J. Indian bot. Soc. e-ISSN:2455-7218, ISSN:0019 - 4468 Vol. 101 (4) 2021: 291-301

FREE RADICAL SCAVENGING AND ANTIOXIDANT POTENTIAL OF SOME ETHNOBOTANICALLY IMPORTANT PLANTS FROM HIMACHAL PRADESH (INDIA)

JYOTI SHARMA, [1]ANIL KUMAR THAKUR AND KAMAL JIT SINGH

Department of Botany, Panjab University, Chandigarh-160014, India. [1]Department of Botany, Rajkiya Kanya Mahavidyalaya, Shimla-171001 (H.P.), India
Email: kamal@pu.ac.in

Date of online publication: 31st December 2021
DOI:10.5958/2455-7218.2021.00043.7

Phytochemicals in the medicinal plants are actually responsible for their healing properties in traditional practices. Present study assessed the antioxidative potential and phytochemical constituents in the leaf and stem extracts of *Viburnum cotinifolium* D. Don, *Isodon rugosus* (Wall. ex Benth.) Codd, *Cotoneaster microphyllus* Wall. ex Lindl., and *Lonicera angustifolia* Wall. ex DC. growing in the wild in Himachal Pradesh. Phytochemical screening confirmed the presence of important secondary metabolites such as flavonoids, phenols, tannins, cardiac glycosides, terpenoids, steroids, saponins, and proteins in all the test plants. *C. microphyllus* showed the presence of a highest amount of total phenolic content in both leaf and stem amongst all the plants analyzed. It also exhibited the maximum quantity of total flavonoid and tannins. Extract of all the plants confirmed concentration-dependent DPPH free radical scavenging and reduction of Fe^{3+} to Fe^{2+} in the reducing power assay. This study corroborates vitality of these plants being used consistently in the herbal medicines.

Keywords: *Cotoneaster microphyllus*, *Isodon rugosus*, *Lonicera angustifolia*, *Viburnum cotinifolium,* traditional medicine, Active compound, Himalayas.

Plants have played a very important role in the socio-cultural and spiritual life of Indian communities. Regular use of the plants in medicinal practices is one of the oldest and richest cultural traditions in India (GOI 2000) and other parts of the world. Himachal Pradesh has rich biodiversity of medicinal and other useful plants (Singh and Thakur 2014). Plants like *Viburnum cotinifolium* D. Don, *Isodon rugosus* (Wall. ex Benth.) Codd (Syn. *Rabdosia rugosa* (Wall. ex Benth.) H. Hara, *Plectranthus rugosus* Wall. ex Benth.), *Cotoneaster microphyllus* Wall. ex Lindl., and *Lonicera angustifolia* Wall. ex DC. have been used traditionally in the treatment of various diseases. *C. microphyllus* leaves and fruits are used for the treatment of diarrhoea, cuts, and wounds (Pala et al. 2010), and especially fruits to regulate the menstrual cycle (Singh and Chauhan 2005). Many Viburnum species are known for their immense medicinal value. Leaf extract of *V. cotinifolium* is used as a remedy of menorrhagia, bark of the stem is used for reducing muscular spasms, and cooked berries are used as laxative and blood purifier (Awan *et al.* 2013). Ethnomedicinal use *I. rugosus* to treat diarrhea also, provides a relief from the generalized body pain (Shuaib and Khan 2015). Amongst the common usage, dried leaves of this plant are placed in mouth to cure toothache and leaf powder used orally for the purification of blood, stomachache, and acidity (Akhtar *et al.* 2013). Such medicinal properties of the plants are due to phytochemicals, and various plant organs such as stem, bark, leaves, root, seeds, fruits contain these active compounds, mostly as secondary metabolites (Tiwari *et al.* 2011). A chemical substance responsible for a physiological activity in the human body hold the therapeutic value of these plants. Free radical scavenging phenols, alkaloids, tannins, carbohydrates, terpenoids, steroids, and flavonoids are some of the most essential bioactive constituents of plants (Edeoga *et al.* 2005).

Most biological systems have developed a natural protective mechanisms that can

Received on May 24, 2021 www.indianbotsoc.org Accepted on August 02, 2021

transform reactive types of the free radicals to non-reactive derivatives (Halliwell and Gutteridge 1989). Sometimes, adverse effect of exceptionally high load of free radicals is so severe that natural defense system feels insufficient for their eradication. Free radicals and other reactive oxygen species are the byproducts of numerous metabolic changes that occur inside a human body. The exposure of vital components of a human body such as lipids, proteins and DNA to these harmful free radicals may cause oxidative damage leading to fatal diseases, such as atherosclerosis, early aging, diabetes, cancer, and other life-threatening maladies (Halliwell 1994, Niki 1997, Poulson *et al.* 1998). Undesirable impact of such oxidative substances on living beings can be alleviated with a more frequent use of antioxidants in our diet (Logani and Davies 1980). Presently, many studies have been undertaken for the search of natural phytochemicals to treat chronic diseases. Roughly, two-thirds of the drugs endorsed worldwide are estimated to be plant-based derivatives (Patridge *et al.* 2016). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), synthetic antioxidants, are being used for many years. However, the consistent use of such synthetic antioxidants is restricted because of their carcinogenic nature (Branen 1975, Ito *et al.* 1983). Therefore, a lookout for the natural antioxidants has increased remarkably during the recent times. Diet rich in natural antioxidants inhibits the oxidative process and ultimately stops the formation of free radicals (Hossain *et al.* 2011). Worldwide, approximately 85% of primary health care medications rely on natural sources (Abbasi *et al.* 2010). Recent researches are focused on the plant sources for antioxidants with effective free radical scavenging potential. Lower cost, easy accessibility, and effectiveness enhance their claim as a potential candidate for a remedial use (Khan *et al.* 2009, Mallikarjun *et al.* 2016). Information on the chemical constituent of plant is very valuable, not just for the sake of discovery of therapeutic operators, but also because dissemination of such knowledge could play a vital role in discovering new resources. The present study was carried out to find the anti-oxidative potential, reducing power and phytochemical constituents of some ethnobotanically important plants growing wild in the hills of Himachal Pradesh (India).

