International Journal of Phytomedicine 9 (2017) 589-596
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Evaluation of free radical scavenging activity of leaf of *Hypericum mysorense* Heyne

Sornalakshmi V[1], Tresina PS[1], Paulpriya K, Mohan VR[1]*

***Corresponding author:**

Mohan VR

[1]PG & Research Department of Plant Biology and Biotechnology V.O. Chidambaram College, Tuticorin – 628 008, Tamil Nadu, India

Received: 19 Jun 2017
Accepted: 10 Oct 2017
Published: 28 Dec 2017

Abstract

Hypericum is a well-known plant genus in herbal medicine. *Hypericum mysorense* is well known in folklore medicine for its varied therapeutic potential. The *in vitro* antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extract of leaf of *Hypericum mysorense* have been tested using various antioxidant model systems viz; DPPH, hydroxyl, superoxide, ABTS and reducing power. The scavenging effect increases with the concentration of standard and samples. Among the solvent tested, ethanol extract exhibited highest DPPH (116.33% inhibition), hydroxyl (101.84%), superoxide (116.09%) and ABTS (119.22%) radical scavenger activity. Methanol extract of *H. mysorense* shows the highest reducing ability. The aim and purpose of this study is to show the significant free radical scavenging potential of *H. mysorense* leaf which can be experimented for the treatment of various free radical mediated ailments.

Keywords: *Hypericum mysorense*, DPPH, free radical, reducing power.

Introduction

The genus *Hypericum* is a large genus of herbs or shrubs with more than 450 species distributed worldwide. The plants grow widely in temperate regions and are used in folklore medicine in many parts of the world. It has attracted much attention in investigation of metabolites, many of which are biologically active compounds with phloroglucinol moiety [1]. It has been reported that it contains some antiviral prenylated phloroglucinol derivatives [2], and variety of compounds such as flavonoids [3], xanthones [4], chromenyl ketones [5], hyperforins derivatives [6], n-alkanes [7], napthodianthrones [8]and essential oil [9]. The phytochemistry of *Hypericum* has attracted the attention of scientists mainly for the two marker compounds, hypericin and hyperforin due to their potent biological activities.

Hypericum mysorense belongs to the family Hypericaceae. *Hypericum mysorense* is a plant native of the Nilgiri Hills in India. It is closely related to *Hypericum perforatum*. *Hypericum mysorense* is mentioned in Ayurvedic texts because it possess anti-viral and nerve calming properties. *Hypericum mysorense* has been used to treat wounds as a part of the Ayurvedic system of traditional medicine [10]. Some research has been organised to check the antiherpetic properties in *Hypericum mysorense* extracts [11][12].

All living organisms contain complex systems of antioxidant enzymes. Some of these systems, like the thioredoxin system, are conserved throughout evolution and are required for life. Antioxidants in biological systems have multiple functions, including defending against oxidative damage and participating in the major signaling pathways of cells. One major action of antioxidants in cells is to prevent the damage caused by the action of reactive oxygen species.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated in our body are quite reactive and harmful to the cells. If generated ROS and RNS are not scavenged, they can damage important molecules, such as proteins, DNA and lipids, which lead to the development of a variety of diseases, including aging, mutagenesis, carcinogenesis, coronary heart disease, diabetes and neurodegeneration [13][14][15].

Reactive oxygen species include hydrogen peroxide, the superoxide anion, and free radicals, such as the hydroxyl radical. These molecules are unstable and highly reactive, and can damage cells by chain reactions, such as lipid peroxidation or formation of DNA adducts that could cause cancer promoting mutations or cell death. In order to reduce or prevent this damage, all cells invariably contain antioxidants. The natural antioxidants may have free radical scavengers, reducing agents, potential complexes of peroxidant metals, quenchers of singlet oxygen etc [16]. The antioxidants can interfere with the oxidation process by reacting with free radicals [17]. Recently interest has been increased considerably in finding natural occurring antioxidants that are used in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [18]. Antioxidants principles from natural

DOI:10.5138/09750185.2137

resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correlating imbalance [19]. Hence, the present study aims to examine the antioxidant capacity of leaf extracts of *H. mysorense* for their *in vivo* antioxidant activity.

Materials and methods

Collection of plant material

Hypericum mysorense Heyne was collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, and Tamil Nadu. The plant samples were identified with the help of local flora and authenticated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, and India. A voucher specimen of collected plants was deposited in the Ethnopharmacological Unit, PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi District, and Tamil Nadu.

