Research Article

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Phytochemical Screening and In Vitro Antioxidant Activities of Ethnomedicinal Plant *Hydrocotyle javanica* Thunb. (Apiaceae)

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ABSTRACT

A therapeutic strategy to elevate the antioxidant defenses of the body in view of the oxidative stress implicated in the pathophysiology of a wide variety of different diseases may attenuate and protect us from diseases and disorders. Biological systems can be balanced by antioxidants that would suppress the formation of active oxygen species (ROS) brought about by reducing hydroperoxides (ROO•) and H_2O_2, and scavenging free radicals among others. An equilibrium between free radical generation and antioxidant supply is thus important. The ethnomedicinal plant H.javanica was evaluated for its radical scavenging and antioxidant activities. The whole plant was extracted in five different solvents and assessed by five different assay methods. The methanol extract evinced higher antioxidant activities in all except DPPH assay with IC_{50} values of 38.26 in SOD, 38.16 in ABTS and 37.92 in hydroxyl radical scavenging assays. The petroleum ether extract had the highest activity in DPPH assay (IC_{50} 24.13). These activities could be attributed to the presence of coumarins, flavonoids, phenols and tannins.

Keywords: Antioxidants; Ethnomedicinal; DPPH; Methanol; SOD.

1. INTRODUCTION

Antioxidants are molecules (or ions, or stable radicals) that retard or prevent oxidation of other molecules. An upsurge in the unique and novel approach for safe and natural antioxidants reflects hope to alleviate 'oxidative stress' related diseases thereby to elevate human health (Halliwell and Gutteridge, 2007; Niki, 2011). Generation of reactive oxygen and nitrogen species (ROS and RNS,respectively) along with high levels of oxidation products, particularly hydroperoxides and DNA fragments manifest due to an imbalance between pro-oxidative and anti-oxidative factors (Sies, 1986; Dotan *et al.* 2004*)*. The various pathophysiological conditions such as neurodegenerative diseases, diabetes mellitus, atherosclerosis, different malignant diseases and virus infections, including AIDS have been associated with oxidative stress. Halliwell and Gutteridge (2007) neuronal and behavioural changes, even in the absence of degenerative diseases have been deciphered to occur with aging. The lower status of dietary antioxidants have been associated with decline in the cognitive function (Satish and Dilipkumar, 2015).

Plants have been explored as a potent and safe antioxidant source due to its rich bioactive phytoconstituents. The role of medicinal plants in augmenting relief from oxidative stress in cancer, aging, inflammation, neurodegenerative diseases, diabetes, and heavy metal toxicity have been emphasized from time and again. Traditional knowledge of these medicinal plants in treatment of various ailments deliberates the exploitation in the drug industry. But a structured and evaluated protocol for every specific plant would be the need of the hour. This forms the basis of the present study in evaluating the antioxidant activities of the ethnomedicinal plant *Hydrocotyle javanica* plant extracts in different solvents subjected to different assay models.

H. javanica Thunb. from the family apiaceae is commonly called as 'java pennywort'. It is a procumbent herb growing in moist shaded places at high altitudinal regions in Nilgiris district, Tamilnadu, India. Traditionally, this plant has been used as an aperient, tonic, stimulant, diuretic, blood purifier and applied as a paste in skin diseases (Khaling Mikawirawng *et al.* 2014). The juice of the whole plant when applied on the chest is said to to cure asthma and fits among the kurichia tribes (Prasad and Shyma, 2013).

2. MATERIALS & METHODS

2.1 Plant Collection and Identification

H. javanica was collected from the Kodanad region of Nilgiris district in Tamilnadu and identified by Dr. M. Murugesan, Scientist B, BSI, Shillong. A voucher specimen (KASC/H/1862) has been deposited at the

herbarium maintained by Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

2.2 Extraction of Plant Material

The washed and air dried whole plant of *H. javanica* were powdered in a pulverizer and about 250 g of this powder were subjected to Soxhlet hot extraction using 750 mL of the series of solvents in the order of polarity - petroleum ether, acetone, ethylacetate, ethanol and methanol. Each of the extracts was reduced to dry residue and was stored in aseptic conditions in the refrigerator for further studies.