MATERIAL AND METHODOLOGY

Plant Collection: Leaves and stem samples of the four plant species *Viburnum cotinifolium* D. Don, *Isodon rugosus* (Wall. ex Benth.) Codd *Cotoneaster microphyllus* Wall. ex Lindl. and *Lonicera angustifolia* Wall. ex DC. (Fig. 1) were collected from high altitude locations like Triund (2842m), Mcleodganj, Boh (2030m) in Dhauladhar range of Himachal Pradesh. Herbarium sheets of these plants were prepared and maintained.

Extract Preparation: Collected plant material was washed under running tap water to remove dust particles. Plants part were cut off into small pieces and air-dried for few days; leaves and stems were powdered separately by using a mechanical blender. Powdered samples were extracted in conical flasks using methanol solvent. Solutes were filtered as slurry after 2 days using Whatman filter paper and filtrate was dried using vacuum rotary evaporator. Collected leaves and stem samples were kept in desiccators for later use. All the measurements were done in triplicate.

Phytochemical Analysis: Presence of various phytochemicals was assayed following the standard method described by Sofowara (1993), Harborne (1973), Audu *et al.* (2007), Obasi *et al.* (2010), and Trease and Evans (1989).

Ferric Chloride Test for Phenols: 0.2 g of plant extract was boiled with 10 ml of distilled water for 5 min, then cooled and filtered. Appearance of dark green colour with the

