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Full Length Research Paper

Phytochemical screening and *in vitro* antioxidant potential of *Cassytha filiformis*

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The present study was aimed to evaluate the antioxidant activity of the whole plants of *Cassytha filiformis*. Antioxidant potential of the *C. filiformis* extracts was studied on different *in vitro* antioxidant assay and the compound was identified by Autobiography. The extracts were subjected to assess their potential antioxidant activities using systems such as A, A Diphenyl- b-Picryl Hydrazyl (DPPH), FTC, TBA, Hydroxyl radical scavenging activity (HRSA), SOD, Phosphomolybdenum assay and Metal chelating activity. The superoxide anion radical-scavenging activity was found to be higher in methanol extract. The DPPH radical and HRSA cation radical-scavenging activities were well proved and related with the ferric-thiocyanate/antioxidant capacity of the extracts. Interestingly, among the various extracts, methanol extract showed significantly higher hydroxyl radical-scavenging activity. Methanol extract exhibited good antioxidant activity against the linoleic acid emulsion system but were significantly lower than the synthetic antioxidant, BHA. Five major compounds were identified by thin layer chromatography. The preliminary phytochemical screening has revealed the presence of phenolics, carbohydrates, glycosides and proteins in high amounts, whereas, amino acid was present in trace amount. Results of our study showed that *C. filiformis* possess significant antioxidant activity. Owing to these properties, this plant has the potential as natural source of antioxidants, capable of protecting against free radical mediated damage and may have applications in preventing and curing various diseases.

Key words: *Cassytha filiformis*, antioxidant activity, 2, 2 - diphenylpicryl - 1 - picryl - hydrazyl (DPPH).

INTRODUCTION

Plants produce a wide range of redox-active secondary metabolites with antioxidant activity, such as ascorbic acid, carotenoids, polyphenols, and enzymes which protect the cells from oxidative damage. Bioactive compounds commonly found in edible plant parts such as fruits, vegetables, flowers, and leaves have been shown to confer health benefits. Interestingly, many of them are known to contain large amounts of phenolic antioxidants (Yen *et al*., 2002). Some of the phenolic compounds present in natural products have higher antioxidant activities than those of synthetic antioxidants.

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Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage, and DNA strand breaking (Ghosal *et al*., 1996). Phenolic compounds in plant-derived foods and beverages have been shown to have important physiological properties and may be responsible for both detrimental and beneficial effects on human health (Chung *et al*., 1998; Singh *et al*., 2003). Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stoke, diabetes, Alzheimer’s diseases and cancer (Devasagayam, 2004).

The most important and prominent species is *Cassytha filiformis, a* vigorous saprophytic climber which

resembles Cuscuta or dodder but with more chlorophyll. An unusual botanical relative in this interesting family of tropical and subtropical distribution is the "woevine," *C. filiformis.* This species actually is a vine resembling the common parasitic dodder which is so prevalent in many parts of California and other subtropical and tropical areas of the world. The present study was carried out to evaluate the antioxidant activity of hexane, ethyl acetate and methanol extracts of this traditional edible species which occurs in wild.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were of analytical grade and purchased from Sigma Chemical Co., St. Louis, MQ, USA, Himedia Laboratories Pvt. Ltd., Mumbai, India, Sd-fine Chemicals. Ltd., Mumbai, India and E. Merck Co., Darmstadt, Germany.

Plant collection

Fresh plants of *C. filiformis* were collected from the fields located in Kelambakkam forest, Chennai, Tamilnadu.

Preparation of *C. filiformis* plant powder

The fresh plant was carefully washed with tap water, rinsed with distilled water, and air-dried for 1 hour. Then it was cut into small pieces & dried in room temperature (25 ± 2ºC). Then they were ground into powder and stored in room temperature and used for solvent extraction.

Solvent extraction

Direct extraction with hexane, ethyl acetate and methanol following the method of (Eloff, 1998) was used as an extraction method. In this method, finely ground plant material was extracted with hexane, ethyl acetate and methanol in the ratio of 1:10 in conical flask in shaking condition. The extract was decanted in to pre-weighed glass vials. The process was repeated 3 times and the same plant material but using fresh solvent. The solvent was removed by placing the extracts in front of a steam of air in a fume hood at room temperature. The extracted residues were weighed and re-dissolved in different solvents to yield 10mg/mL solutions ready for further analysis.