2.3 Phytochemical Studies

The whole plant extracts in each of the solvents were investigated qualitatively for the presence of phytoconstituents following the standard methods of Harborne (1998) and Wagner *et al*. (1996).

2.4 In Vitro Antioxidant Assays

2.4.1 DPPH* Radial Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant assay was performed by the method of Blios (1958). A methanol solution of the sample extract at various concentrations (100-1600 µg/dL) were added to 5 ml of 0.1 mM methanolic solution of DPPH* and allowed to stand for 20 min at 27 ºC. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula

% DPPH radical scavenging activity = (Control OD-sample /Control OD) x 100 (1)

2.4.2 Hydroxyl Radical Scavenging Activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell (2000). Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM), deoxyribose (10 mM), were prepared in distilled deionized water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml H_2O_2, 0.36 ml of deoxyribose, 1.0 ml of the extract of different concentration (100, 200, 400, 800, 1600 µg /dL) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, PH-7.4), 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 hr. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA 1.0 ml of 0.5% TBA (in 0.025 M NaoH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using eq. (1).

2.4.3 Antioxidant Activity by Radical Cation ($ABTS^{\cdot+}$)

$ABTS^{\cdot+}$(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium per sulphate in the dark for 12-16 hr at room temperature. Prior to assay, this solution was diluted in ethanol and equilibrated at 30 ºC to give an absorbance of 0.70 ±0.02 at 734 nm. The stock solution of the sample extracts were diluted such that after introduction of 10µl aliquots into the assay, they produced between 20-80 % inhibitions of the blank absorbance. After the addition of 1ml of diluted $ABTS^{\cdot+}$ solution to 10µl of sample or Ascorbic acid standards in ethanol, absorbance was measured at 734 nm, at 30 ºC exactly 30min after the initial mixing (Re *et al*. 1999). Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated.

2.4.4 Super Oxide Radical Scavenging Activity (SOD)

SOD (Super oxide dismutase) radical scavenging activity was measured by the reduction of Nitro Blue Tetrazolium NBT (Beauchamp and Fridovich, 1999). The non-enzymatic phenazinemethosulfate - nicotinamide adenine dinucleotide (PMS / NADH) system generates superoxide radicals which reduce NBT to a purple formazan. The one ml reaction mixture contained phosphate buffer (20 mM pH 7.4), NADH (7µM), NBT (50µM), PMS (15µM) in various concentrations (100-1600 µg /dL) of sample solution. At ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of Formazan generated. All tests were performed in triplicates. Ascorbic acid was used as a standard.

2.4.5 Reducing Power of Copper Ions (CUPRAC)

The cupric reducing antioxidant capacity (CUPRAC) of the extracts of *H.javanica* was determined according to the method of Apak *et al*. (2004). 7.5 mmol of neocuprine and NH_4Ac buffer (1 M, pH 7.0) solutions were added to a test tube containing 1mL each of 10 mM Cu(II). Extracts were added to the initial mixture so as to make the final volume of 4.1 mL. The tubes were stoppered and the absorbance at 450 nm was recorded against a reagent blank after 30 min.

2.5 Statistical Analysis

Data are reported as the mean ± S.E.M. of three measurements. Statistical analysis was performed by the student t-test and by ANOVA. IC_{50} (50% antioxidant activity) values for all the above experiments were determined by the linear regression method. A *p*-value less than 0.05 was considered as indicative of significance.

3. RESULTS & DISCUSSION

The preliminary qualitative phytochemical analysis evinced the presence of Coumarins, Flavonoids, Phenols, Tannins, terpenoids and saponins. The phytochemicals such as alkaloids were present in moderate amounts whereas glycosides, steroids and phytosteols were present in trace amounts in the methanol extract of *H.javanica*. (Table 1).