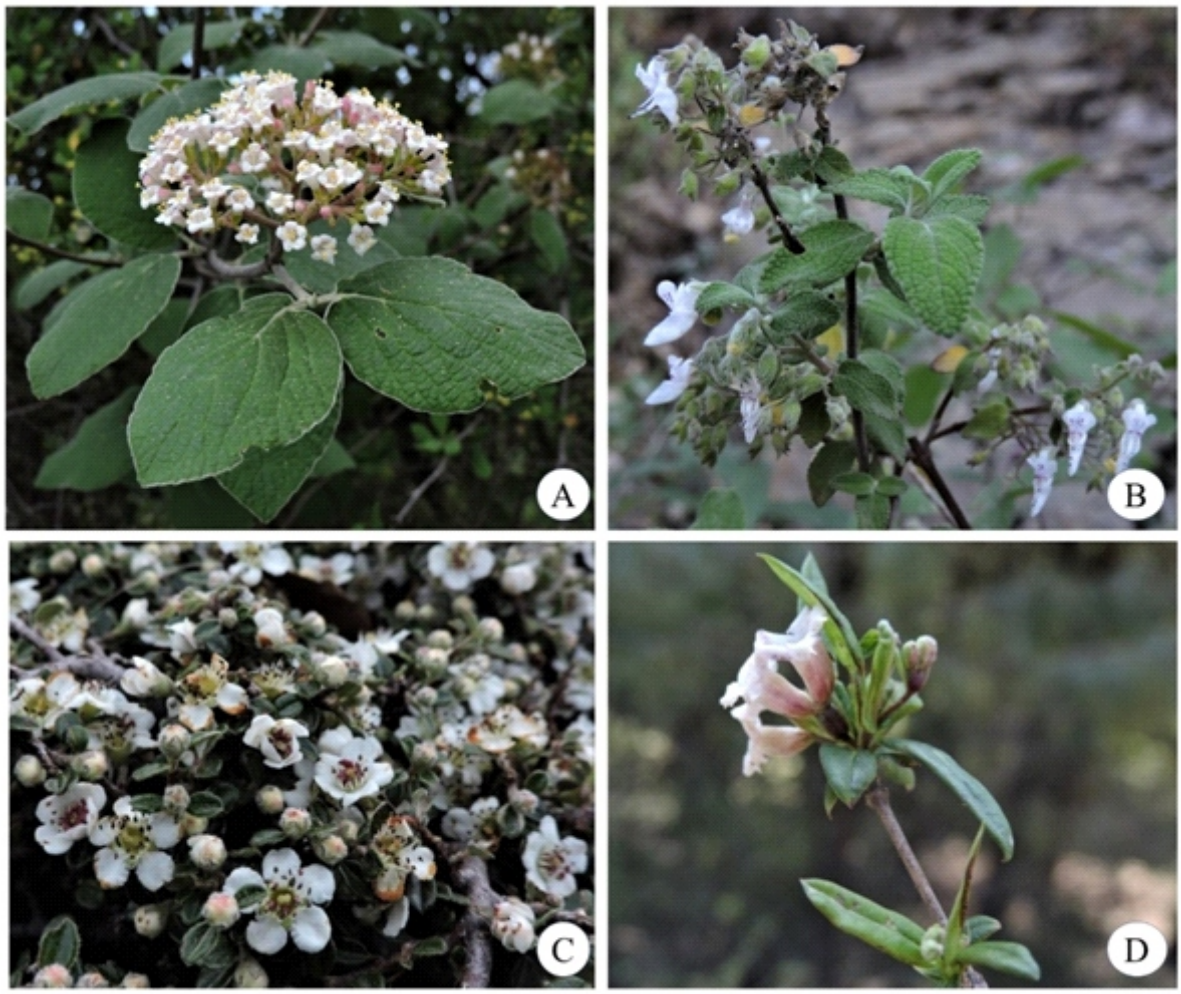

Fig. 1 (A-D): A. *Viburnum cotinifolium*, **B.** *Isodon rugosus,* **C.** *Cotoneaster microphyllus* and **D.** *Lonicera angustifolia*

addition of a few drops of $FeCl_3$ (5%) solution to 1 ml of extract, confirmed the presence of phenol.

Lead Acetate Test for Tannin: 0.2 g of plant extract with 10 ml of distilled water was boiled (5 min), cooled and filtered. To 1 ml of filtrate 10 ml of distilled water and a few drops of 1% lead acetate solution were mixed that allowed the appearance of white precipitates indicating presence of tannins.

Ferric Chloride Test for Tannin: 1 ml of the filtrate was diluted with distilled water followed by addition of 2-3 drops of 10% alcoholic ferric chloride. The formation of transient greenish to black colour indicated the presence of tannin.

Sodium Hydroxide Test for Flavonoid: Few drops of 20% NaOH added to 2 ml filtrate of the plant extract, appearance of a deep yellow coloured solution, followed by mixing of a few drops of 70% HCl, the disappearance of yellow colour confirmed the presence of flavonoid.

Froth Test for Saponins: After mixing 100 mg of the dried extract with 2 ml of distilled water, the solution was shaken thoroughly and the persistence of foam produced for 10 min indicated the presence of saponins.

Keller Killiani Test for Cardiac Glycoside: 1 ml of extract filtrate 0.5 ml of glacial acetic acid mixed with few drops of 1% aqueous $FeCl_3$, 1 ml of concentrated H_2SO_4 was added

from the sides of test tube. Appearance of a purple ring at the interface indicated the presence of cardiac glycosides.

Salkowski Test for Terpenoids: 1 ml of the extract filtrate mixed with 2 ml of chloroform, 3 ml of conc. H_2SO_4 was added from the sides of test tube. Appearance of a layer of reddish-brown colour interface indicated the presence of terpenoids.

Test for Steroids: 0.1 g of filtrate mixed with 1 ml of acetic anhydride and added 1 ml conc. H_2SO_4. Change of colour from violet to blue indicated the presence of steroids.

Biuret Test for Proteins: 2 ml of filtrate treated with 10% NaOH solution and mixed with a few drops of 0.1% $CuSO_4$. Formation of violet colour indicated the presence of proteins.

Determination of DPPH Radical Scavenging Activity: DPPH free radical scavenging activity in plant extract was calculated following the method of Manzocco *et al.* (1998) with little modifications. Different concentrations of samples prepared through serial dilutions were mixed with 0.5 ml of 0.2 mM DPPH prepared in methanol. Reaction mixture was incubated in dark at room temperature for 25-30 min after thorough shaking. Absorbance was measured at 517 nm using spectrophotometer. Ascorbic acid was used as standard. DPPH scavenging activity was calculated as:

% free radical scavenging potential = $[(A_c - A_s) / A_c] \times 100$
A_c = Absorbance of Control
As = Absorbance of sample

Reducing Power Determination: Ability of the plants to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) was calculated using the procedure given by Yen & Chen (1995) with few modifications. Different concentrations (25-800 µg/ml) of samples and standard (ascorbic acid) were prepared in methanol and 1 ml was mixed in the reaction mixture consisting of phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. Reaction mixture was incubated at 50°C for 20 minutes followed by mixing of 2.5 ml of 10% TCA. After vigorous shaking, 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ added to 2.5 ml of the reaction mixture and incubated at room temperature for 5 min. Absorbance was measured at 700 nm using spectrophotometer.

