 ± 0.044
SCW	150.6 ± 0.6	18.6 ± 0.6	-	94.2 ± 1.0	0.751 ± 0.029
Gallic acid	-	94.3 ± 1.3	2 ± 0	-	1.213 ± 0.046
Ascorbic acid	-	92.6 ± 1.0	3 ± 0	-	-
Quercetin	-	87.3 ± 0.6	5 ± 1	-	-

[a]mg GAE/g of extract; [b]at 500 µg/ml; [c]equivalent to $FeSO_4.7H_2O$ (µM); [d] absorbance at 695 nm; - = not calculated. *P. roxburghii* : in methanol (PRM), in n-hexane (PRH), in chloroform (PRC), in ethyl acetate (PRE), in butanol (PRB) and in water (PRW); *C. bonariensis:* in methanol (CBM), in n-hexane (CBH), in chloroform (CBC), in ethyl acetate (CBE), in butanol (CBB) and in water (CBW); *W. fruticosa:* in methanol (WFM), in n-hexane (WFH), in chloroform (WFC), in ethyl acetate (WFE), in butanol (WFB) and in water (WFW); *S. chrysanthemoids*: in methanol (SCM), in n-hexane (SCH), in chloroform (SCC),in ethyl acetate (SCE), in butanol (SCB) and in water (SCW).

bonariensis, P. roxburghii, W. fruiticosa and *S. chrysanthemoids* measured by DPPH, FRAP and phosphomolybdate methods were found correlated with total phenols as indicated by the R^2 values: *P. roxburghii*, R^2 = 0.7875, 0.9627, 9152; *W. fruiticosa*, R^2 = 0.9207, 0.8736, 0.9364; *C. bonariensis*, R^2 = 0.7578, 0.7025, 0.7506; *S. chrysanthemoids*, R^2 = 0.8917, 0.8715, 0.854 for DPPH, FRAP and phosphomolybdate methods, respectively (Table 2).

DISCUSSION

An imbalance between ROS (reactive oxygen species)