Plants contain polyphenols, flavonoids and phenolic compoundsthat act asfree radical scavengers and natural antioxidants in inhibiting or preventing the deleterious consequences of oxidative stress (Tripathi *et al.* 2016). The capacity of an antioxidant is measured by Spectrophotometric ET-based assays as a degree of colour change (either an increase or decrease of absorbance at a given wavelength) in correlation to the concentration of antioxidants in the sample. The antioxidants in the DPPH assay donate hydrogen to reduce the stable purple coloured radical DPPH to the yellow-coloured non-radical diphenyl-picryl hydrazine (DPPH-H). A single-electron transfer process causes the bleaching of a preformed solution of the blue-green radical cation $ABTS^{-\cdot}$ that can be used to measure the antioxidant capacities of complex mixtures and individual compounds. Hydroxyl radical (·OH) is extremely reactive, more toxic than other radical species and attacks biologic molecules such as DNA, proteins and lipids. ·OH is widely believed to be generated from the Fe^{2-} (or Cu^{-}) / H_2O_2 Fenton reaction system, by simply incubating $FeSO_4$ and H_2O_2 in aqueous solution. Thus, ·OH scavenging activity of antioxidants can be accomplished through direct scavenging or preventing of ·OH formation. The scavenging ability of antioxidants can be determined by Gutteridge method, which is monitored in the Fe^{3-}–EDTA–H_2O_2–deoxyribose system (Gutteridge, 1987; Jian-Ming Lu *et al.* 2010).

Table 1. Preliminary qualitative phytochemical analysis of *H. javanica* extracts

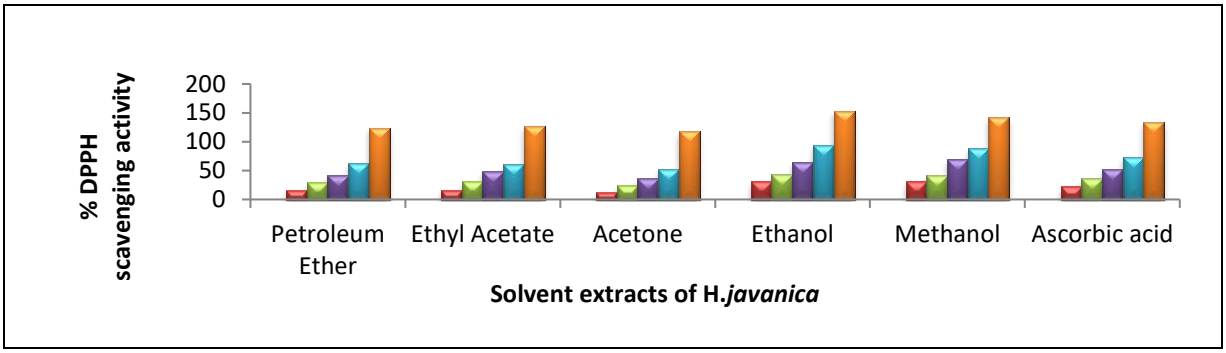
Phytochemicals	Tests	Petroleum ether	Acetone	Ethyl acetate	Ethanol	Methanol
Alkaloids	Mayer's, Wagner's	-	-	+	-	+
Coumarins	Fluorescence test	++	+	-	++	+++
Cardiac glycosides	Keller-kiliani	++	-	-	-	-
Flavonoids	Shinoda, Ferric chloride	++	++	++	+++	+++ +++
Glycosides	Liebermann	-	-	-	+	+
Phenols	Ferric Chloride	+	++	++	+++	+++
Terpenoids	Salkowski	+	-	++	+	+++
Tannins	Braemer's	-	-	-	++	+++
Saponins	Foam	++	-	-	++	+++
Steroids & Phytosterols	Lieberman-Burchard	++	+	++	-	+