Phenolic Content Determination: Total phenolic content was determined with Folin-Ciocalteu's phenol reagent using the modified method of Singleton & Rossi (1965). Gallic acid (0.01-0.1 mg ml^{-1}) was used as a standard. Plant extract (1 mg/ml) was added to 1 ml Folin-Ciocalteu's reagent (ten-fold dilution) and mixed thoroughly and kept for 5 minutes followed by addition of 1 ml of 2% sodium carbonate. Reaction mixture was incubated at 25°C for 2 hours. Absorbance was measured at 765 nm using spectrophotometer. The results were expressed as mg gallic acid equivalent per g dry weight using formula:

$T = C \times V/m$
T = Total Phenolic Content
V = Volume of the extract (ml) used in the assay
C = Gallic acid equivalent derived from the calibration curve in (mg/ml)
m = Weight of the plant extract (g) used in the assay

Determination of Tannin Content: Tannin content was determined by the method of Mbaebia *et al.* (2012) with some modifications. 0.2 g of sample extract was dissolved in 20 ml of 50% methanol followed by vigorous shaking; mixture was kept in water bath for 1 hour at 80°C. The reaction mixture was filtered and mixed with 20 ml of distilled water, 2.5 ml of Folin-Ciocalteu reagent, and 10 ml of 17% sodium carbonate and allowed to stand for 20 min. Absorbance of bluish-green colour of reaction mixture was measured at 760 nm and expressed mg tannic acid equivalent per g of dry weight using formula:

$T = C \times V/m$
T= Total tannin content
V= Volume of extract (ml)
C= Tannic acid equivalent (mg/ml)
m= Weight of leaf extract (g)

Determination Flavonoid Content: Total

flavonoid content was quantified according to the procedure of Chang *et al.* (2002) with some modifications. Rutin (0.01- 0.1 mg ml^{-1}) was used as a reference standard. To leaf and stem extracts (1mg ml^{-1}), 0.1 ml of aluminium chloride (10%) and 0.1 ml of potassium acetate (1M) were added after mixing 3 ml of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was recorded at 415 nm with spectrophotometer. Distilled water with methanol, 10% aluminium chloride, and potassium acetate was used as a blank. The results were expressed in milligrams of rutin equivalent per gram of dry leaf or stem extract. TFC was determined by using the formula

$T = C \times V/m$
T = Total flavonoid content
V = Volume of extract (ml)
C = Rutin equivalent (mg/ ml)
m = Weight of the leaf extract (g)

Statistical analysis: All estimations were carried out in triplicate and results were expressed as mean ± standard error. Microsoft Excel was used for the determination of regression analysis. The regression equation is used for the calculation of IC_{50} values i.e, the concentration needed for 50% inhibition.

RESULTS

Present study was carried out in four ethnobotanically important medicinal plants from the Dhauladhar range of Himachal Pradesh. Many important metabolites like flavonoid, phenols, tannins, saponins, terpenoids, cardiac glycosides and steroids were confirmed almost in all samples with few exceptions (Table 1). In *Viburnum cotinifolium,* both stem and leaves lacked saponins whereas, terpenoids were noticed in shoots. Leaves of *Isodon rugosus* lacked steroids while, cardiac glycosides were missing in the shoots of *Cotoneaster microphyllus.*

DPPH Radical Scavenging Assay: DPPH assay of the plant extracts (Fig. 2.1 and 2.2) exhibited a concentration-dependent free radical scavenging activity with a reference to their antioxidant nature. Results revealed that samples of all the four plants contain active compounds that donates hydrogen to free radicals to make them non-reactive. It suggested thereby, that leaf and stem extracts could be useful against a pathological damage caused by free radicals, especially at higher concentrations. IC_{50} values of leaf extract (µg/ml) in *V. cotinifolium, C. microphyllus, L. angustifolia* and *I. rugosus,* concentrations that can scavenge 50% of the radical, were 140.96, 129.25, 201.97 and 236.73 respectively. Similarly, these values in stem extracts were 128.234, 187.180, 140.902 and 191.314, respectively.

Reducing Power: The power to reduce ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}) denoting antioxidant potential of the compound was found to be concentration-

Table 1: Phytochemical constituents in the leaves and stems of tested plants.

Name of plant	Part used	Flavonoid	Phenol	Tannin	Saponin	Terpenoids	Cardiac glycoside	Protein	Steroid
Viburnum cotinifoliu m	Leaf	+	+	+	-	-	+	+	+
	Stem	+	+	+	-	+	+	+	+
Cotoneaste r microphyll us	Leaf	+	+	+	+	+	+	+	+
	Stem	+	+	+	+	+	-	+	+
Isodon rugosus	Leaf	+	+	+	+	+	+	+	-
	Stem	+	+	+	+	+	+	+	+
Lonicera angustifolia	Leaf	+	+	+	+	+	+	+	+
	Stem	+	+	+	+	+	+	+	+

Table 2: DPPH Scavenging activity of plant leaf extract.

S.No	Concentration(µg/ml)	*V. cotinifolium*	*L. angustifolia*	*I. rugosus*	*C. microphyllus*
1.	31.25	13.068 ± 3.23	15.01 ± 6.009	12.478 ± 2.430	25.576 ± 0.566
2.	62.50	24.765 ± 2.76	27.307 ± 0.287	22.644 ± 0.182	39.968 ± 0.195
3.	125	46.847 ± 0.786	41.404 ± 2.806	30.058 ± 0.928	49.944 ± 0.996
4.	250	66.811 ± 4.763	48.076 ± 1.626	49.074 ± 1.989	58.555 ± 0.210
5.	500	90.180 ± 0.892	65.120 ± 5.638	62.417 ± 1.918	73.423 ± 0.728
6.	1000	92.418 ± 0.382	87.216 ± 0.534	86.571 ± 0.958	83.473 ± 0.829

Table 3: DPPH Scavenging activity of plant stem extract.