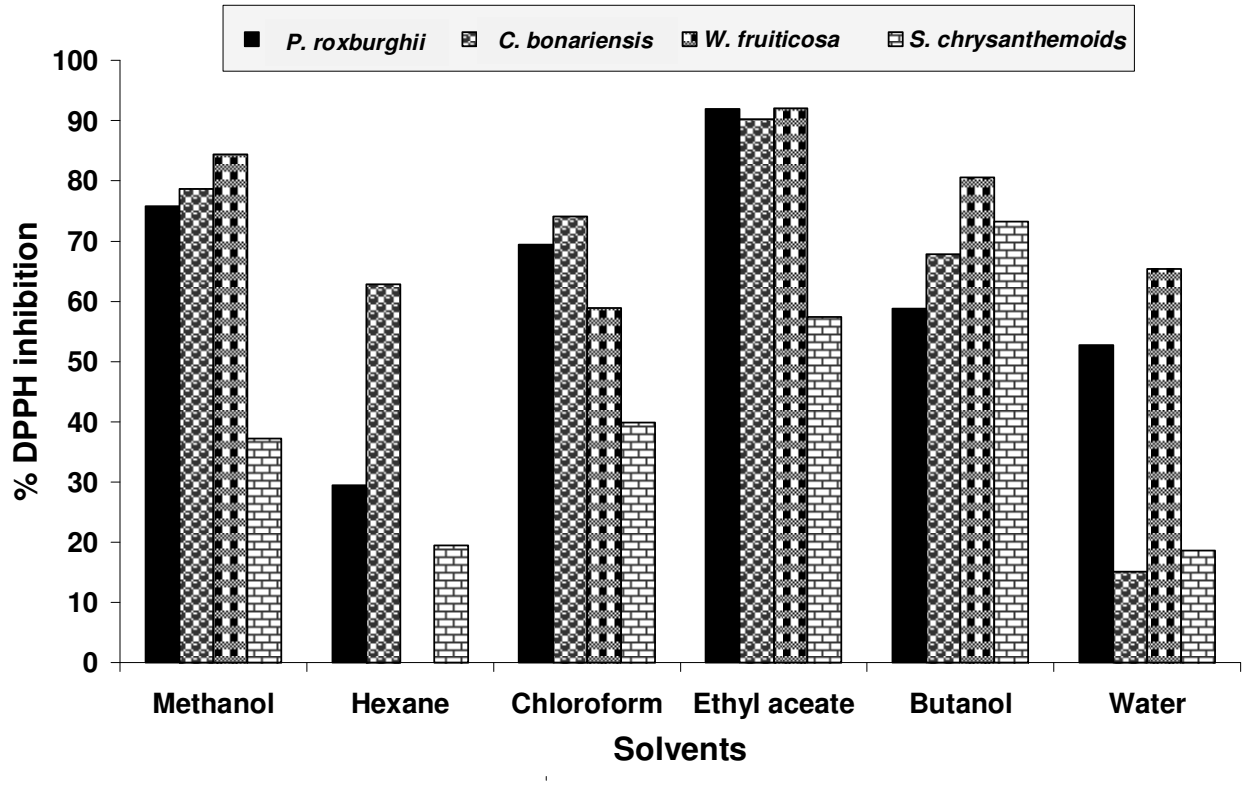

Figure 1. % DPPH inhibition activity of the extracts of *P. roxburghii, C. bonariensis, W. fruiticosa* and *S. chrysanthemoids*.

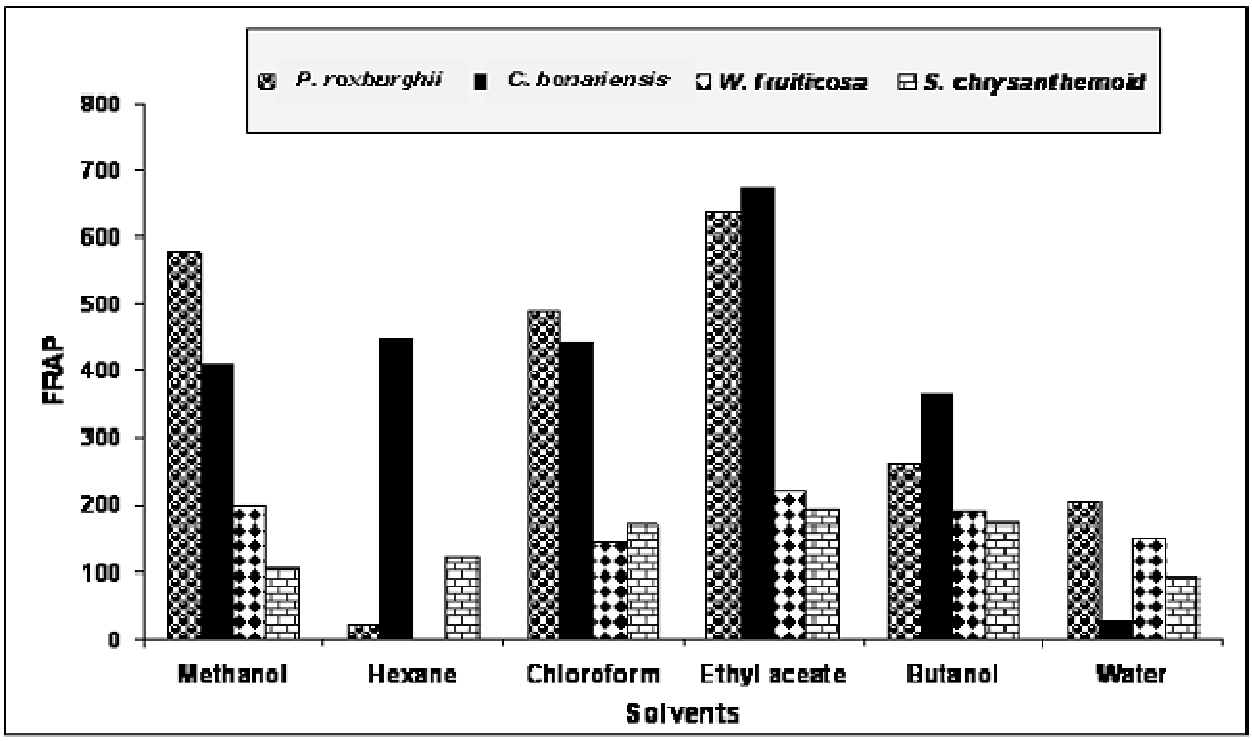

Figure 2. FRAP activity of the extracts of *P. roxburghii, C. bonariensis, W. fruiticosa* and *S. chrysanthemoids*.

