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ANTIOXIDANT ACTIVITY OF *SCHLEICHERA OLEOSA*

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KEYWORDS

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ABSTRACT

The present study was undertaken to determine total antioxidant activity and the concentration of total phenolics and flavanoids present in aquatic and methanolic leaf extracts of *Schleichera oleosa*. The extracts showed a maximum concentration of total phenolics and flavanoids up to 20.0% and 168.89 % respectively in methanolic extract and 4.90% and 9.33% respectively in aqueous extract. The total antioxidant activity of these extracts has been determined by estimation of DPPH radical, hydroxyl radical, superoxide anion radical and nitric oxide radical scavenging activity. From our observations, it was confirmed that the Methanolic and aqueous extracts of *Schleichera oleosa* showed a significant antioxidant activity which reduces the DPPH radical to 54.94% and 5.92%, hydroxyl radical to 35.83% and 8.19%, superoxide anion radical to 22.58% and 3.23% and Nitric oxide radical up to an extent of 33.81% and 31.43% respectively for a concentration of 100µg/ml of the extract. Also increase in concentration decreases the radical formation.

INTRODUCTION

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules get damaged. Free radicals such as superoxide (O₂), hydroxyl (OH) and peroxy (OOH, ROO) are generated in the body in several biochemical reactions that cause oxidative changes (Peiyuan, *et al.*, 2010). These free radicals accelerate many degenerative and pathological processes of various serious diseases (Burns, *et al.*, 2001) like cancer, coronary heart diseases, Alzheimer's disease etc., (Smith, *et al.*, 1996). There are antioxidants produced within the body to scavenge these free radicals' but these endogenous antioxidants are not adequate to remove them fully and to maintain a balance. Therefore antioxidants should be taken in from outside as dietary supplies to counteract surplus free radicals. (Lim, *et al.*, 2007, Scalbert, *et al.*, 2005). Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are effective in their role as antioxidants, and are commercially obtainable and currently used to counteract the free radicals. However, they are suspected to have carcinogenic actions and other side effects, hence their use in food, cosmetic and pharmaceutical products have been decreasing (Namiki, 1990, Scalbert *et al.*, 2005, Namiki, 1990, Pliteo, *et al.*, 2007, Tepe, *et al.*, 2005, Ku, *et al.*, 2007, Gulcin *et al.*, 2003).

Plants are rich source of antioxidants which is made obvious through many reports on medicinal plants with antioxidant potential. (Atawodi, 2005, Katalinic, *et al.*, 2006). An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may therefore have health-promoting effects in the prevention of degenerative diseases (Shahidi, 1997). It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). Thus, there has been an increase of interest in naturally occurring antioxidants from vegetables, fruits, leaves, oilseeds, cereal crops, tree barks, roots, spices and herbs (Gámez-Meza, *et al.*, 2009).

Schleichera is a genus of plants in the soapberry family, Sapindaceae and is generally found in Indian subcontinent and in mixed deciduous forests and in Southeast Asia. It is used in traditional medicine systems and its leaf, seed, oil and bark are used for curing itch, burns, acne pain in the back and loins etc., and it promotes hair growth, treats rheumatism, head ache, skin diseases, malarial fever and is prophylactic against cholera (Palanuvej, Vipunngun, 2008]. Scientific study of free radical scavenging capacity, antioxidant activity and phenolic and flavonoid content of the leaf extracts of *Schleichera oleosa* have not been done.

The present study was undertaken to determine the antiradical and total antioxidant activities of the leaf extracts of *Schleichera oleosa* in four in vitro models, including DPPH, Hydroxyl radical scavenging assay, super oxide anion radical scavenging assay and nitric oxide radical scavenging assay. The total phenolic and flavonoid contents (TP, FT) were studied and the relationship between the TP and FT content and antioxidant activities were also investigated.

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MATERIALS AND METHODS

Collection of plant material

The fresh mature leaves of the plant were collected, dried in shade under room temperature for six to seven days and then crushed into coarse powder using electric grinder. The powder was sieved to get fine powder using fine plastic sieve which was stored in air tight bottle in the laboratory until required.