Estimation of Radical Scavenging Activity (RSA) using the DPPH assay

The Radical Scavenging Activity of different extracts was determined by using DPPH assay according to Nenadi's (2002), with a small modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2960µl of 0.1mM ethanolic DPPH solution mixed with 40µl of 20 to 200 µg/mL of plant extract and vortexed thoroughly. The setup was left at dark in room temperature and the absorption was monitored after 20minutes. Ascorbic acid and Butylated hydroxytoluene (BHT) were used as references. The ability of the plant extract to scavenge DPPH radical was calculated by the following equation:

$$\% \text{ of DPPH Radical Scavenging Activity } (\% \text{ RSA}) = \frac{\text{Abs. control} - \text{Abs. sample} * 100}{\text{Abs. control}}$$

Abs. control is the absorbance of DPPH radical + ethanol; Abs. sample is the absorbance of DPPH radical + plant extract. Measurements were performed in triplicates. Absorbance values were corrected for radicals decay using blank solutions.

Ferric thiocyanate (FTC) method (Osawa and Namiki, 1981)

The plant sample of 4 mg in 99.5% ethanol were mixed with 2.51% linoleic acid in 99.5% ethanol (4.1 mL), 0.05 M phosphate buffer, pH 7 (8 mL) and distilled water (3.9 mL) and kept in screw cap containers under dark conditions at 40°C. To 0.1 mL of this solution, 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate were added. After 3 min, 0.1 mL of 2M ferrous chloride in 3.5% HCl was added to the reaction mixture and the absorbance of the red color was measured at 500 nm each 24 h until one day after absorbance of the control reached maximum. The control and the 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 4 mg of sample were replaced with 4 mg of α - tocopherol or Butylated hydroxytoluene.

Thiobarbituric acid (TBA) test: (Kikuzaki and Nakatani, 1993)

The same samples as prepared for the FTC method were used in TBA test. To 1 mL of sample solution, 2 mL each of 20% aqueous trichloroacetic acid were added. This mixture was then incubated in a boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and the absorbance of supernatant was measured at 532 nm. Antioxidative activity was recorded based on absorbance on the eighth day.

Superoxide Anion Radical Scavenging Assay (Nishikimi *et al.*, 1992)

1ml of NBT solution (156µM NBT in 100mM phosphate buffer, pH8) mixed with 1ml of NADH solution