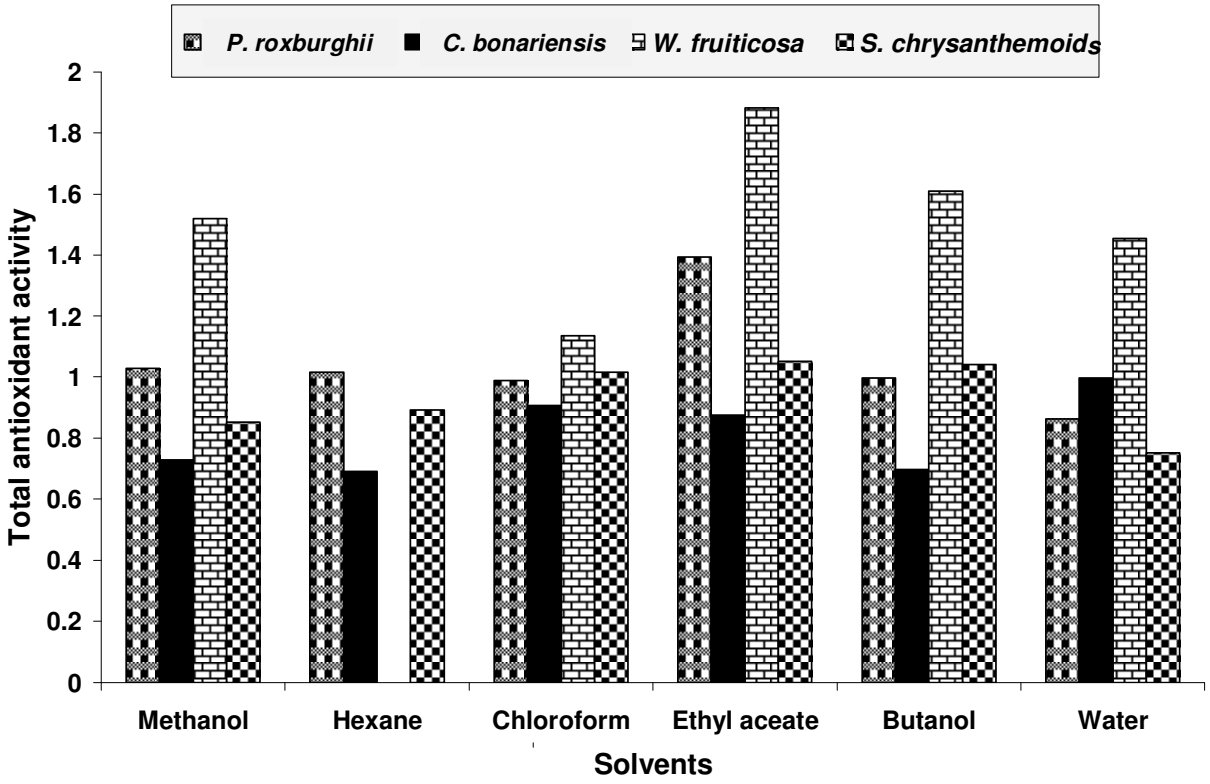

Figure 3. Total antioxidant activity of the extracts of *P. roxburghii, C. bonariensis, W. fruiticosa* and *S. chrysanthemoids*.

Table 2. Correlation studies between total phenols and antioxidant activities of the extracts of *P. roxburghii*, *C. bonariensis*, *W. fruiticosa* and *S. chrysanthemoids*.

Assays	*P. roxburghii*	*C. bonariensis*	*W. fruiticosa*	*S. chrysanthemoids*
	R^2 Values			
DPPH	0.9627 (y=0.1128x +51.835)	0.7578 (y=0.0817x +54.833)	0.9207 (y=0.0876x +12.466)	0.8917 (y=0.0616x + 8.8166)
FRAP	0.7875 (y=1.2805x +230)	0.7025 (y=0.8806x +253.85)	0.8736 (y=0.2052x +32.178)	0.8715 (y=0.1173x +81.107)
Total antioxidant capacity	0.9152 (y=0.0014x +0.8265)	0.7506 (y=0.0008x +0.5865)	0.9364 (y=0.0017x +0.2569)	0.854 (y=0.0003x +0.7552)