Chemicals

All the chemicals and reagents used in the experiments were of analytical grade and were obtained from BDH (England and India), E. Merck (Germany), Sigma Chemical Company (U.S.A.), Sarabhai, M. Chemicals (India) and LOBA–Chemie Indo Austranol Co., (India). Whenever necessary, the solvents were redistilled before use.

Preparation of plant extract

H. mysorense leaf was cut into small pieces, washed and dried at room temperature; the dried leaf was powdered in a Wiley mill. The coarse leaf powder (100g) of *H. mysorense* was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filter paper. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for estimation of total phenolic and flavonoid.

Estimation of total phenolic content

Total phenolic contents were estimated using Folin-Ciocalteau reagent based assay as previously described [20] with little modification. To 1mL of each extract (100µg/mL) in methanol, 5mL of Folin-Ciocalteau reagent (dluted ten-fold) and 4mL (75g/L) of Na_2CO_3 were added. The mixture was allowed to stand at 20^{0}C for 30min and the absorbance of the developed colour was recorded at 765nm using UV-VIS spectrophotometer. 1mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of flavonoids

The flavonoids content was determined according to Eom *et al* . [21] An aliquot of 0.5ml of sample (1mg/mL) was mixed with 0.1mL of 10% aluminium chloride and 0.1mL of potassium acetate (1M). In this mixture, 4.3ml of 80% methanol was added to make 5mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H.

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method [22]. Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts at different concentration (50,100,200,400 & 800µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the average of the results were calculated.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell [23]. Stock solutions of EDTA (1mM), $FeCl_3$ (10 mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM) were prepared in distilled deionized water.

The assay was performed by adding 0.1mL EDTA , 0.01mL of $FeCl_3$, 0.1mL H_2O_2, 0.36mL of deoxyribose, 1.0mL of the extract of

different concentration (50,100,200,400 &800µg/mL) dissolved in distilled water,0.33mL of phosphate buffer (50mM , pH 7.9), 0.1mL of ascorbic acid in sequence . The mixture was then incubated at 37^0C for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al.* [24]. The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, P^H 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 mL NADH (0.936mM) solution, 1.0 mL extract of different concentration (50,100,200,400 & 800µg/mL), and 0.5 mL Tris – HCl buffer (16mM, P^H 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula

Antioxidant activity by radical cation (ABTS +)

ABTS assay was based on the slightly modified method of Huang *et al.* [25]. ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Reducing power

The reducing power of the extract was determined by the method of Kumar and Hemalatha [26]. 1.0 mL of solution containing 50,100,200,400 &800µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH6.6) and potassium ferricyanide (5.0 mL, 1.0%): The mixture was incubated at 50°C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was repeated thrice and the average of the results were calculated.

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

Results and Discussion

Total phenolic and total flavonoid contents

The total phenolic and total flavonoid contents of the methanol extract of *H. mysorense leaf* was found to be 0.98g $100g^{-1}$ and 1.02g $100g^{-1}$ respectively.

Phenolic compounds are considered as the most important antioxidative components of herbs and other plant materials, and good correlation between the concentration of plant phenolics and the total antioxidant capacity has been reported [27]. The polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants [28] and their epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent [29].

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. Among those substances, the flavonoids widely distributed in plants have the ability to scavenge free radicals, superoxide and hydroxyl radicals by single-electron transfer [30]. Depending on their structure, flavonoids are able to scavenge practically all known Reactive Oxygen Species. According to our present investigation, the high contents of phenols and flavonoids in *H. mysorense* can explain its high radical scavenging activity.

DPPH radical scavenging activity

DPPH have been used extensively as a free radical to evaluate reducing substance. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability [31]. A freshly prepared DPPH solution exhibits a deep purple colour with absorption maxima at 517nm. This purple colour generally fades or disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals and convert them to a colourless product, resulting in a decrease in absorbance at 517nm. DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, and methanol and ethanol extracts of leaf of *H. mysorense* is shown in Figure 1. The scavenging effect increases with the concentration of standard

and samples. Among the solvent tested, ethanol extract exhibited highest DPPH radical scavenging activity at 800 µg/ml concentration (116.33% inhibition) followed by methanol extract (109.28% inhibition). The lowest inhibition was shown by petroleum ether extract (76.16%). IC_{50} values of ethanol extract of leaf of *H. mysorese* and standard ascorbic acid were 25.67µg/ml and 20.11µg/ml respectively (Table 1). Chandrashekar *et al.* [32] evaluated antioxidant activity of *H.perforatum, H.japonicum,* and *H. patulum*. Extract of *H. mysorense* was found to scavenge DPPH radicals more efficiently than other *Hypericum* species.