Extract preparation

50g of the powder was subjected to extraction by soxhlet using methanol and distilled water separately. The extracts obtained were filtered, concentrated after dryness in rotary flash evaporator maintained at 45°C., percentage yield of each extract was calculated and the dried extracts were stored in airtight containers at room temperature for further studies.

Phytochemical analyses

Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses to find the presence of the following phyto constituents such as flavanoids, alkaloids, carbohydrates, glycosides, polysaccharides, tannins, saponins, steroids, proteins, lipids, oils by standard methods. [Trease and Evans, 2002; Sofowara, 2008].

Estimation of total phenolics

Known amount of sample were pipetted out in series of test tubes and volume was made up to 3 ml with distilled water. Folin-Ciocalteu reagent (0.5ml) was added to each tube and incubated for 3 min. at room temperature Sodium carbonate (20%; 2ml) solution was added, mixed thoroughly and the tubes were incubated for 1 min. in boiling water bath. Absorbance was measured at 650nm against a reagent blank. Standard curve using different concentrations of standard phenolic -catechol was prepared. From the standard curve, concentration of phenols in the test samples was determined and expressed as mg of catechol equivalent. (Malick, and Singh, 1980). The values are furnished in Fig.1.

Estimation of flavonoids

Known volume of samples was pipetted out in series of test tubes and volume was made up to 0.5 ml with distilled water. Sodium nitrite (5%; 0.03ml) was added to each tube and incubated for 5 min. at room temperature Aluminium chloride solution (10%; 0.06ml) solution was added and incubated

for 5 min. at room temperature. Sodium Hydroxide solution (1 M; 0.2ml) solution was added and total volume was made up to 1 ml with distilled water. Absorbance was measured at 510nm against a reagent blank. Standard curve using different concentrations of rutin was prepared. From the standard curve, concentration of flavonoids in the test samples was determined and expressed as mg of rutin equivalent. (Helmja, et al., 2007) The values are furnished in Fig.1.

Estimation of free radical scavenging activity

Different concentrations (10 μ g, 50 μ g and 100 μ g) of samples in Dimethyl sulfoxide (DMSO), were taken in a series of test tubes. The volume was adjusted to 500 μ l by adding Methanol. Five milliliters of a 0.1 mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH; from Sigma -Aldrich, Bangalore) was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at RT for 20 min. The absorbance of the samples was measured at 517 nm. Butylated Hydroxy Anisole (BHA) was used as reference standard. (Kumar S, et al., 2008). Free Radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = (\text{control OD} - \text{sample OD}) \times 100.$$

control OD

Estimation of hydroxyl Radical Scavenging Activity

Various concentrations (10 μ g, 50 μ g, and 100 μ g) of samples in DMSO were taken in different test tubes and made up to 250 μ l with 0.1M phosphate buffer. One milliliter of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of Dimethyl sulphoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. These reaction mixtures were incubated at room temperature for 15 min. The reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (150 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent

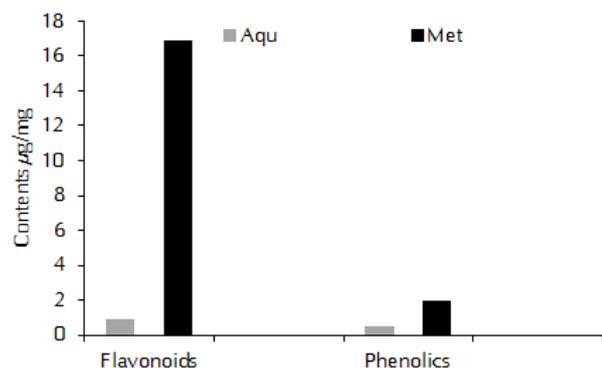


Figure 1: Phenolic and flavonoid contents of Methanolic and aqueous extracts of *S. oleosa*.