generating and scavenging system which results in cellular damage has been implicated in the pathology of a range of human disorders, (Atawodi, 2005). Chemo-preventive capacities of ethnobotanicals with radical scavenging potential has been demonstrated on ulcer (Borelli and Izzo, 2000), diabetes (Sabu and Kuttan, 2002), memory and cognitive function (Howes et al., 2003), Alzheimer's disease (Howes et al., 2003; Perry et al., 1998), age related neurological dysfunction (Delanty and Dichter, 2000), cardiovascular and renal disorders (Anderson et al., 1999). Hence, therapy using anti-oxidants (free radical scavengers) may have potential to prevent or delay many of these disorders. The use of traditional medicinal plants with significant antioxidant activity provides an effective strategy to prevent these disorders (Shahwar et al., 2010c).

In the present study, four different species of medicinal plants had been selected on the basis of their traditional uses. Antioxidant activity of the selected plant extracts was determined using DPPH, FRAP and phos-phomolybdate methods. According to the results shown in Table 1 for different antioxidant assays, the selected

plants can be ranked as; DPPH, *W. fruiticosa* > *P. roxburghii* > *C. bonariensis* > *S. chrysanthemoids*: FRAP, *C. bonariensis* > *P. roxburghii* > *W. fruiticosa* > *S. chrysanthemoids*: TAC, *W. fruiticosa* > *P. roxburghii* > *S. chrysanthemoids* > *C. bonariensis*. A literature survey revealed that *W. fruiticosa* has been recommended for peptic ulcer diseases (Das et al., 2006). Recently, a wide range of compounds including flavonoids and polyphenols, have been isolated from this species (Kalidhar et al., 1981; Kadota et al., 1990). High R^2 values of the correlation studies (Table 2) suggested that the phenolic compounds are responsible for strong antioxidant activity of this plant. Therefore, it can be suggested that the use of *W. fruiticosa* in the prevention of peptic ulcer is correlated with the polyphenols and antioxidant activity (Sumbul et al., 2011).

The extracts of *P. roxburghii* were found second in the order of results of antioxidant activities. Traditionally this plant has been claimed to possess antidiabetic activity (Amit et al., 2010). Many recent studies revealed the involvement of free radicals in the pathogenesis of diabetes (Matteucci and Giampietro, 2000). Antioxidants are effectively used for the prevention of experimentally induced diabetes in animal models as well as diabetes complications (Kubish et al., 1997; Lipinski, 2001; Naziroglu and Cay, 2001). According to results of our studies, antiradical and reducing properties of *P. roxburghii* may be suggested to be directly related to the prevention of diabetes. Moreover, antimicrobial activity of *C. bonariensis* has been reported (Chaudhary et al., 2001), but no significant work related to the phytochemistry of this plant is found in the literature. The extracts of *C. bonariensis* showed a weak correlation with the phenolic contents (Table 2). Therefore, significant results of the hexane extract in the FRAP assay (CBH = 449.5 ± 1.3) can be attributed to the presence of some non polar constituent.

Furthermore, the aqueous extracts of *S. chrysanthemoids* are used in the folk medicine as antipyretic and calmative (Qureshi et al., 2007). The results of antioxidant activity of *S. chrysanthemoids* were found to correlate with total phenols (Table 2). However, higher IC_{50} values in the DPPH assay could be due to the slow rate of reaction of the antioxidants contained by the extracts (Table 1). In several studies, it has been demonstrated that cyclooxygenase-2(COX-2) catalysis the synthesis of prostaglandins which mediates fever. The role of antioxidants to control the activity of COX-2 is also well established (Jiang et al., 2000). Therefore, the traditional use of *S. chrysanthemoids* in fever can be attributed to its antioxidant activity.

Conclusion

The results of this study provide some scientific credence to the indigenous uses of the selected medicinal plants evaluated for the treatment of the diseases induced by the overproduction of free radicals such as diabetes, peptic ulcer, inflammation and Alzheimer's disease. The antioxidant activity of the extracts is attributed to the phenolic contents. Consequently, our results suggested that these plant extracts can be utilized as an effective and safe source of natural antioxidants with consequent health benefits. It is proposed that the beneficial effects of these plants in traditional medicine results from their action as antioxidant. Hence, further investigation of the plant extracts that exhibited the highest antioxidant activity need to be carried out, including fractionation, to isolate active constituent and subsequent pharmacological evaluation

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