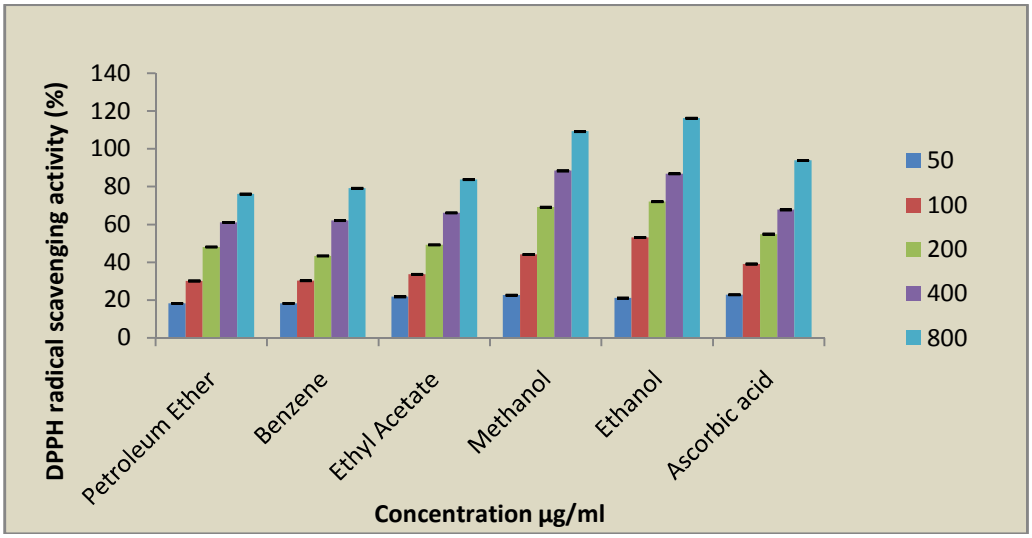

Figure 1. DPPH radical scavenging activity of different solvent extracts of leaf of *H. mysorense*

Table 1. IC_{50} value of different solvent extract of whole plant of *H. leschenaultiana* (µg/ml)

Different solvent extract	DPPH assay	Hydroxyl assay	Superoxide assay	ABTS assay
Petroleum ether	18.75	19.89	22.05	20.06
Benzene	19.38	17.23	20.53	20.98
Ethylacetate	19.84	19.05	20.97	21.34
Methanol	24.22	22.11	24.15	22.53
Ethanol	25.67	23.16	25.88	24.98
Standard (Ascorbic acid)	20.11	19.46	22.18	-
Standard (Trolox)	-	-	-	20.67

Hydroxyl radical scavenging activity

Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals (such as Fe^{2+}) and H_2O_2, which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate cell damage *in vivo* [33]. These oxygen radicals induce some oxidative damage to biomolecules like carbohydrates, proteins, lipids [34][35] and nucleic acids, and this damage causes aging, cancer, and several diseases [36][37][38]. The cell damaging action of hydroxyl radical is well known, as it is the strongest among free radicals [39]. Thus, removing OH^- is very important for the protection of living systems.

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *H. mysorense* leaf is shown in Figure 2. Of these, the ethanol extract (101.84±0.078% inhibition) was found to be more effective in quenching the hydroxyl radicals produced in the reaction mixture. The lowest inhibition was shown by benzene extract (68.39±0.093). IC_{50} values of ethanol extract of leaf of *H. mysorese* and standard ascorbic acid were 23.16µg/ml and 19.46µg/ml respectively (Table 1). All the leaf extracts of *H. mysorense* scavenge hydroxyl radicals in a concentration dependent manner. The scavenging of the hydroxyl radicals may be due to the presence of phenolic compounds in the extracts [40].