S.No	Concentration(µg/ml)	*V. cotinifolium*	*L. angustifolia*	*I. rugosus*	*C. microphyllus*
1.	31.25	18.313 ± 0.344	15.703 ± 0.8743	7.158 ± 1.117	13.225 ± 1.907
2.	62.5	21.29 ± 1.0861	44.081 ± 0.840	16.204 ± 1.513	26.805 ± 1.646
3.	125	34.773 ± 1.949	53.971 ± 0.450	37.302 ± 3.028	40.544 ± 2.032
4.	250	53.428 ± 3.514	56.516 ± 1.666	55.381 ± 2.783	53.074 ± 1.936
5.	500	91.081 ± 0.104	69.029 ± 3.275	81.068 ± 1.903	72.791 ± 0.478
6.	1000	92.764 ± 0.0645	84.319 ± 2.058	90.589 ± 0.993	86.261 ± 0.526

dependent. It was also noticed that the reducing power of standard ascorbic acid was much higher than in any of tested plant. At 800µg/ml absorbance values of *V. cotinifolium, C. microphyllus, L. angustifolia,* and *I. rugosus*, leaf extracts values were 0.932 ± 0.031, 1.890 ± 0.073, 1.462 ± 0.204, and 1.846 ± 0.037, respectively, and of stem extracts were 1.1043 ± 0.087 , 2.249 ± 0.113, 0.9 ± 0.038, and 2.1073 ± 0.067, respectively (Fig. 3.1 and 3.2).

Total Phenolic Content: It was noticed that leaves of *Cotoneaster microphyllus* showed maximum phenolics (107.103 ± 2.621) in comparison to *L. angustifolia*, *I. rugosus* and *V. cotinifolium* (24.48 ± 0.223, 82.90 ± 6.249 and 41.67 ± 0.087 respectively) while, the same was higher in stem extract of *I. rugosus* (106.189 ± 2.621) than in *C. microphyllus*, *V. cotinifolium,* and *L. angustifolia* (88.937 ± 2.55, 81.989 ± 7.960 and 70.848 ± 5.431 respectively) (Fig. 4).

Total Flavonoid Content: Total flavonoid content of *Cotoneaster microphyllus* leaves was highest (76.29 ± 5.069) followed by *L. angustifolia, I. rugosus,* and *V. cotinifolium* 43.553 ± 1.853, 35.725 ± 4.201 and 5.46 ± 0.413 respectively while, in stem extracts, the decreasing trend noticed in *C. microphyllus I. rugosus, L. angustifolia*, and *V. cotinifolium* 68.468 ± 2.940, 51.413 ± 5.748, 31.717 ± 1.402 and 3.51 ± 2.207 respectively (Fig. 5).

Total Tannin Content: Similarly, tannin content of leaves was highest in *Cotoneaster microphyllus* (27.09 ± 1.194) followed by *I. rugosus*, *L. angustifolia,* and *V. cotinifolium* 22.460 ± 2.833, 21.41 ± 0.171 and 19.209 ± 0.909 respectively and; the descending sequence in stem extract was in *C. microphyllus* (31.962 ± 0.579) followed by *V. cotinifolium, L. angustifolia,* and *I. rugosus* 26.611 ± 1.935, 24.566 ± 1.638 and 20.342 ± 0.099 respectively (Fig. 6).

DISCUSSION

Generally, the usability of medicinal plants is evaluated by correlating their biological activities with phytochemical constituents (Belkacem *et al.* 2013). The screening of plant extracts of *Viburnum cotinifolium* D. Don, *Isodon rugosus* (Wall. ex Benth.) Codd, *Cotoneaster microphyllus* Wall. ex Lindl., and *Lonicera angustifolia* Wall. ex DC. confirmed the presence of some important bioactive chemicals like flavonoids, phenols, tannins, saponins, terpenoids, cardiac glycosides, and proteins in these plants from Himachal Pradesh. Phenols, a key component of our food, primarily act as anti-oxidants, anti-septic and anti-inflammatory agents to eradicate many human ailments (Okwu 2004).

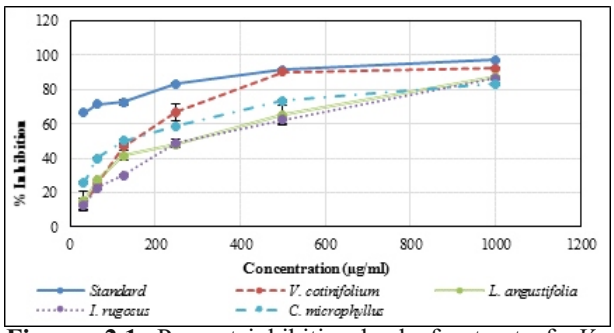

Figure 2.1: Percent inhibition by leaf extract of *V. cotinifolium*, *L. angustifolia*, *I. rugosus,* and *C. microphyllus*