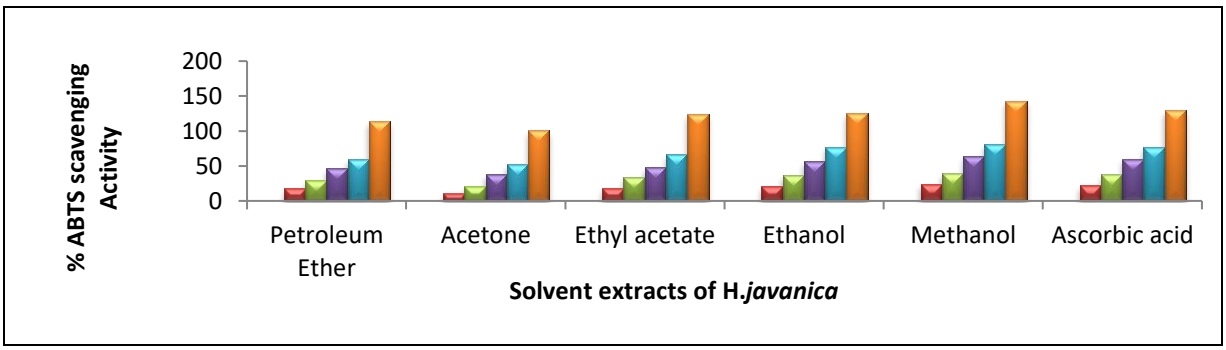
(+ indicates present, ++ indicates moderately present, +++ highly present, - indicates absence)

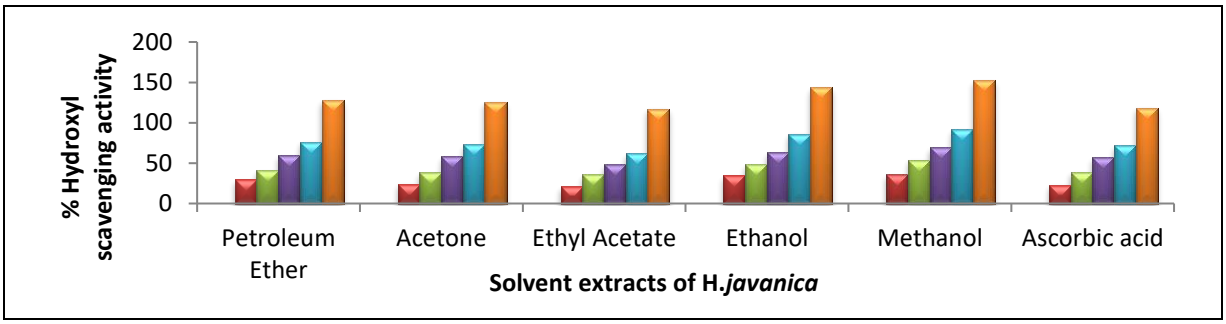
Table 2. IC 50 values of various extracts of *H. javanica* in different antioxidant assay

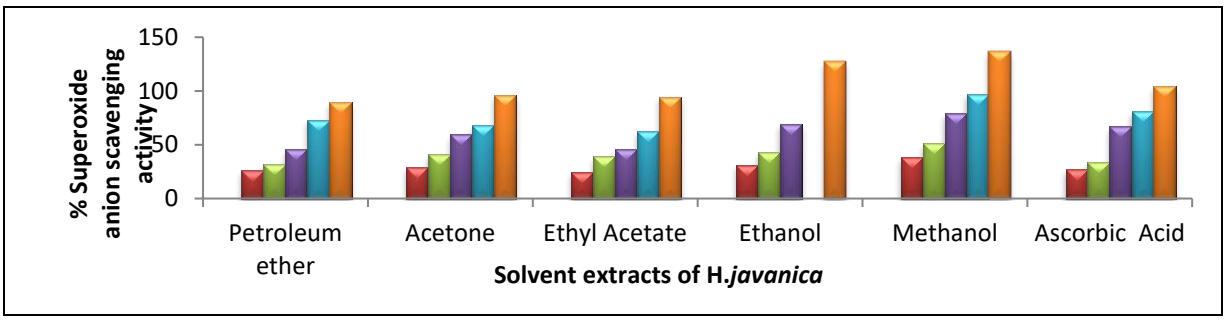
Radical scavenging assay	Ethanol	Methanol	Petroleum ether	Acetone	Ethyl acetate	Standard Ascorbic acid
DPPH	39.11	31.15	24.13	27.13	29.84	31.16
ABTS	36.81	38.16	30.16	31.65	27.88	29.84
SOD	32.92	38.26	22.16	25.93	25.16	28.16
Hydroxyl	31.38	37.92	29.47	23.28	30.36	32.11