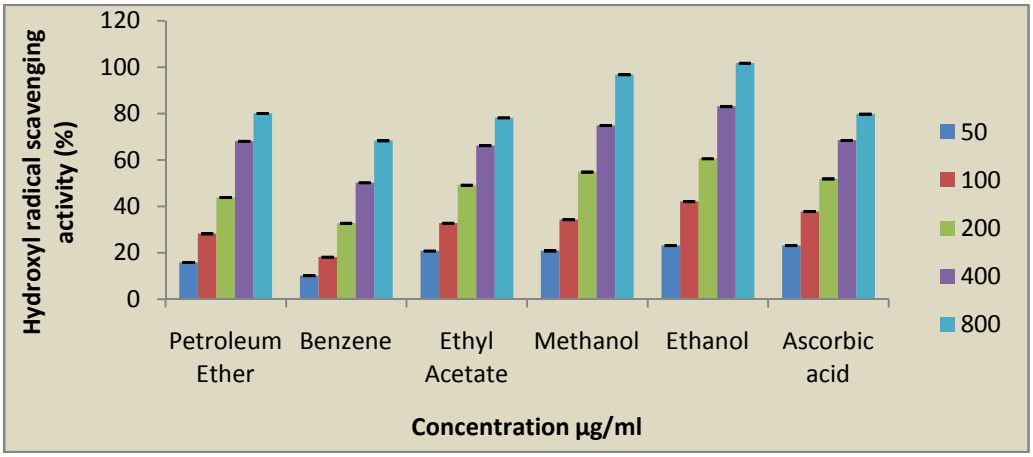

Figure 2. Hydroxyl radical scavenging activity of different solvent extracts of leaf of *H. mysorense*

Superoxide radical scavenging activity

Superoxide anion is a weak oxidant produced during various biological reactions is highly toxic [41]. Superoxide anion radical is known as an initial radical and plays an important role in the formation of other reactive oxygen-species, such as hydrogen peroxide or singlet oxygen. Superoxide is generated in vivo by several oxidative enzymes, including xanthine oxidase. A decrease in absorbance indicated the antioxidant activity of the extracts which may be due to the inactivation or consumption of superoxide anion radicals produced in the reaction mixture [40].

Superoxide anion radical scavenging activity of leaf extracts of *H. mysorense* was denoted in Figure.3. It indicates that, ethanol extract of *H. mysorense* exhibited the maximum superoxide radical scavenging activity of 116.09% which is higher than the standard ascorbic acid whose scavenging effect is 93.51%. IC_{50} values of ethanol extract of leaf of *H. mysorense* and standard ascorbic acid are 25.88µg/ml and 22.18µg/ml respectively (Table 1).

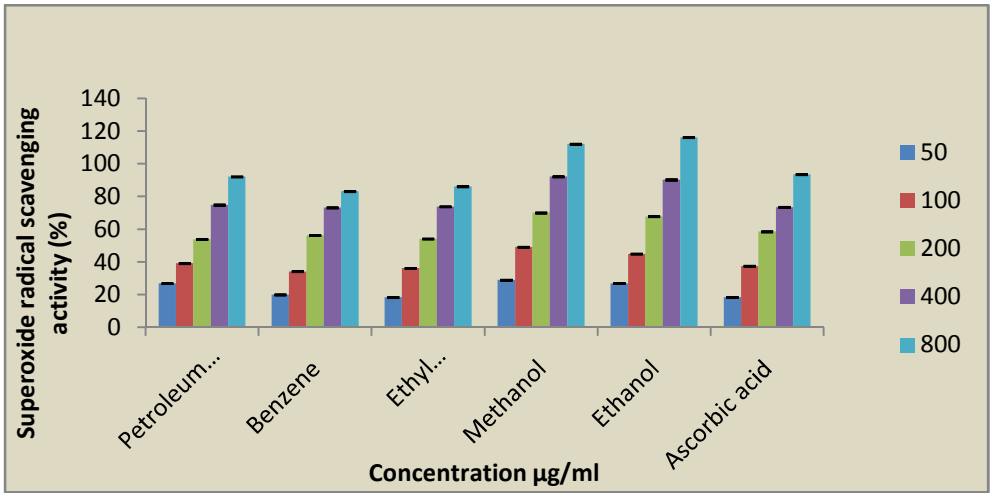

Figure 3. Superoxide radical scavenging activity of different solvent extracts of leaf of *H.mysorense*

ABTS radical cation scavenging activity

Actually, the ABTS radical cation scavenging activity also reflects hydrogen-donating ability. Hagerman *et al.* [42] reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals (ABTS). *H. mysorense* extracts were subjected to ABTS radical cation scavenging activity and the results were shown in Figure 4. The ethanol extract exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800 µg/ml concentration, ethanol extract of *H. mysorense* possessed 119.22 % scavenging activity on ABTS which is higher than the standard trolox whose scavenging activity is 79.16%. IC_{50} values of ethanol extract of *H. mysorense* and standard trolox were 24.98µg/ml and 20.67µg/ml respectively (Table 1).

