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EVALUATION OF ANTIOXIDANT ACTIVITY OF *CASSYTHA FILIFORMIS*

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ABSTRACT: The study was aimed to evaluate the antioxidant activity of *Cassytha filliformis* for the first time. The antioxidant activity of *cassytha filiformis* extracts in hexane, ethyl acetate and methanol were assessed based on their radical scavenging activity (RSA) using the DPPH assay. Methanolic extract showed good antioxidant activity when compared with standard Butylated hydroytoluene(BHT). Further, the methanolic extracts were evaluated by Ferric thiocyanate(FTC) method, Thiobarbituric acid(TBA) test and Superoxide anion radical scavenging assay. The results obtained suggest that methanolic extract of *cassytha filiformis* have promising therapeutic potential and could be considered as potential source for drug development by pharmaceutical industries.

Keywords: DPPH, FTC, TBA, *Cassytha filiformis*

INTRODUCTION

Plants are potential sources of natural antioxidants. They absorb the sun's radiation and generate high levels of oxygen as secondary metabolites of photosynthesis. Oxygen is easily activated by ultra violet (UV) radiation and heat from the sunlight to produce toxic, reactive oxygen species (ROS). Plants produce various antioxidative compounds to counteract these ROS in order to survive (Lu & Foo, 1995). Antioxidants play an important role in defending the body against free radicals damage. Antioxidants refer to a group of compounds that are able to delay or inhibit the oxidation of lipids or other biomolecules and thus, prevent or repair the damage of the body cells that is caused by oxygen (Shahidi & Naczk, 2004; Tachakittirungrod, Okonogi, & Chowwanapoonpohn, 2007). They work by preventing the formation of new free radical species, converting existing free radicals into less harmful molecules and preventing radical-chained reactions (Rodriguez et al., 2007).

Cassytha filiformis Linn., is perennial, parasitic, herbaceous and leaf less plant belonging to family Lauraceae. This plant is distributed throughout India and used medicinally in China, Indochina, Madagascar and South Africa. Cassytha filiformis is medicinally used as antiplatelet, Vesorelaxant (Versiani Mohammed Ali, 2004), alpha-adrenoreceptar antagonist (Hoet,S. et al., 2004) and antitrypnosomal (Chang ,C.W. et al., 1997).

Consumers, all over the world, are now more conscious of nutritional value and safety of food ingredients. Therefore, it is interesting and worthwhile to investigate and identify natural antioxidants from edible plants even though they may not be comparable, in efficiency, to synthetic agents.

The objective of this study is to evaluate the antioxidant activity of *Cassytha filiformis* extracts in hexane, ethyl acetate and methanol on the basis of Radical Scavenging activity using DPPH assay. And further evaluating the extracts by Ferric thiocyanate (FTC) method, Thiobarbituric acid (TBA) test and Superoxide anion radical scavenging assay.

MATERIALS AND METHODS

Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), ascorbic acid, was purchased from Himedia Laboratories Pvt. Ltd. (India). All other chemicals and solvents used were of standard analytical grade and HPLC grade, respectively.

Plant material

Cassytha filiformis was collected from the fields located in Kelambakkam and Aanaimalai forest. Collected specimens were shade dried, powdered, sieved and stored until further use.

Extraction

Extraction was carried by the Direct extraction methods of Eloff, 1998 with hexane, ethyl acetate, and methanol. Finely ground plant material (1 gm) was extracted with 10 ml of hexane, ethyl acetate, and methanol in conical flask under shaking condition. The extracts were then decanted into pre-weighed glass vials. The process was repeated thrice with the same plant material but using fresh solvent each time. The solvent was removed by keeping them in a steam of air in a fume hood at room temperature. The dried extracts were weighed to determine the yield of soluble constituents and stored in vacuum desiccators for further analyses.

Determination of antioxidant activity

DPPH radical-scavenging assay

Measurement of DPPH radical-scavenging activity was performed according to recommendations by Nenadis and Tsimidou (2002). Conditions consisted of an approximately 20 min reaction period and a molar ratio between DPPH and antioxidant that permits 60–80% radical-scavenging activity for the most potent antioxidant. Briefly, 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol (250 lM,2 ml) was added to 2 ml of the test compounds at different concentrations in ethanol. The final concentrations of the test compounds in the reaction mixtures were 0.5, 5, 10, 25 and 50 µM. Each mixture was then shaken vigorously and held for 30 min at room temperature in the dark. The decrease in absorbance of DPPH at 517 nM was measured. Ethanol was used as a blank solution. DPPH solution (2 ml) in ethanol (2 ml) served as the control. All tests were performed in triplicate. The radical-scavenging activity of the samples was expressed as % inhibition of DPPH absorbance:

$$\%\text{Inhibition} = [(A\text{control} - A\text{test})/A\text{control}] \times 100$$

where, Acontrol is the absorbance of the control (DPPH solution without test sample) and Atest is the absorbance of the test sample (DPPH solution plus compound). Ascorbic acid and α-tocopherol were used as reference compounds.