Each value is expressed as percentage of activity mean ± standard deviation (n=3)









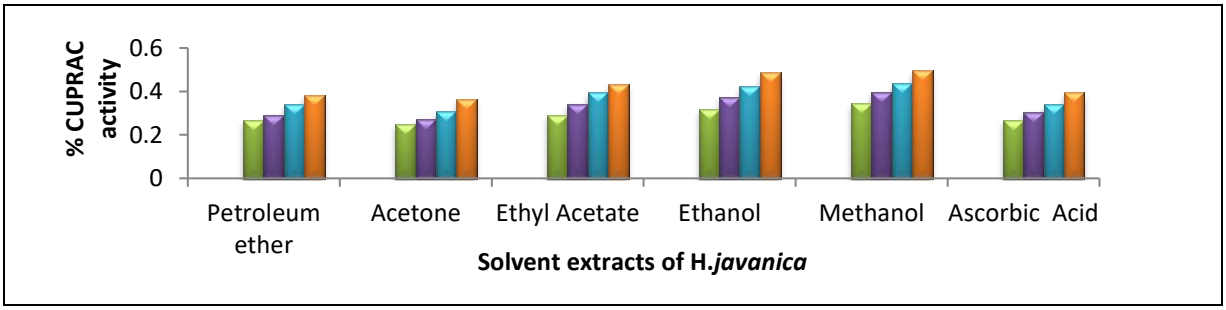


Fig. 1: Antioxidant activity at different concentrations of *H. javanica* (Concentrations- 100 ,200, 400, 800, 1600µg/dL)

Oxidative stress is known to be aggravated by transition metals such as copper and iron (Cu^{-} & Fe^{2-}) when present in higher amounts. Moreover, these metal chelators and antioxidants have been demonstrated to be favourable in the treatment of neurodegenerative diseases in earlier studies such as Alzheimer's disease.Fe^{2-} and Cu^{-} are oxidized to Fe^{3-} and Cu^{2-}, respectively.

The dismutation reaction of O_2 by SOD results in the production of H_2O_2, to which these metals react to produce highly reactive $^{\cdot}OH$. NADH, a cellular reductant and oxidized metal ions ($Fe^{3-}$and Cu^{2-}), in the presence of reducing agents such as ascorbic acid ($AscH^{-}$) get reduced so as to enable recycling to react with another molecule of H_2O_2 to generate ·OH radical. Hydroxyl radical is highly reactive wherein the large absorption bands of the complex in the UV-vis range give a direct evidence of antioxidant-metal coordination (Battin *et al*. 2006). The SODs remove the two toxic reactants superoxide radical and hydrogen peroxide with the help of catalase and ultimately converted to oxygen and water.

Copper reduction assay (CUPRAC), recently introduced as a variant of FRAP assay is based on the reduction of Cu(II) to Cu(I) by the combined action of all antioxidants (reducing agents) in a sample (Mustafa *et al*. 2011). Copper, in the form of free and phenanthro line complexes, has a lower redox potential than iron, so its reactions are more selective that enhances redox cycling. Thus copper reduction may be an even more sensitive indicator of potential pro-oxidant activity with faster kinetics.