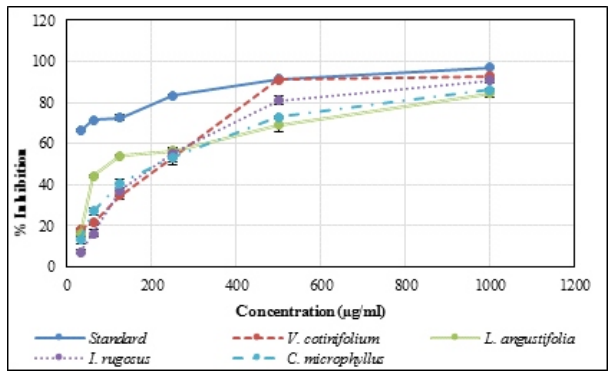

Figure 2.2: Percent inhibition by stem extract of *V. cotinifolium*, *L. angustifolia*, *I. rugosus,* and *C. microphyllus*

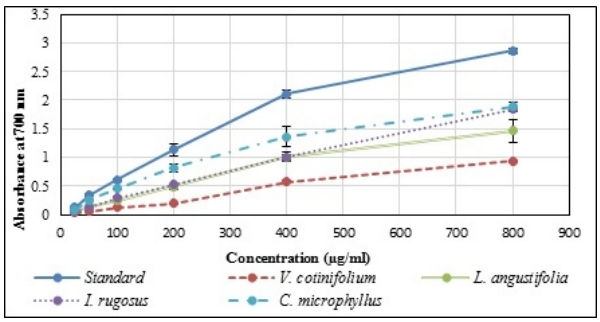

Figure 3.1: Reducing power by leaf extract of *V. cotinifolium*, *L. angustifolia*, *I. rugosus* and *C. microphyllus.*

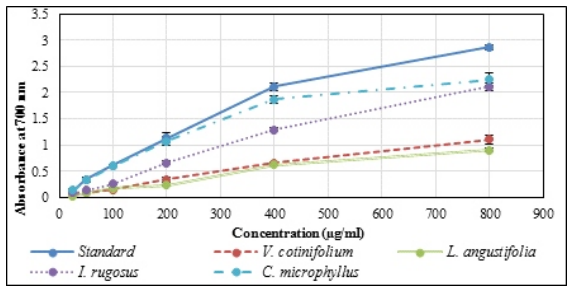

Figure 3.2: Reducing power by stem extract of *V. cotinifolium*, *L. angustifolia*, *I. rugosus* and *C. microphyllus.*

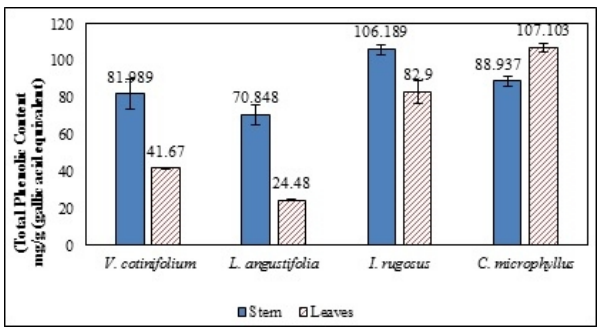

Figure. 4: Total phenolic content in leaf and stem extract of *V. cotinifolium*, *L. angustifolia*, *I. rugosus,* and *C. microphyllus*

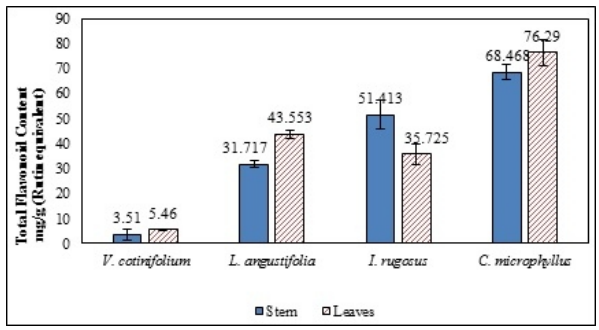

Figure 5: Total flavonoid content in leaf and stem extract of *V. cotinifolium*, *L. angustifolia*, *I. rugosus,* and *C. microphyllus.*

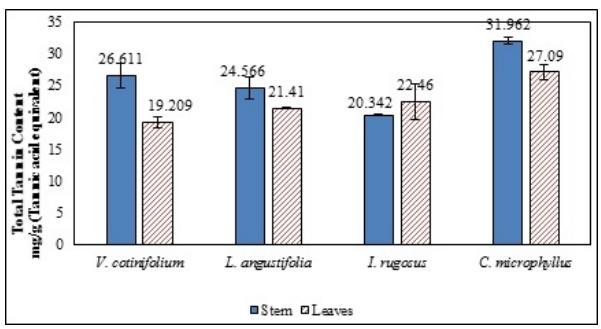

Figure 6: Total tannin content in leaf and stem extract of *V. cotinifolium*, *L. angustifolia*, *I. rugosus,* and *C. microphyllus*.