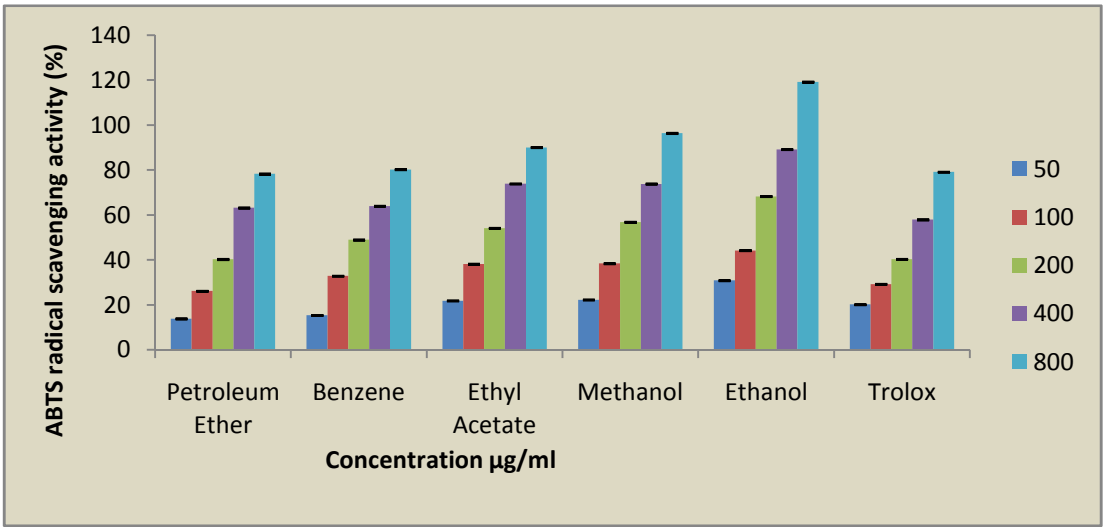

Figure 4. ABTS radical cation scavenging activity of different solvent extracts of leaf of *H.mysorense*

Reducing power

Several reports indicated that the reducing power of bioactive compounds were associated with antioxidant activity [43]. Therefore, it is necessary to determine the reducing power of phenolic constituents contained in the plant extracts to elucidate the relationship between their antioxidant effect and their reducing power. Figure 5 shows the reducing ability of different solvent extracts of leaf of *H. mysorense* when compared to ascorbic acid. It is seen that, when the concentration of the solution is increased, the absorbance of the solution also gets increased. A higher absorbance indicates a higher reducing power. Among the solvent tested, it is evident that methanol extract exhibited higher reducing activity but it is lesser than the standard ascorbic acid.

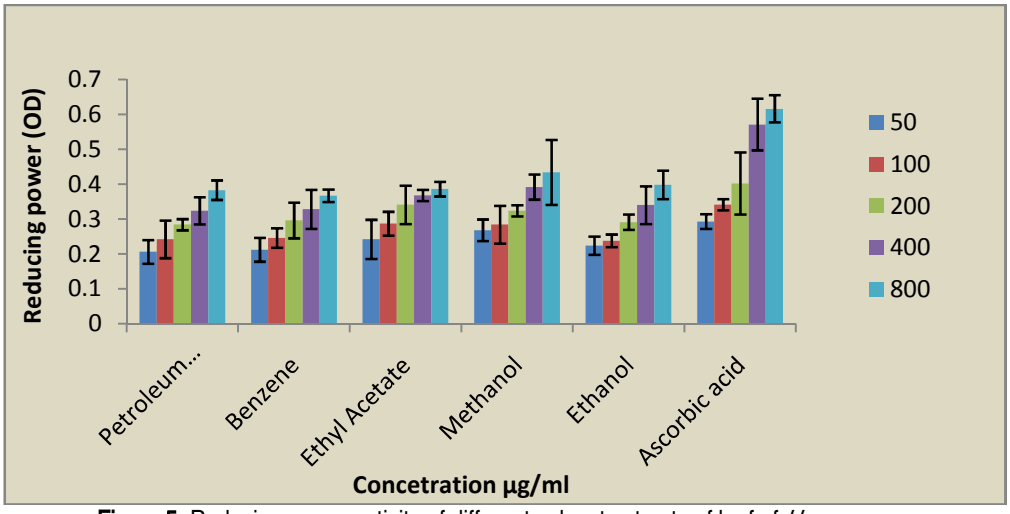

Figure 5. Reducing power activity of different solvent extracts of leaf of *H.mysorense*