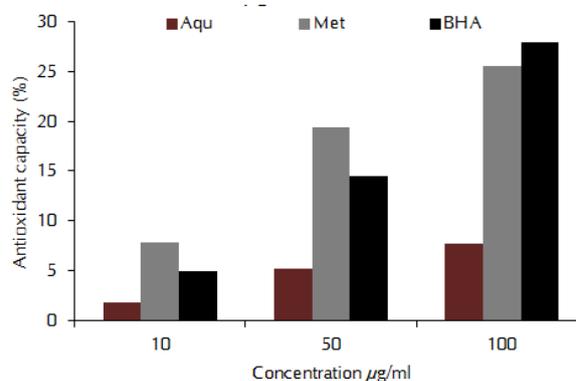


Figure 2: Total antioxidant capacity of *Schleicheria oleosa* leaf

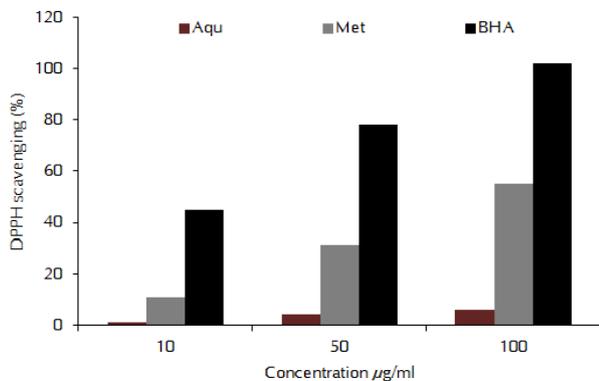


Figure 3: DPPH radical scavenging activity of *Schleicheria Oleosa* leaf

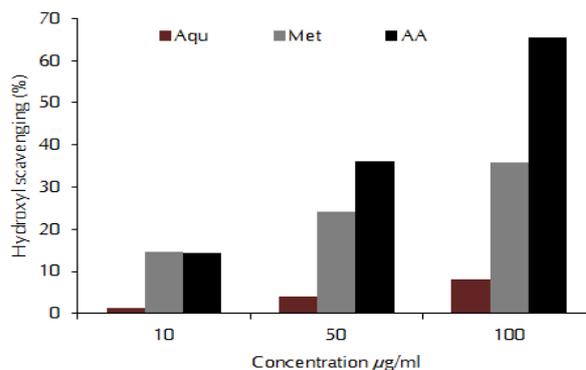


Figure 4: Hydroxyl scavenging capacity of *Schleicheria oleosa* leaf

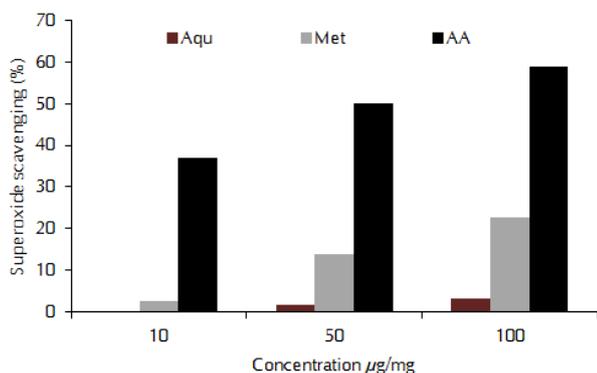


Figure 5: Superoxide anion scavenging capacity of *Schleicheria oleosa* leaf

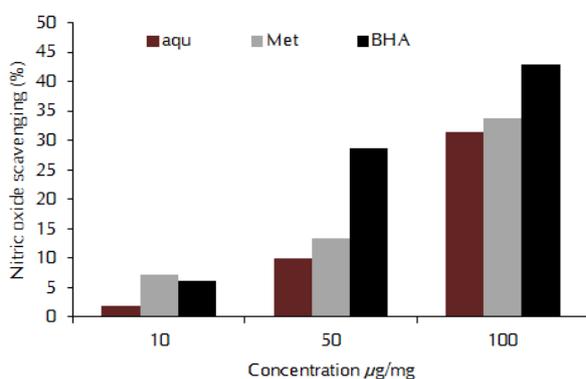


Figure 6: Nitric oxide scavenging capacity of *Schleicheria oleosa* leaf

blank. Ascorbic acid (AA) was used as reference standard. [Klein, et al (1991)]. The percentage hydroxyl radical scavenging activity was calculated by the following formula:

$$\% \text{ hydroxyl radical scavenging activity} = \frac{1 - \text{difference in absorbance of sample}}{\text{difference in absorbance of blank}} \times 100$$

Estimation of super oxide anion Radical Scavenging Activity

Nitroblue tetrazolium (NBT; 150 µM in Tris-HCl buffer 16mM pH 8.0; 1ml) and Nicotinamide adenine dinucleotide (NADH; 234 µM in Tris-HCl buffer 16mM pH 8.0; 1ml) were mixed in a series of test tube. Various concentrations (10µg, 50µg and 1000µg) of samples and Ascorbic acid (AA) were added to these test tubes and made up to 3ml with Tris-HCl buffer (16mM; pH 8.0). Ascorbic Acid (AA) was used as reference standard for comparison. Phenazine methosulphate solution was added (40 µM; 1ml) to each test tube. The reaction mixture was incubated for 5 min at RT. A control without the test compound was maintained. The absorbance of the samples was measured at 560 nm. (Gulcin. *et al.*, 2005). Super oxide radical scavenging activity was calculated using the following formula:

$$\% \text{ NO radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}} \times 100$$

Estimation of nitric oxide radical Scavenging Activity

Various concentrations (10µg, 50µg and 100µg) of samples and Butylated hydroxy anisole (BHA) were taken in different test

tubes and made up to 3ml with 0.1M phosphate buffer (pH 7.2). Sodium Nitroprusside (5mM) prepared in buffered saline (pH 7.2) was added (1 ml) to each tube. The reaction mixture was incubated for 30 min at RT. A control without the test compound, but with an equivalent amount of methanol was maintained. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1- Naphthylethylenediamine dihydrochloride). The absorbance of the samples was measured at 546 nm. (Kumar, *et al.*, 2008). Nitric oxide radical scavenging activity was calculated using the following formula

$$\% \text{ NO radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}} \times 100$$

Estimation of total antioxidant activity

The antioxidant properties of plant samples were determined by Spectrophotometric quantitation method (Prieto *et al.*, 1999). Various concentrations of samples (5 µg, 50 µg, 100µg) were taken in a series of test tubes. The 1.9mL of reagent solution (0.6m Sulphuric acid, 28mm Sodium phosphate and 4mm Ammonium molybdate) was added to the test tubes. The tubes were incubated at 95°C for 90 min and allowed to cool down. The absorbance of aqueous solution of each was measured at 695nm against blank. Antioxidant capacities were expressed as equivalents of ascorbic acid. Butylated hydroxylanisole (BHA) was used as reference standard. (Prieto,

et al., 1999).

RESULTS AND DISCUSSION

Phytochemical analyses

Quantitative evaluation of phytochemicals, known for their roles in providing antioxidant properties to plants, from leaf extracts of *S.oleosa* indicated the plant as a rich source of carbohydrates, glycosides, polysaccharides, proteins, steroids, alkaloids, triterpenoids, tannins, lipids, oils, saponins and flavanoids. These constituents are responsible for the curative nature of *Schleichera oleosa* against itching, head ache, malaria, skin diseases etc. which could make the plant useful for treating different ailments and having a potential of providing useful and safe drugs and drug leads for human use. (Sushmita, et al., 2012).