Ferric thiocyanate method (FTC)

The FTC method was adapted from Osawa and Namiki (1981). Samples (4 mg or 4 ml) in 99.5% ethanol were mixed with 2.51% linoleic acid in 99.5% ethanol (4.1 ml), 0.05M phosphate buffer, pH 7.0 (8 ml), and distilled water (3.9 ml) and kept in screw cap containers under dark conditions at 40ºC. To 0.1 ml of this solution was added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after addition of 0.1 ml of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red colour was measured at 500 nm each 24 h until one day after absorbance of the control reached maximum. The control and standard were subjected to the same procedure as the sample except for the control, where there was no addition of sample, and for the standard, where 4 mg of sample were replaced with 4 mg of α-tocopherol or BHT.

Thiobarbituric acid (TBA) test

TBA test (Mackeen et al., 2000) was conducted instantly after the control sample from FTC test reached its maximum absorbance value. In brief, 1.0 ml of 20% aqueous trichloroacetic acid and 2.0 ml of 0.67% aqueous thiobarbituric acid were added to 2 ml of sample solutions acquired from FTC test. The mixtures were then placed in boiling water bath for 10 min. After cooling under the running tap water, the mixtures were centrifuged at 3000g for 30 min. Finally, the absorbance of supernatants at 532 nm was measured by using a spectrophotometer.

Superoxide radical scavenging activity

The superoxide radical was generated with an enzymatic reaction according to a modified version of the method explained by Nishikimi, Rao, and Yagi (1972). The reaction mixture contained 1 mL of each of 3 mM hypoxanthine, xanthine oxidase (100 mIU), 12 mM diethylenetriaminepentaacetic acid, 178 mM nitro blue tetrazolium and the sample. The absorbance of the medium was read at 560 nm over a 60 min period at 10 min intervals. The absorbance values were corrected by subtracting 0 min readings from those obtained subsequently. Superoxide radical scavenging activity (at 10 min) of additives was calculated using the following equation:

Superoxide Radical Scavenging Capacity (%) = 100 - [(absorbance of medium containing the additive of concern) / (absorbance of the control medium)] x 100.

RESULTS AND DISCUSSION

DPPH radical-scavenging assay

The DPPH assay is a simple method to measure the ability of antioxidants to trap free radicals. All the three extracts showed a concentration dependent scavenging of DPPH radicals as shown in Figure 1. Methanolic extract was found to be more active as a radical scavenger than Hexane and ethyl acetate extract. The results also revealed that the other extracts might prevent reactive radical species from damaging biomolecules such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars in susceptible biological and food systems (O.I. Aruoma (1995). The activity was compared to ascorbic acid which is employed as the standard.

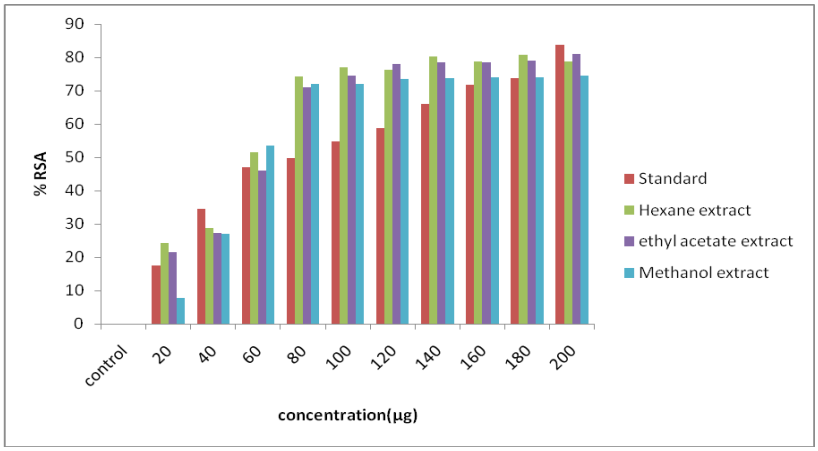

Figure1. Antioxidant activity of hexane, ethyl acetate and methanol extracts of *cassytha filiformis* measured as %RSA using the DPPH assay. Comparatively, methanol extract is found to exhibit significant antioxidant activity.

Ferric thiocyanate method (FTC)

The FTC method was used to measure the peroxide level during the initial stage of lipid oxidation. Low absorbance values indicate high levels of antioxidant activity. Figure 2 shows the absorbance (at 500nm) values of standard, methanol extract and control against time in days.

