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ANTIOXIDANT ACTIVITY OF *SCHLEICHERA OLEOSA*

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KEYWORDS

Schleichera oleosa
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Total phenolic compounds
Total flavonoids
NO radical
DPPH radical
Hydroxyl radical

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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 100ìg/ml of the extract. Also increase in concentration decreases the radical formation.

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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 peroxyl (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 , Vipunngeun, 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.

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 45ºC., 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-Ciocalteau 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 boling 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 (10ìg, 50ìg and 100ìg) of samples in Dimethyl sulfoxide (DMSO), were taken in a series of test tubes. The volume was adjusted to 500ìl 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:

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

control OD

Estimation of hydroxyl Radical Scavenging Activity

Various concentrations (10ìg, 50ìg, and 100ìg) of samples in DMSO were taken in different test tubes and made up to 250ìl 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

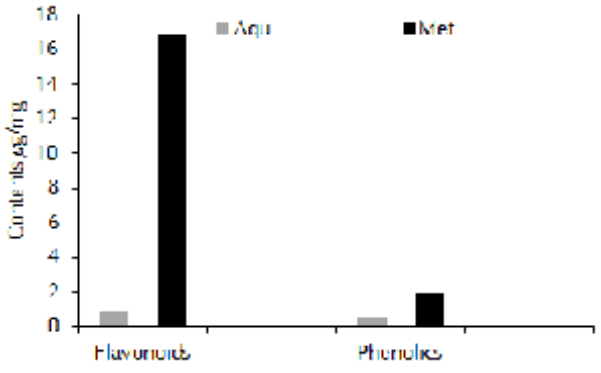

Figure 1: Phenolic and flavonoid contents of Methanolic and aqueous extracts of *S. oleosa*.

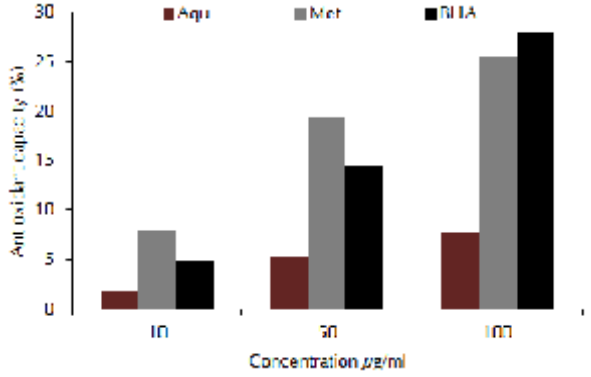

Figure 2: Total antioxidant capacity of *Schleichera oleosa* leaf

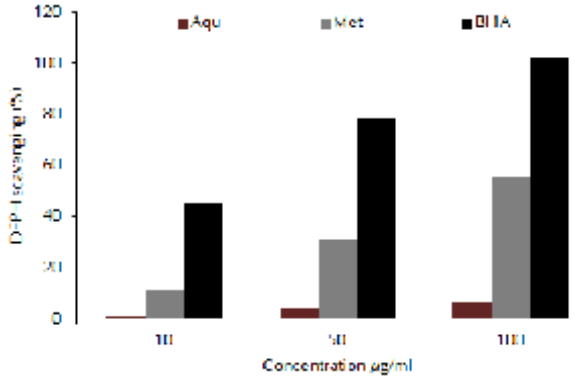

Figure 3: DPPH radical scavenging activity of *Schleichera Oleosa* leaf

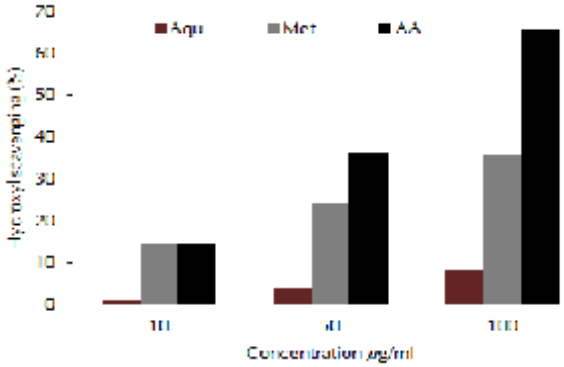

Figure 4: Hydroxyl scavenging capacity of *Schleichera oleosa* leaf

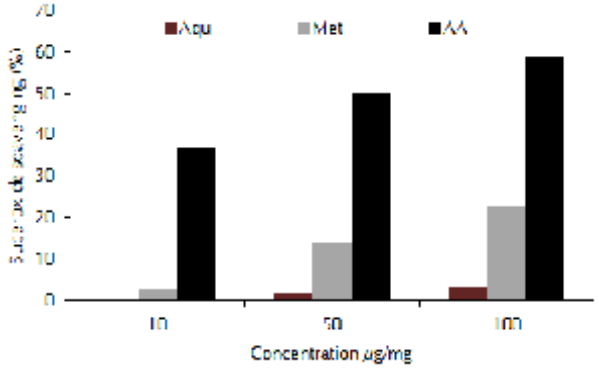

Figure 5: Superoxide anion scavenging capacity of *Schleichera oleosa leaf*

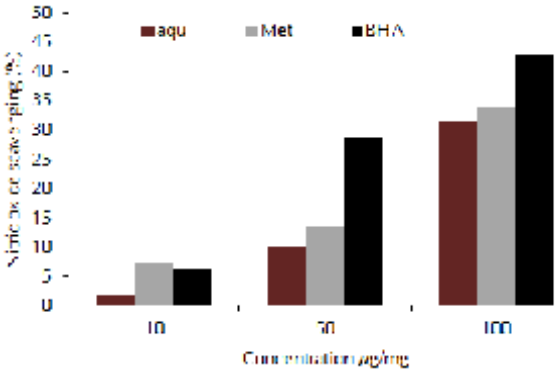

Figure 6: Nitric oxide scavenging capacity of *Schleichera oleosa leaf*

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:

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

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 (10ìg, 50μg and 1000ìg) 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 tubeThe 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:

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

Estimation of nitric oxide radical Scavenging Activity

Various concentrations (10ìg, 50ìg and 100ìg) 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 (pH7.2) 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

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

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 ìg, 50 ìg, 100ìg) were taken in a series of test tubes. The 1.9mL of reagent solution (0.6m Sulphuric acid, 28mm Sodium phosphate and4mm Ammonium molybdate) was added to the test tubes. The tubes were incubated at 95ºC 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 *Schelichera 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 ± 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 Lakshmidevi, 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 concentration 100μg/ml.

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	-	+

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 aquous 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 ± 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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