The total phenolic content was found to be 2.0 ± 0.490 mg/g and the flavonoid content of the sample was $16.8.89 \pm 0.933$ mg/g, which are of moderate range. Phenolic compounds and flavonoids, found in the edible and inedible parts of plants portray antioxidant activity, and hence are of immense importance (Premanath and Lakshmidivi, 2010). The antioxidant capacity of phenols and flavonoids is mainly due to their redox properties, which allows them to cut as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators (Kanimozhi et al., 2011)

Total antioxidant capacity

Several reports have shown a correlation between higher amounts of total polyphenols in plants and correspondingly higher antioxidant potential. (Gul, et al., 2011, Lee, et al., 2002, Das, et al., 2012, Dureja and Dhiman, 2012, Nagmoti et al., 2012, Talukdar, 2013). Earlier works on the antioxidant activity of methanolic leaf extracts of various plants species like *Dipterocarpus turbinatus*, *Garuga pinnata*, *Mallotus tetracoccus* etc., was found to be 10.76%, 65.1%, 31.42% respectively. (Rajendra and Shakti, 2009). Our results also largely supported these conclusions. The total antioxidant capacity of the methanolic and aqueous leaf extracts was investigated (Fig. 2). And both extracts exhibited moderate concentration-dependent antioxidising abilities. Methanolic extract was found to be the more powerful antioxidising agent, with an inhibition of up to 25.5 % at a concentration of 100µg/ml. The aqueous extract showed an inhibition of 7.7% at a

Table 1: Proximate Phytochemical composition of Methanolic and aqueous extracts of *S.Oleosa*

Phytochemicals	Methanolic	Aqueous
Carbohydrates	+	+
Glycosides	+	+
Polysaccharides	+	-
Proteins	+	+
Alkaloids	+	+
Steroids	+	+
Triterpenes	+	-
Flavanoids	+	-
Tannins	+	-
Lipid	-	+
Oils	+	+
Saponins	-	+

concentration 100µg/ml.

Free radical scavenging assay

Interception of free radicals is mainly by radical scavenging, The scavengers include various antioxidants like carotenoids, flavonoids, etc. (Sies, 1996; Cadenas and Packer, 1996; Halliwell and Aruoma, 1993) In the present study, aqueous and methanolic extracts were found to be effective scavengers against DPPH radical. Their activities increased in a concentration dependent manner (Fig. 3). The methanolic extract showed the higher DPPH radical scavenging activity, than the aqueous extract. The DPPH radical scavenging activity of methanolic extracts was shown to be 54.94% at 100µg/ml and of aqueous extract was 5.92 % at 100 µg/mg .

Hydroxyl radical scavenging activity

Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and enormous biological damage (Aurand, et al., 1977, Sunil, et al., 2008)The hydroxyl radical scavenging activity of the methanolic and aqueous extracts was studied (Fig. 4). Both extracts exhibited a concentration-dependent scavenging abilities for the hydroxyl radical. Methanolic extract was found to be the more powerful scavenger of the hydroxyl radical, with an inhibition of up to 35.83 % at a concentration of 100µg/ml. It is worth mentioning that aqueous extract showed an inhibition of 8.19 % at a concentration 100µg/ml.

Superoxide anion scavenging activity

Earlier work on the percentage inhibition of superoxide generation by *Citrullus colocynthis* at 2500 mg mL⁻¹ concentration was found to be $71.3 \pm 3.2\%$ ($p < 0.005$), (Sunil et al., 2008).The Superoxide anion scavenging activity of the methanolic and aqueous extracts was explored (Fig. 5). Both extracts exhibited concentration-dependent scavenging abilities for the superoxide anion radical. Methanolic extract was found to be the more powerful scavenger of the superoxide anion radical, with an inhibition of up to 22.58 % at a concentration of 100µg/ml and aqueous extract showed an inhibition of 3.23 at a concentration 100µg/ml .

The nitric oxide scavenging activity

Earlier works have shown nitric oxide scavenging capacity of different plant parts. (Wankupar, et al., 2015) Methanolic and aqueous leaf extracts of *Schleichera oleosa* exhibited concentration-dependent scavenging abilities for the nitric oxide radical. Methanolic extract was found to be the more powerful scavenger of the nitric oxide radical, with an inhibition of up to 33.81 % at a concentration of 100µg/ml and aqueous extract showed an inhibition of 31.43% at a concentration 100µg/ml.(Fig. 6).