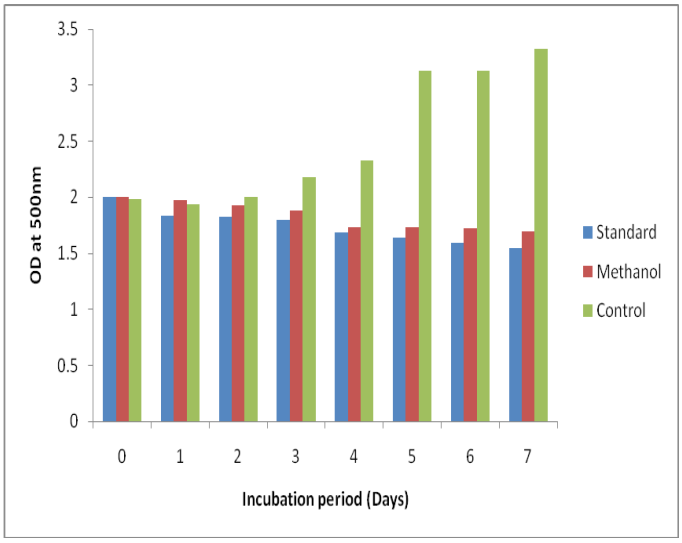

Figure 2. Antioxidant activity of methanol extracts of *cassytha filiformis* as measured by FTC method.

Thiobarbituric acid (TBA) test

TBA test measures the thiobarbituric acid reactive substances content at a later stage of lipid oxidation, involving the quantification of the secondary products formed from lipid oxi- dation. Figure 3 shows the absorbance values of methanol extract for TBA test.

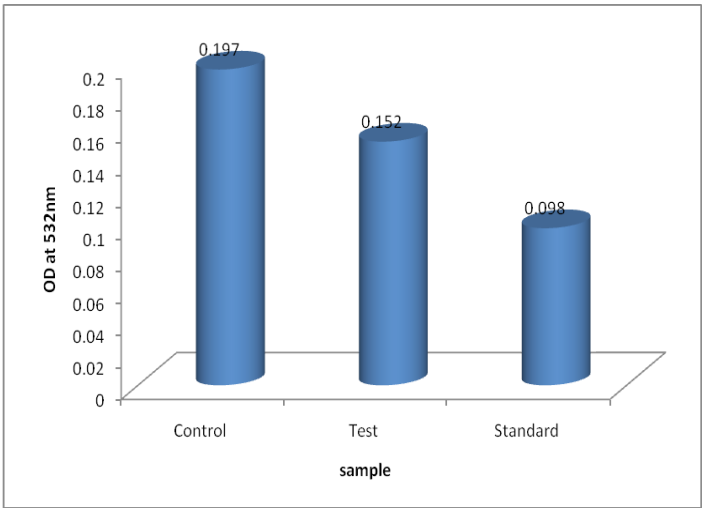

Figure 3. Antioxidant activity of methanol extracts of *cassytha filiformis* as measured by Thiobarbituric acid (TBA) test.

Superoxide radical scavenging activity

The methanolic extract of Cassytha filliformis could scavenge free radicals. Figure 4 shows the antioxidant activity of methanol extracts of *cassytha filiformis* as measured by Superoxide radical scavenging activity.

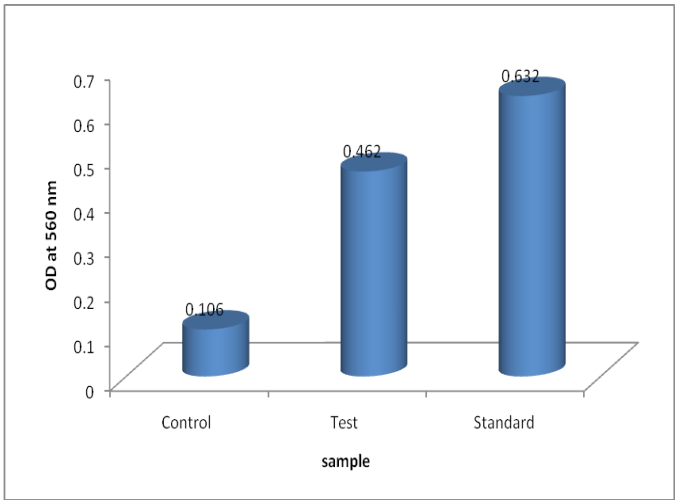

Figure 4. Antioxidant activity of methanol extracts of *cassytha filiformis* as measured by Superoxide radical scavenging activity.

Based on the results obtained, it is highly possible that several compounds of different polarity may contribute to the antioxidative activity of *cassytha filiformis*.Methanol extracts may include phenolic and hydroxy-phenolic compounds with acids, alcohols,sugars or glycosides, as reported by Kim and Pratt (1993).

In summary,antioxidant activity is observed in the methanolic extracts of *cassytha filiformis*. Thus, the extract can be considered as new sources of natural antioxidants for food and nutraceutical products, potentially.Further studies on the identification and purification of components responsible for the antioxidative activities in *cassytha filiformis* is are now in progress.

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