The present study reports highest content of total phenolic acids in leaves of *Cotoneaster microphyllus* and in stems of *Isodon rugosus*. Flavonoids, a group of polyphenols, are free radical scavengers and effective antioxidants with anti-inflammatory properties that stop cell damage caused by oxidation due to their water-soluble tendency, and also exhibit anti-cancer activity (Salah *et al.* 1995, Gurib-Fakim 2006). We have reported highest flavonoid and total tannin content in the leaves as well as in stems of *Cotoneaster microphyllus* of the four plants investigated in this study. Antioxidant activity of flavonoids, phenolic acid, and tannins is reported to be responsible for the anti-inflammatory, anti-carcinogenic, and anti-atherosclerotic properties of medicinal plants (Li *et al.* 2007). Phytochemicals like saponins have hypotensive and cardio depressant properties (Olaleye 2007). Glycosides are recognized as naturally occurring cardioactive drugs that help in the treatment of congestive heart failure and cardiac arrhythmia (Brian *et al.* 1985). The redox potential of such compounds is responsible for the free radical scavenging which allows them to donate hydrogen and act as reducing agents (Hall and Cuppett 1997).

DPPH is a free radical that is commonly used for testing radical scavenging activity of plant-based phytochemicals, specifically has a centrally located nitrogen in its structure. DPPH changes its colour from violet to yellow when reduced by the electron donation capacity of antioxidant (Brand-williams *et al.* 1995). IC_{50} values obtained from DPPH assay shows that least amount of *C. microphyllus* leaf extract is required to scavenge half of the free radicals amongst all the plant species in this study. Ferric-reducing antioxidant ability of a plant organ determines its potential to reduce Fe^{3+} to Fe^{2+} and is connected with the amount of reductant as free radical scavenger. Reducing power is commonly related to the presence of reductones, responsible for free radical chain termination, they also prevent formation of peroxide exhibiting their antioxidant potential (Matsushige *et al.* 1996). Ferric trichloride reacts with antioxidants forming ferric ferrocyanide complex whose absorbance is measured at a maximum of 700 nm (Guder and Korkmaz 2012). The reducing power assay confirmed that any increase in absorbance corresponds to enhanced phytochemical content and ascorbic acid responsible for reducing the oxidative stress. The observations clearly revealed that these plants can specifically be used as electron donors for termination of the radical chain reactions, specifically stem and leaf extracts of *C. microphyllus* show highest value of absorbance and highest content of total phenolic compounds, total flavonoids and total tannins in this this study. The leaf and stem extracts of all selected plant species exhibited free radical scavenging activity besides having an adequate pool of phenols, tannins, and flavonoids. Based on our results, it can be proposed that flavonoid, phenols and tannin act as free radical scavengers in DPPH assay and contribute to the reducing potential of the selected plant species.

CONCLUSION

Leaf and stem extract of *C. microphyllus, I. rugosus, L. angustifolia,* and *V. cotinifoliu*m contains a good amount of flavonoid, tannins, and phenolic compound that exhibited significant antioxidant and free radical scavenging potential. The ability of polyphenols to donate hydrogen atom from their hydroxyl group qualifies them as a strong antioxidant and free radical scavenger. As such, these plants are a noteworthy resource of antioxidants that may be helpful in preventing diverse type of oxidative stresses. Our findings support the belief that a diet comprising herbaceous plants can reduce oxidation and also defend against many disorders. For pharmaceutical applications and efficacy, research can be expanded towards isolating active components from these plant species.

Financial assistance by the CSIR, New Delhi

for this study is duly acknowledged.

REFERENCES

Abbasi AM, Khan M, Ahmad M, Zafar M, Jahan S and Sultana S 2010 Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North West Frontier Province, Pakistan. *J Ethnopharmacol* **128** 322–335.

Akhtar N, Rashid A, Murad W and Bergmeier E 2013 Diversity and use of ethno-medicinal plants in the region of Swat, North Pakistan. *J Ethnobiol Ethnomed* 9 25-38.

Audu SA, Mohammed I and Kaita HA 2007 Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae). *Life Sci J* **4(4)** 75-79.

Awan Z I, Rehman H, Awan AA and Minhas F A 2013 Ethnobotanical importance of some highly medicinal plants district Muzaffarabad, Pakistan with special reference to the species of the Genus Viburnum. *J Pharm Biol Sci* ***6*** 53-66.

Belkacem N, Djaziri R, Lahfa F, El-Haci I A and Boucherit Z 2013 Phytochemical screening and in vitro antioxidant activity isolated bioactive compounds from Tridax procumbens Linn. *Pak J Biol Sci* 1**6** 1971–1977.

Brand-williams W, Cuvelier M and Berset C 1995 Use of a free radical method to evaluate antioxidant activity. LWT-*Food Sci Technol* **28** 25-30.

Branen A L 1975 Toxicology and biochemistry of butylated hydroxyl anisole and butylated hydroxy toluene. *J Amer Oil Chem Soc* **52** 59-63.

Brian FH, Thomas-Bigger J and Goodman G 1985 The Pharmacological Basis of Therapeutics, 7th edition. Macmillan, New York: NY, USA.

Chang CC, Yang MH, Wen HM and Chern JC 2002 Estimation of total flavonoid content in propolis by complementary colorimetric methods. *J Food Drug Anal* **10** 178-182.

Edeoga HO, Kwa D and Mbaebia BO 2005 Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol 7* 685-688.