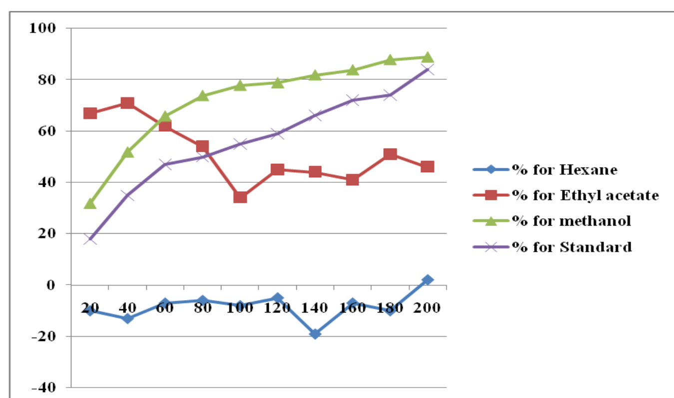

Figure 1. Estimation of Radical Scavenging Activity (RSA) Using DPPH Assay

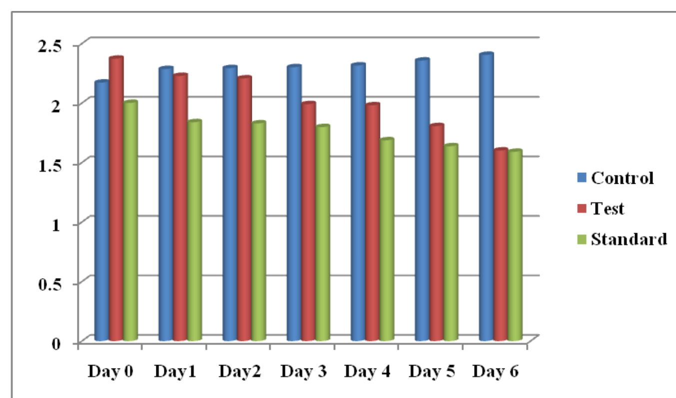

Figure 2. Ferric thiocyanate (FTC) method

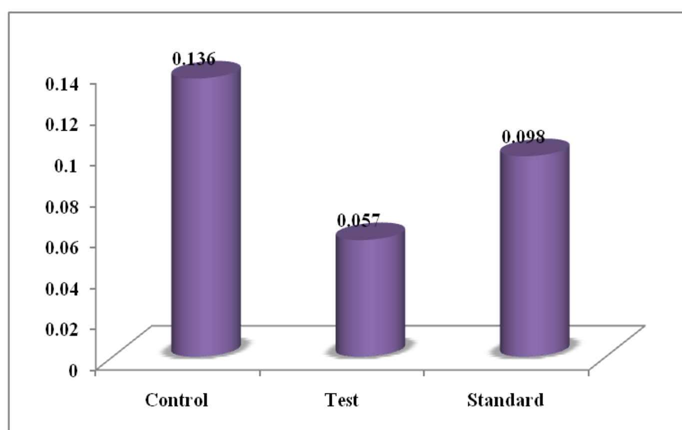

Figure 3. Thiobarbituric acid (TBA) test

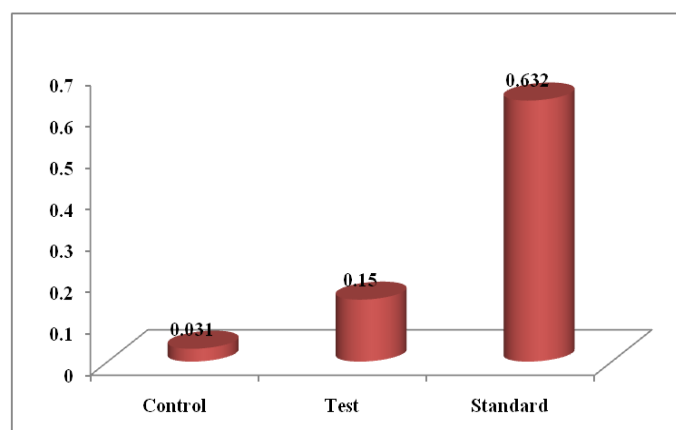

Figure 4. Superoxide Anion Radical Scavenging Assay

(468µM in 100mM phosphate buffer, pH8). Then it mixed with 0.1ml of sample solution (10mg/mL). The reaction was started by adding 100µl of PMS solution (60µM PMS in 10mM, Phosphate buffer, Ph 8). The mixture was incubated at 25ºC for 5minutes. A control was performed with reagent mixture but without the sample. Absorbance was measured spectrophotometrically at 560 nm.

Hydroxyl radical scavenging activity

The scavenging activity of methanol extract of *C. filiformis* on hydroxyl radical was measured according to the method of Klein *et al*. Various concentrations (50, 100, 150 and 200µg/mL) of extracts were added with 1.0ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA solution (0.018%), and 1.0mL of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1M phosphate buffer, pH 7.4).The reaction was initiated by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80–90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of1.0mL of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0g of ammonium acetate, 3.0mL of glacial acetic acid, and 2mL of acetyl acetone were mixed and raised to 1L with distilled water) was added and left at room temperature for 15min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity is calculated by the following formula:

% HRSA = from [(A0 - A1)/A0] X100,

where A0 is the absorbance of the control and A1 is the absorbance of the extract/standard.

Metal chelating activity

The chelating of ferrous ions by methanol extract of *C. filiformis* was estimated by the method of Dinis *et al.* Briefly the extract samples (250µl) were added to a solution of 2mmol/L $FeCl_2$ (0.05mL). The reaction was initiated by the addition of 5mmol/L ferrozine (0.2mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution

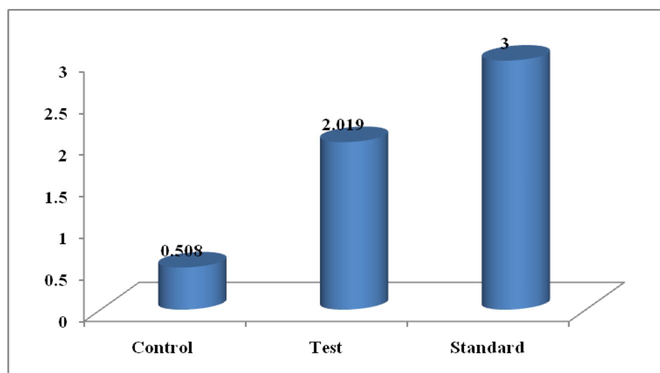

Figure 5. Phosphomolybdenum assay

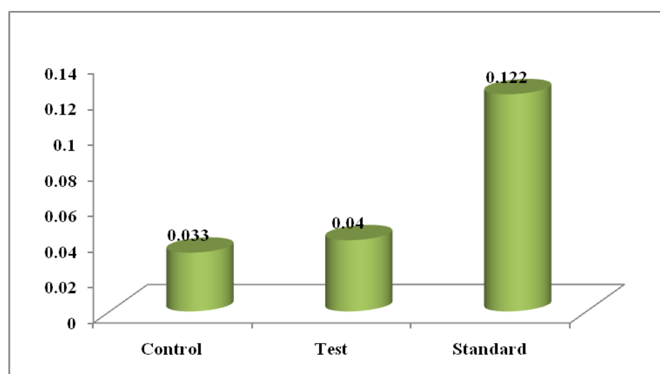

Figure 7. Metal chelating activity

was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al* (1999). An aliquot of 100µl of sample solution was combined with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in a 4ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents/100g extract.

Antioxidant Activity Evaluation by Thin-Layer Chromatography (TLC) (Eloff, 1998):

It is used to separate the compound present in the crude extract. The separation of the compound also depends on the usage of the solvent. Here the solvent used are 20% methanol in chloroform. The concentration (10mg/mL) of the drug was spotted on the TLC plates and dried. It was then run with different solvent ratio the spots were identified both in the UV light, far light and in the iodine chamber. Then R*f* value was calculated. R*f* value is distance travelled by the solute to the distance traveled by the solvent. The compounds from the spots were scrabbed and used for further screening.

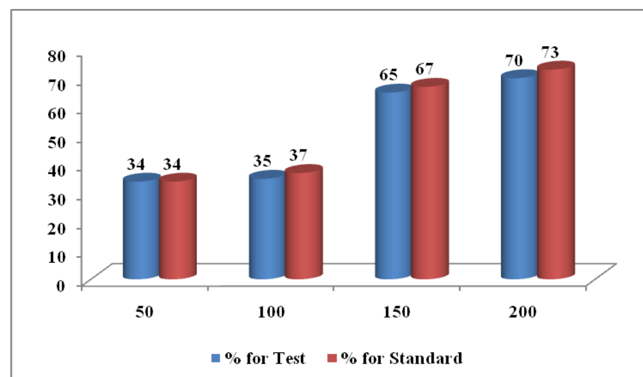

Figure 6. Hydroxyl radical scavenging activity

Table 1. Phytochemical screening

Detection of phytochemicals	Test performed	Result
Alkaloids	Mayer's	-
Carbohydrates and glycosides	Fehling's	++
	Benidict's	+
Glycoside	Borntrager's	-
Saponins	Foam's	-
Protein and Aminoacids	Millon's	-
	Biuret	+
Phenolic compound	Ferric Chloride	++
	Lead Acetate	+

++ Present in high concentration;
+ Present in trace concentration;
- Constituents not detectable using the specified assay method.

Bioautography

Bioautography is a rapid aid in the bioassay guided isolation and fractionation of anti-oxidant compounds and fractions. In this approach, the activity of plant extract against free radical DPPH. Developed standardized chromatography plates of crude extract was sprayed with free radical of DPPH (dissolved in Ethanol) and the zone of inhibition was seen immediately after sprayed. The specific compound (band) which has antioxidative properties shows clear zone. This method was chosen for its simplicity, low cost, accuracy and rapid results make it ideal for bioassay guided isolation (Eloff, 1998).