Conclusion

The present study observed a marked radical scavenging effect of leaf extracts of *H. mysorense* was observed in the present study. The observed bioactivities of extracts could be ascribed to the presence of secondary metabolites present in the extracts in particular phenolic compounds. The plant appears to be promising resource for bioactive agents which can be exploited for the prevention and treatment of oxidative stress diseases. However, further investigation is required for the purification and characterization of the active metabolites which may lead to the discovery of novel bioactive molecules of industrial and pharmaceutical importance.

References

[1]. Decosterd LA, Hoffmann E, Kyburz R, Bray D, Hostettmann K. A New Phloroglucinol Derivative from *Hypericum calycinum* with Antifungal and *in vitro* Antimalarial Activity. Planta med. 1991; 7: 548-551.

[2]. Tada M, Chiba K, Yamada H, Maruyama H. Phloroglucinol derivatives as competitive inhibitors against thromboxane A2 and leukotriene D4 from *Hypericum erectum.* Phytochem. 1991; 30: 2559-2562.

[3]. Chung MI, Lai MH, Yen MH, Wu RR, Lin CN. Phenolics from *Hypericum geminiflorum.* Phytochem. 1997; 44: 943-947.

[4]. Wu QL, Wang SP, Du LJ, Zhang SM, Yang JS, Xiao PG. Chromone glycosides and flavonoids from *Hypericum japonicum*. Phytochem. 1998a; 49:1417-1420.

[5]. Wu QL, Wang SP, Du LJ, Yang JS, Xiao, PG. Xanthones from *Hypericum japonicum* and *H.henryi*. Phytochem. 1998b; 49: 1395-1402.

[6]. Decosterd LA, Stoeckli-Evans H, Chapuis JC, Msonthi JD, Sordat B, Hostettmann K. New Hyperforin Derivatives from *Hypericum revolutum* VAHL with Growth-Inhibitory Activity against a Human Colon Carcinoma Cell Line. Helv Chim Acta. 1989; 72: 464-471.

[7]. Brondz I, Greibrokk J, Aasen AJ. *n*-Alkanes of *hypericum perforatum*: a revision. Phytochem.1983; 2: 295-296.

[8]. Kitanov GM. Hypericin and pseudohypericin in some *Hypericum* species. Biochem Syst Ecol. 2001; 29: 171-178.

[9]. Cakir A, Duru ME, Harmandar M, Ciriminna R, Passannanti S, Piozzi F. Comparison of the volatile oils of *Hypericum scabrum* L. and *Hypericum perforatum* L. from Turkey. Flavour Frag J. 1997; 12: 285-287.

[10]. Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P J. Ethnopharmacological approaches to wound healing--exploring medicinal plants of India Ethnopharmacol. 2007; 114:103-113.

[11]. Vijayan P, Raghu C, Ashok G, Dhanaraj SA, Suresh B. Antiviral activity of medicinal plants of Nilgris. Indian J. Med. Res. 2004; 120: 24-29.

[12]. Khan MTH, Ather A, Thompson KD, Gambari R. Extract and molecules from medicinal plants against herpes simplex viruses. Antiviral Res. 2005; 67(2):107-119.

[13]. Ben-Yoseph O, Boxer PA, Ross BD. Assessment of the role of the glutathione and pentose phosphate pathways in the protection of primary cerebrocortical cultures from oxidative stress. J. Neurochem. 1996; 66: 2329–2337.

[14]. Harman D. Free radical theory of aging: Origin of life, evolution, and aging. Age 1980; 3:100–102.

[15]. Moskovitz J, Yim KA, Choke PB. Free radicals and disease. Arch. Biochem. Biophys. 2002; 397:354–359.

[16]. Ebadi M. pharmacodynamic basis of herbal medicines. CRC press. Boca Raton. 2002; 86.

[17]. Gupta M, Mazumdu UK, Gomathi P, Kumar RS. Antioxidant and free radical scavenging activity of *Ervatamia coronaria* Stapf leaves. Iranian J. Pharm. Res. 2004; 2:119.