In this present study higher radical scavenging activities of *H.javanica* was by the petroleum ether extract in DPPH assay, methanol extract in ABTS, SOD and hydroxyl assays. In the DPPH assay the methanol extract had an IC_{50} value of 31.15 which was comparable to that of the standard ascorbic acid (31.16). The acetone and ethyl acetate extracts had moderate and intermediate radical scavenging activities in all the assays when compared to other solvent extracts. In all the assays there was a concentration dependent antioxidant activity by all the *H.javanica* solvent extracts (fig.1). The antioxidant activities might offer some lead clues that would explain the pathologic mechanism of abnormal free radical metabolism.

The data obtained in this study evince notable antioxidant activities exhibited by different solvent extracts of the ethnomedicinal plant *H.javanica* in various assays. On the whole methanol extract of *H.javanica* proved to be a capable free radical scavenger. This was well supported by the presence of phytochemicals such as coumarins, flavonoids, phenols and tannins. Flavonoids are secondary plant phenolics with powerful antioxidant properties (Pieta, 2000; Yao *et al.* 2004). They act as scavengers of superoxide and hydroxyl radicals by terminating the chain radical reaction and result in the formation of flavonoids radical that react with free radicals (Ferreira *et al.* 2010). They have been reported to inhibit the generation of ROS and quench them once they are formed (Agati *et al.* 2012). Flavonoids such as quercetin, rutin and kaempferol identified from *Centella asiatica* and *Hydrocotyle sibthorpioides* were shown to be responsible for theantioxidant activities in the DPPH assay (Maulidiani *et al.* 2014). The methanol extract of dry seeds of few apiaceae plants (*Foeniculum vulgare, Anethum graveolens, Coriandrum sativum, Carum carvi*) have been reported to have high phenolic and flavonoid content with high antioxidant activities (Bagdassarian *et al*. 2014). The inquisitiveness to probe the importance and role of non-nutrient compounds, particularly phenolic acids, flavonoids and high molecular tannins as natural antioxidants has greatly increased (Sahoo *et al*. 2013). A linear relationship between the antioxidant activities and phenolic compounds in the methanolic extracts of *Halenia elliptica* have been reported (Huang *et al.* 2010). The methanol extracts of aerial parts and roots of *Eryngium palmate* (Apiaceae) exerted moderate DPPH radical scavenging activity and hydroxyl radical scavenging activity as correlated to the HPLC determination of flavonoids apigenin and apigenin 7-O-glucoside (Marcetic *et al*. 2014). Coumarins are heterocyclic compounds with antioxidant properties (Guilherme *et al*. 2013). Laboratory synthesized coumarins have been shown to have *in vitro* antioxidant activities in the DPPH and hydrogen peroxide radical scavenging methods against the standard ascorbic acid (Abdul *et al*. 2011; Patel Rajesh and Patel Natvar 2011; Shivani *et al*. 2013). In a comparative study of the antioxidant capacities of *Heracleum persicum* Desf., *Prangos ferulacea* (L.) Lindl., *Chaerophyllumm acropodum* Boiss. species from apiaceae family by DPPH radical scavenging, and lipid peroxidation inhibition it was evaluated that *C. macropodum* and *H. persicum* were less effective antioxidants than *P. ferulacea* due to their respective phenolics content (Oruh, 2007).

4. CONCLUSION

The selected plant *H.javanica* is an ethnomedicinal herb with powerful antioxidant activities that may aid in various biological and pharmacological functions.The methanol extract evinced remarkable antioxidant and radical scavenging activities. Hence it can be proposed as a natural and potent antioxidant.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Abdul Amir H Kadhum, Ahmed, A., Al-Amiery, Ahmed Y. Musa and Abu Bakar Mohamad, The antioxidant activity of new coumarin derivatives, *Int. J. Mol. Sci*., 12, 5747-61(2011). https://doi.org/10.3390/ijms12095747