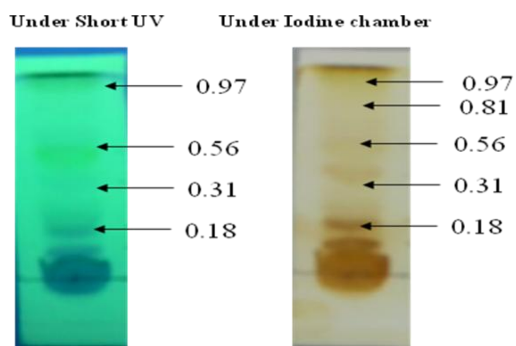

Figure 9. Thin Layer Chromatography

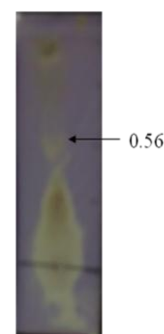

Figure 10. Autobiography

RESULTS AND DISCUSSION

DPPH radical scavenging activity

DPPH radical scavenging activity has been widely used to evaluate the antioxidant activity of plant extracts and foods (Soares *et al*., 1997). The presence of antioxidant in the sample extract react with DPPH, which is a stable free radical, and convert it to 1,1-diphenyl-2-(2,4,6- trinitrophenyl) hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant compounds which can be detected spectrophotometrically at 517 nm. Figure. 1 shows the DPPH radicals scavenging capacity of various extracts of *C. filiformis* with α-tocopherol as references. Concentration of the sample necessary to decrease initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was calculated. Therefore lower value indicates a higher antioxidant activity. The experimental data indicate that methanol extract of *C. filiformis* plant displayed the highest DPPH scavenging effect (89%) followed by the ethyl acetate and hexane (46 and 2% respectively). The radical scavenging activity of the extracts could be related to the nature of phenolics and their hydrogen donating ability (Shimada *et al*., 1992).

In vitro antioxidant activities of *C. filiformis* plant extract (FTC method)

The FTC method was used to measure the peroxide levels during the initial stage of lipid oxidation. Low absorbance values have indicated high levels of antioxidative activity. Figure. 2 details the absorbance values of different methanol extracts of *C. filiformis*. It is interesting to note that the methanol extract exhibited higher antioxidant activity.

In vitro antioxidant activities of *C. filiformis* plant extract (TBA method)

During the oxidation process, peroxides are gradually decomposed to lower molecular weight compounds, like malonaldehyde, which can be measured by TBA method on the final day of the incubation period. The antioxidative activity of *C. filiformis* Methanol was high on 7th day of incubation (Fig .3).

Superoxide anion radical and hydroxyl radicalscavenging activities

The effects of methanolic extract of *C. filiformis* on superoxide anion radical scavenging activity were estimated by the nitro blue tetrazolium (NBT) assay method and the results are compared to tannic acid and quercetin (Siddhuraju and Becker, 2007). Methanol extract had a scavenging activity on the superoxide radicals in a dose dependent manner (1000µg in the reaction mixture). Nonetheless, when compared to tannic acid, the superoxide radical-scavenging activity of methanol extract was found to be low (Fig . 4). This could be due to the presence of relative concentration of bioactive constituents and mixture of impurities/ other nutrients in the extracts.

Hydroxyl radical scavenging activity

Scavenging of OH is an important antioxidant activity because of its very high reactivity which can easily cross the cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing OH is very important for the protection of living systems (Yang *et al*., 2008). Fig . 5, shows the OH scavenging effect of *C. filiformis* extracts at different dose levels (50, 100, 150 and 200µg/mL). All the extracts generally registered good hydroxyl radical scavenging activity in a concentration dependent manner. The methanol extract showed the highest OH scavenging potential (70% at 200µg/mL concentration). The ability of the *C. filiformis* extract to quench hydroxyl radicals seems to directly relate to the prevention of propagation of the process of lipid peroxidation, and seem to be good scavengers of active oxygen species thus reducing the rate of chain reaction.

Metal chelating activity

Presence of transition metal ions in a biological system could catalyse the Haber–Weiss and Fenton-type reactions, resulting in generation of hydroxyl radicals (OH). However, these transition metal ions could form chelates with the antioxidants, which result in the suppression of OH generation, and inhibit ion of peroxidation processes of biological molecules. In this assay, the presence of chelating agents in the extracts of *C. filiformis* disrupts the ferrozine – Fe_2+ complex formation, thus decreasing the red colour (Fig . 6). It is reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gardner, 2000). The data presented in this study indicated that *C. filiformis* ability for iron binding and could reduce the generation of hydroxyl radicals.