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REFERENCES

McLeod, M. N. 1974. Plant tannins-their role in forage quality. *J. Nutr Abstr Rev.* **44**: 803e815.

- Burns, J., Gardner, P. T., Matthews, D., Duthie, G. G., Lean, M. E. and Crozier, A. 2001. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. *J. Agric. Food Chem.* **49**: 5797-5808.
- Smith, M. A., Perry, G., Richey, P. L., Sayre, L. M., Anderson, V. E. and Beal, M. F. 1996. *J. Oxidative damage in Alzheimer's. Nature* **382**: 120-121.
- Peiyuan Li, Lini Huo, Wei su, Rumei lu, Chaocheng Deng, Liangquan Liu, Yongkun Deng, Nana Guo, Chengsheng Lu and Chunling He. Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica*. 2010 *J. Serb. Chem. Soc.* **76(5)**: 709-717.
- Lim, Y. Y. and Murtijaya, J. 2007. Antioxidant properties of Phyllanthus amarus extracts as affected by different drying methods *LWT-Food Sci. Technol.* **40**: 1664.
- Scalbert, A., Manach, C., Morand, C. and Remesy, C. 2005. Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.* **45**: 287.
- Namiki, M. 1990. Anti oxidants/antimutagens in food *Crit. Rev. Food Sci.* **29**: 273.
- Politeo, O., Jukic, M. and Milos, M. 2007. Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil *Food Chem.* **101**: 379.
- Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., Polissiou, M. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae) *Food Chem.* **90**: 333.
- Ku, C. S. and Mun, S. P. 2007. Characterization of seed oils from fresh Bokbunja (*Rubus coreanus* Miq.) and wine processing waste. *Bioresour. Technol.* **99**: 2852.
- Gulcin, I., Buyukokuroglu, M. E., Oktay, M., Kufrevioglu, O. I. 2003. On the in vitro antioxidative properties of melatonin. *J. Ethnopharmacol.* **86**:
- Atawodi, S. E. 2005. Antioxidant potential of African medicinal plants, *Afr. J. Biotechnol.* **4(2)**: 128-133.
- Katalinic, V., Milos, M., Kulisic, T. and Jukic, M. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols, *Food Chem.* **94**: 550-557.
- Shahidi, F. 1997. Natural antioxidants: Chemistry, health effects and applications. Urbana, IL: AOCS Press
- Cook, N. C. and Samman, S. 1996. Flavonoids: chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nutr. Biochem.* **7**: 66-76.
- Gómez-Meza, N., Noriega-Rodríguez, J. A., Leyva-Carrillo, L., Ortega-García, J., Bringas-Alvarado, L., García, H. S., Medina-Juárez, L. A. 2009. *J. Food Process. Pres.* **33**: 110.
- Palanuvej, C., Vipunngun, N. 2008. Fatty acid constituents of *Schleichera oleosa* (Lour) Oken seed oil. *J. Health Res.* **22**: 203-2.
- Trease, G. E. and Evans, W. C. 2002. Pharmacognosy. 15th Ed. Saunders Publishers, London. p. 42.
- Sofowora, A. 2008. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 3rd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria. p. 134.
- Prieto, P. and Pineda, M. 1999. Aguilar m. Spectrophotometric quantitation of antioxidant capacity through the formation of a phospho molybdenum complex: specific application to the determination of Vitamin E1. *Analytical Biochemistry.* **269**: 337-341.
- Sushmita Choudhury, Latika Sharan and Sinha, M. P. 2012. Antibacterial efficacy and phytochemistry of methanolic leaf extracts of *Mangifera indica* linn. *J. The Ecoscan: Special issue.* **1**: 419-423. 2012.