Agati, G., Azzarello, S., Pollastri, S. and Tattini, M., Flavonoids as antioxidants in plants: location and functional significance, *Plantscience*, 196, 67-76(2012). https://doi.org/10.1016/j.plantsci.2012.07.014

Apak, R., Guclu, K., Ozyurek, M. and Karademir, S. E., A novel total antioxidant capacity index for dietary polyphenols, vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method, *J. Agric Food Chem.*, 52, 7970-7981(2004). https://doi.org/10.1021/jf048741x

Battin, E. E., Perron, N. R. and Brumaghim, J. L., The central role of metal coordination in selenium antioxidant activity, *Inorg. Chem.*, 45, 499-501(2006). https://doi.org/10.1021/ic051594f

Beauchamp, C. and Fridovich, I., Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels, *Anal. Biochem. Rev*., 44, 276-87(1971). https://doi.org/10.1016/0003-2697(71)90370-8

Blios, M. S., Antioxidant determinations by the use of a stable free radical, *Nature*, 29, 1199-1200(1958). https://doi.org/10.1038/1811199a0

Dotan, Y., Lichtenberg, D. and Pinchuk, I., Lipid peroxidation cannot be used as a universal criterion of oxidative stress, *Prog. Lipid Res*., 43(3), 200-227(2004). https://doi.org/10.1016/j.plipres.2003.10.001

Ferreira, J. F. S., Luthria, D. L., Sasaki, T. and Heyerick, A., Flavonoids from *Artemisia annua*as antioxidants and their potential synergism with Artemisinin against malaria and cancer, Molecules, 15(5), 3135-3170(2010). https://doi.org/10.3390/molecules15053135

Guilherme Borges Bubols, Damiana da Rocha Vianna, Alexander Medina R, Gilsane von Poser, Rosa Maria Lamuela-Raventos, Vera Lucia Fifler-Lima and Solange Cristina Garcia, The antioxidant activity of coumarins and flavonoids, *Mini. Rev. Med. Chem.*, 13(3), 318-334(2013). https://doi.org/10.2174/1389557511313030002

Gutteridge, J. M., Ferrous-salt-promoted damage to deoxyribose and benzoate, the increased effectiveness of hydroxyl-radical scavengers in the presence of EDTA, *Biochem. J.*, 243(3), 709-714 (1987).

Halliwell, B. and Gutteridge, J., Free Radicals in Biology and Medicine, 4th ed. New York; Oxford University Press(2007).

Halliwell, B., The antioxidant paradox. *Lancet*, 355, 1179-1180(2000). https://doi.org/10.1016/S0140-6736(00)02075-4

Harborne, J. B., Phytochemical methods: A guide to modern techniques of plant analysis, 3rd ed., London, UK; Chapman and Hall(1998).

Huang, B., Ban, X., He, J. S., Zeng, H., Zhang, P. and Wang, Y. W., Hepatoprotective and antioxidant effects of the methanolic extract from *Haleniaelliptica*, *Journal of Ethnopharmacology*, 131(2), 276-281(2010). https://doi.org/10.1016/j.jep.2010.06.029

Jian-Ming Lu, Peter, H. Lin, Qizhi Yao and Changyi Chen, Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems, *J. Cell. Mol. Med.*, 14(4), 840-60(2010).

Khaling Mikawirawng, Suresh Kumar and Vandana, Current scenario of urolithiasis and the use of medicinal plants as antiurolithiatic agents in Manipur (Northeast India): A review, *Int. J. Herbal. Med.*, (1), 1-12(2014).

Marcetic, M. D., Petrovic, S. D., Milenkovic, M. T., Antioxidant potential of two Apiaceae plant extracts: A comparative study focused on the phenolic composition, *Cent. Eur. J. Biol*., 9, 149 (2014). https://doi.org/10.2478/s11535-013-0247-0.