Government of India 2002 Report of the Task Force on Conservation and Sustainable use of Medicinal Plants. New Delhi: Planning Commission, Govt. of India.

Guder A and Korkmaz H 2012 Evaluation of in-vitro antioxidant properties of hydroalcoholic solution extracts Urtica dioica L., Malva neglecta Wallr. and their mixture. *Iran J Pharm Res* **11(3)** 913–923.

Gurib-Fakim A 2006 Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol Aspects Med* **27 (1**) 1-93.

Hall C and Cuppett S 1997 Structure-activities of natural antioxidants. *Antioxidant Methodology: In Vivo and in Vitro Concepts,* eds. OI Aruoma, SL Cuppett AOCS Press and Champaign, IL, p. 141-172.

Halliwell B 1994 Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* **344** 721–724.

Halliwell B and Gutteridge J M C 1989 Free Radicals in Biology and Medicine. Clarendon Press: Oxford, U.K.

Harborne JB 1973 Phytochemical Methods. Chapman and Hall Ltd, London. Pp. 49-188.

Hossain MA, Shah MD, Gnanaraj C and Iqbal M 2011 In vitro total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant Tetrastigma from Sabah. *Asian Pac J Trop Med* **4** 717-721.

Ito N, Fukushima S, Hasegawa A, Shibata M and Ogiso T 1983 Carcinogenicity of butylated hydroxy anisole in F344 rats. *J Natl Cancer Inst* **70** 343-347.

Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, Siddiqui M and Khan AU 2009 Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules **14** 586–597.

Li HB, Wong CC and Cheng KW 2007 Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT- *Food Sci Technol* **41** 385-90.

Logani MK and Davies RE 1980 Lipid oxidation: biologic effects and antioxidants- A review. *Lipids* **15** 485-495.

Mallikarjun S, Ashwini R, Rajesh G, Ramya S and Mithun P 2016 Antimicrobial efficacy of Tulsi leaf (Ocimum sanctum) extract on periodontal pathogens: An in vitro study. J Indian Soc Periodontol **20** 145–150.

Manzocco L, Anese M and Nicoli M C 1998 Antioxidant properties of tea extracts as affected by processing. LWT- Food SciTechnol **31** 694-698.

Matsushige K, Basnet P, Hase K, Kadota S, Tanaka K and Namba T 1996 Propolis protects pancreaticells against the toxicity of streptozotocin (STZ). *Phytomed* **3(2)** 203– 209.

Mbaebia BO, Edeoga HO and Afolayan AJ 2012 Phytochemical analysis and antioxidants activities of aqueous stem bark extract of Schotia latifolia Jacq. *Asian Pac J Trop Biomed* **2** 118-24.

Niki E 1997 Free radicals, antioxidants, and cancer. In: Food Factors for Cancer Prevention, eds. Ohigashi H, Osawa T, Terao J, Watanabe S and Yoshikawa T, Springer, Tokyo. Pp. 55–57.

Obasi NL, Egbuonu ACC, Ukoha PO and Ejikeme PM 2010 Comparative phytochemical and antimicrobial screening of some solvent extracts of Samanea saman pods. *Afr J Pure Appl Chem* **4(9)** 206-212.

Okwu DE 2004 Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. J *Sustain Agric Environ* **6** 30-34.

Olaleye MT 2007 Cytotoxicity and antibacterial activity of methanolic extract of Hibiscus sabdariffa. *J Med Plant Res* **1** 9–13.

Pala N A, Negi A K and Todaria N P 2010 Traditional uses of medicinal plants of Pauri Garhwal, Uttarakhand. *Nat Sci* **8(6)** 57-61.

Patridge E, Gareiss P, Kinch MS and Hoyer D 2016 An analysis of FDA approved drugs: Natural products and their derivatives. Drug Discov Today **21(2)** 204-207.

Poulson HE, Prieme H and Loft S 1998 Role of oxidative DNA damage in cancer initiation and promotion. *Eur J Cancer Prev* **7** 9–16.

Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell G and Rice- Evans C 1995 Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch Biochem Biophys* **322(2)** 339-346.

Shuaib M and Khan I 2015 Study of Medicinal Plants of Lower Dir, Timergara, Tehsil alambat, Khyber Paktunkhaw-Pakistan. *Am Eurasian J Agric Environ Sci* **15** 2088–2094.

Singh KJ and Thakur AK 2014 Medicinal Plants of the Shimla Hills, Himachal Pradesh: A Survey. *Int J Herbal Med* **2 (2)** 118-127.

Singh V and Chauhan NS 2005 Traditional practices of herbal medicines in the Lahul valleys, Himachal Himalayas. *Indian J Tradit*

Know **4 (2)** 208-220.

Singleton VL and Rossi JA 1965 Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* **16** 144-158.

Sofowara A 1993 Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. Pp. 191-289.

Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H 2011 Phytochemical screening and extraction: A Review. *Int Pharm Sci* **1** 98-106.

Trease GE and Evans WC 1989 *Pharmacognosy*, 11th edition. Bailliere Tindall, London. Pp. 45-50.

Yen GC and Chen HY 1995 Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem* **43**(1) 27–32.