- Premanath, R. and Lakshmidevi, N. 2010. Studies on Anti-oxidant activity of *Tinospora Cordifolia* (Miers.) Leaves using *in vitro* models. *J. American Sci.* **6(10)**: 736-743.
- Kanimozi, D., Kandhymathi, K., Bharathidasan, R., Mahalingam, R., Deepa, S. and Panneerselvam, A. 2011. Antioxidant activity, estimation of total phenolic content and tannin of *Lecuasaspera* and *Sassiaariculata*. *World J. Sci. and Tech.* **19(9)**: 11-17.
- Kumar, S., Kumar, D., Manjusha, Saroha, K., Singh, N. and Vashishta, B. 2008. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract, *Acta. Pharm.* **58**: 215-220.
- Klein, S. M., Cohen, G. and Cederbaum, A. I. 1991. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. *Biochemistry.* **20**: 6006-6012
- Gulcin, I., Alici, H. A. and Cesur, M. 2005. Determination of *in vitro* antioxidant and radical scavenging activities of Propofol. *Chem. Pharm. Bull.* **53(3)**: 281-285.
- Malick, C. P. and Singh, M. B. 1980. In: *Plant Enzymology and Histoenzymology*. Kalyani Publishers. New Delhi. p. 286.
- Helmja, K., Vaheer, M., Gorbatošova, J. and Kaljurand, M. 2007. Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis. *Proc. Estonian Acad. Sci. Chem.* **56(4)**: 172-186.
- Rajendra Kshirsagar and Shakti Upadhyay 2009. Free radical scavenging activity screening of medicinal plants from Tripura, Northeast India. *J. Natural Product Radianc.* **8(2)**: 117-122.
- Gul, M., Bhakshu, L., Ahmed, F., Anand, K., Qureshi, I. and Ghazi, I. 2011. Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using *in vitro* assays. *BMC Complement Altern Med.* **11**: 64.
- Lee, J. C., Kim, H. R., Kim, J. and Jang, Y. S. 2002. Antioxidant property of an ethanol extract of the stem of *Opuntia ûcus-indica* var. *saboten*. *J. Agric Food Chem.* **50**: 6490e 6496.
- Das, M., Sarma, B., Ahmed, G., Balasubramaniam, N., Choudhury, M. 2012. In vitro antioxidant and total phenolic content of *Dillenia indica* and *Garcinia penducalata*, commonly used fruits in Assamese cuisine. *Free Rad Antiox.* **2**: 30e36.
- Dureja, A. G. and Dhiman, K. 2012. Free radical scavenging potential and total phenolic and flavonoid content of *Ziziphus mauritiana* and *Ziziphus nummularia* fruit extracts. *Int J. Green Pharm.* **6**: 187e192.
- Nagmoti, D. M., Khatri, D. K., Juvekar, P. R. and Juvekar, A. R. 2012. Antioxidant activity and free radical-scavenging potential of *Pithecellobium dulce* Benth seed extracts. *Free Rad Antiox.* **2**: 37e43.
- Talukdar, D. 2013. In vitro antioxidant potential and type II diabetes related enzyme inhibition properties of traditionally processed legume based food and medicinal recopies in Indian Himalayas. *J Appl Pharm Sci.* **3**: 26e32.
- Aurand, L. W., Boonme, N. H. and Gidding, G. G. 1977. Superoxide and singlet oxygen in milk lipid peroxidation, *J. Dairy Sci.* **60**: 363-369.
- Sunil Kumar, Dinesh Kumar, Manjusha, Kamal Saroha, Nidhan Singh, Bhoodev Vashishta. 2008. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. *J. Acta. Pharm.* **58**: 215-220.
- Wankupar Wankhar, Sakthivel Srinivasan, Ravindran Rajan, Sheeladevi Rathinasamy. 2015. Evaluating *in vitro* antioxidant activity and GC-MS analysis of *Scoparia dulcis* Linn (Scrophulariaceae). *J. Applied Pharmaceutical Science.* **5(07)**: 029-034.