Maulidiani, Faridah Abas, AlfiKhatib, Khozirah Shaari, Nordin H. Lajis, Chemical characterization and antioxidant activity of three medicinal Apiaceae species, *Industrial Crops and Products*, 55, 238-247(2014). https://doi.org/10.1016/j.indcrop.2014.02.013

Mustafa E. Ozyeurek, Kubilay GeucLeu, EsmaTeutem, Kevser Seozgen Basxkan, A comprehensive review of CUPRAC methodology, *Ana. methods.*, (2011). https://doi.org/10.1039/c1ay05320e

Niki, E., Do free radicals play causal role in atherosclerosis? Low density lipoprotein oxidation and vitamin E revisited, *J. Clin. Biochem. Nutri.*, 48, 3-7(2011). https://doi.org/10.3164/jcbn.11-007FR

Oruh, N. C., Sagdıcoglu Celep, A. G., Ozgokce, F., Antioxidant properties of *Prangosferulacea* (L.) Lindl., *Chaerophyllummacropodum* Boiss. And *Heracleum persicum* Desf. from Apiaceae family used as food in Eastern Anatolia and their inhibitory effects on glutathione-S-transferase, *Food Chem.*, 100(3), 1237-1242(2007). https://doi.org/10.1016/j.foodchem.2005.12.006

Patel Rajesh, M. and Patel Natvar, J., *In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods, *J. Adv. Pharm. Edu. Res.*, 1, 52-68(2011).

Pieta, P. G., Flavonoids as antioxidants, *J. Nat. prod.*, 63, 1035-1042(2000). https://doi.org/10.1021/np9904509

Prasad, A. G. and Shyma, T. B., Medicinal plants used by the tribes of VythiriTaluk, Wayanad district (Kerala state) for the treatment of human and domestic animal ailments, *J. Med. Plants Res.*, 7(20), 1439-1451(2013). https://doi.org/10.5897/JMPR12.841

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Biol. Med.*, 26(9-10), 1231-37(1999).

Sahoo, H. B., Bhattaisra, S. K., Biswas, U. K. and Sagar, R., Estimation of total phenolics and flavonoidal contents as well as in vitro antioxidant potential of *Apiumleptophyllum* Pers. *HerbaPolonica*, 59(3), 37-50 (2013). https://doi.org/10.2478/hepo-2013-0015

Satish Balasaheb Nimse and Dilipkumar Pal, Free radicals, natural antioxidants and their reaction mechanisms, *RSC Adv*., 35(5), 27986-28006(2015). https://doi.org/10.1039/C4RA13315C

Shivani Choudhary, Suvarna G. Kini and Muhammad Mubeen, Antioxidant activity of novel coumarin substituted benzothiazole derivatives, *Der Pharma Chemica.*, 5(4), 213-222(2013).

Sies, H., Biochemistry of Oxidative Stress, *Angewandte Chemie*, 25(12), 1058-1071(1986). https://doi.org/10.1002/anie.198610581

Tripathi, I. P., Vandana Pathak, Pushpendra Kumar Shukla and Ruchi Dwivedi, Antioxidant, antidiabetic potential and quantification of lupeoll in methanolic extract of Anethum sowaLinn. (seed), *Int. J. Curr. Res. Chem. Pharm. Sci.*, 3(3), 29-36(2016).

Valentina Lubomirova Christova - Bagdassarian, Kristine Samvel Bagdassarian, Maria Stefanova Atanassova and Ayaz Ahmad, M., Comparative analysis of total phenolic and total Flavonoid contents, rutin, tannins and antioxidant capacity in Apiaceae and Lamiaceae families, *Indian Horti. J.* 4(3/4), 131-140(2014).

Wagner, H., Bladt, X. S., Gain, Z. and Suie, E. M., Plant drug analysis, Germany: Springer Verlag(1996).

Yao, L. H., Jiang, Y. M., Shi, J., Tomas-Barberan, F. A., Datta, N., Singanusong, R. and Chen, S. S., Flavonoids in food and their health benefits, Plant Foods for Human Nutrition, 59(3), 113-122(2004). https://doi.org/10.1007/s11130-004-0049-7