[18]. Kumaran A, Karunakaran JK. In vivo antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT Food Sci. Technol. 2007; 40:344.

[19]. Shirwaikar A, Kuppusamy R, Punitha ISR. In vivo antioxidant studies on the Benzyl tetra isoquinoline alkaloid Berberins. Biol. Pharm. Bull. 2006; 29:1906.

[20]. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001; 73:73-84.

[21]. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu C, Cho DH. Far infra red ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries, Korean J. Med. Crop Sci. 2007; 15:319-323.

[22]. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity *in vitro* of selenium-contained protein from the se-enriched. *Bifodobacterium animalis* 01. Anaerobe. 2010; 16:380-386.

[23]. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. Anal. Biochem. 1987; 65:215-219.

[24]. Srinivasan R, Chandrasekar MJN, Nanjan MJ. Suresh B. Antioxidant activity of *Caesalpinia digyna* root. J. Ethnopharmacol. 2007; 113:284-291.

[25]. Huang MH, Huang SS, Wang BS, Sheu MJ, Hou WC. Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo* and *in vivo*. J. Ethnopharmacol. 2011; 133:743-750.

[26]. Kumar RS, Hemalatha S, *In vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites, J. Chem. Pharm. Res. 2011; 3:259-267.

[27]. Pellegrini N, Simonetti P, Gardana C, Brenna O, Brighenti F, Pietta P. Polyphenol content and Total antioxidant activity of *Vini Novelli* (Young Red Wines). J Agric Food Chem. 2000; 48: 732-735.

[28]. Brown JE, Rice-Evans CA. Luteolin-rich artichoke extract protects low density lipoprotein from oxidation *In vitro.* Free Rad Res. 1998; 29: 247-255.

[29]. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence. Lancet. 1994; 344: 721-724.

[30]. Chang WC, Sei CK, Soon SH, Bong KC, Hye JA, Min YL, Sang HP, Soo KK. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 2002; 163: 1161-1168.

[31]. Wang H, Gao XD, Zhou GC, Cai L, Yao WB. *In vitro* and *in vivo* antioxidant activity of aqueous extract from Choerospondias axillaris fruit. Food Chem 2008; 106: 888-895.
[32]. Chandrashekhar RH, Venkatesh P, Arumugam M, Vijayan P. Estimation of total phenols with special emphasis to antioxidant potentials of few *Hypericum* species. Pharmacologyonline. 2009; 1: 680-687.
[33]. Duan X, Wu G, Jiang Y. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules. 2007; 12:759–771.
[34]. Lai CS, Piette LH. Hydroxyl radical production involved in lipid peroxidation of rat liver microsomes. Biochem Biophys Res Commun. 1977;78:51-59.
[35]. Kellog EW, Fridvoch I. Superoxide, hydrogen peroxide and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J. Bioi. Chem. 1975; 250:8812-8817.
[36]. Suematsu T, Kamada T, Abe H, Kikuchi S, Yagi K. Serum lipoperoxide level in patients suffering from liver diseases. 1977. Clin.Chim. Acta, 1977; 79:267-270.
[37]. Meerson FZ, Kagan VE, Kozlov Yu P, Belkina LM, Arkhipenko Yu V. Role of lipid peroxidation in pathogenesis of ischemic damage and antioxidant protection of the heart. Basic Res. Cardiol. 1982; 77:465-485.
[38]. Fong KL, McCay PB, Poyer JL, Keele PB, Misra H. Evidence that peroxidation of lysosomal membranes is initiated by hydroxyl free radicals produced during enzyme activity. J. Bioi. Chem. 1973; 248:7792-7797.
[39]. Koppenaol WH, Liebman JF. The oxidizing nature of the hydroxyl radical. A comparison with the ferryl ion (FeO2+). J. Phys. Chem. 1984; 88:99–101.
[40]. Vadivukkarasi S, Pavithra K. Evaluation of free radical scavenging activity of various leaf extracts from *Kedrostis foetidissima* (Jacq.) Cogn.Biochem Anal Biochem. 2014; 3:2.
[41]. Stief TW. The physiology and pharmacology of singlet oxygen. Med Hypotheses. 2003; 60:567-572.
[42]. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 1998; 46:1887–1892.
[43]. Siddhuraju P, Mohan PS, Becker K, Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves flowers and fruit pulp. Food Chem. 2002; 79:61-70.