Phosphomolybdenum assay

Methanol extract of *C. filiformis* was used to determine their antioxidant capacities by the formation of green phosphomolybdenum complex. The formation of the complex at 95°C was measured by the intensity of absorbance (695 nm) in extracts at the concentration of 100µg/ml as shown in Figure .7. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. In the present study, the phosphomolybdenum reduction potential of *C. filiformis* extract was in the order of test – 2.019, control – 0.508. The results indicate that the methanol extract of *C. filiformis* plant has powerful antioxidant activity. This may be explained by the fact that the transfer of electrons/hydrogen from antioxidants occurs at different redox potential in various assay systems and the transfer also depends on the structure of the antioxidants (Loo *et al.,* 2008).

Phytochemical screening of methanol extract of *C. filiformis:*

The preliminary phytochemical screening of *C. filiformis* has revealed the presence of phenolics, carbohydrates, glycosides and proteins in high amounts, whereas, amino acid was present in trace amount. (Figure. 8). The plant phenols are important group of natural antioxidants and have the ability to scavenge active oxygen species and electrophiles. Alkaloids are plant derived compounds with many physiological activities. They contain nitrogen in a heterocyclic ring with complex structure, which posses' potent antioxidant activity (Chung and Shin, 2007). Generally, glycosides are nonvolatile and fragrant. Glycosides serve as defense mechanism against predation by many microorganism and insects.

Thin Layer Chromatography:

The chromatogram developed with 20% methanol in chloroform revealed the presence of five major compounds at R_f value of 0.18; 0.31; 0.56; 0.81 and 0.97 as visualized under iodine vapour and UV illumination (Fig . 9).

Autobiography

The specific compound (band) which has anti oxidative properties shows in the Rf value of 0.56, which was chosen as effective compound and scrabbed that particular compound yields partial purified compound. The purity of the compound was checked by TLC with 20% methanol in chloroform (Fig .10). This method was chosen for its simplicity, low cost, accuracy and rapid results make it ideal for bioassay guided isolation (Eloff, 1998).

CONCLUSION

Flavanoids are a class of polyphenolics that can be synthesized from the amino acid phenylalanine. Phenolic compounds are a large group of antioxidant compounds found in many food systems (Sun *et al*., 2002). According to Rice – Evans *et al* (1997) the number of hydroxyl groups and the amount and types of conjugation are two important factors in the antioxidant potential of phenolic compounds. The better antioxidants are generally more conjugated and have numerous hydroxyl groups present (n=2 to 5), which enables the antioxidant to scavenge several radicals as shown in the table. The finding results supported by Norhaiza *et al* (2009) who reported that, *C. filiformis* showed significantly higher phenol and flavanoid content.

Natural antioxidants constitute a broad range of compounds including phenolics, nitrogen compounds (Velioglu, 1998). Among bioactive compounds naturally occurring phenolic flavanoids have gained a particular interest because of their broad pharmacological activity. The whole plant is the main source of antioxidants, which have shown radical scavenging activity and reducing potential. In the overall, the synthetic antioxidants such as butylated hydroxyytoluene (BHT) and ascorbic acid have been widely used for many years to retard lipid oxidation. However, the safety of using these synthetic antioxidants in food industry has become a concern among scientists and leading to current interest in uncovering natural antioxidants (Karimi *et al*., 2010).

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REFERENCES

Chung KT, Wong TY, Wie CI, Huang YW, Lin Y. (1998). Tannins and human health: A review. Crit. Rev. Food Sci. 38: 421 - 464.

Chung, H.S and Shin, J.C, 2007. Food chemistry. 104, 1670 - 1677.

Devasagayam TPA, Tilak JC, Boloor KK. 2004. Review: Free radical and antioxidants in human health. Curr Stat Fut Pros JAPI 53: 794 - 804.

Dinis TCP, Madeira VMC, Almeida LM. 1994. Action of phenolic derivatives (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Pharm. Res. 315: 161 - 169.

Eloff, J.N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica* 64: 711 - 713.

Gardner PT, White TC, McPhail DB, Duthie GG. 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chem. 68: 471 - 474.

Ghosal S, Tripathi VK, Chauhan S. 1996. Active constituents of Emblica officinalis. Part I. The chemistry and antioxidant effects of two new hydrolysable tannins, emblicanin A and B. Ind. J. Chem. 35B: 941 - 948.

Kikuzaki, H. & Nakatani, N. 1993. Antioxidant effects ofsome ginger constituents. J Food Sci 58, 1407 - 141.

Klein SM, Cohen G, Cederbaum AI. 1991. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. Biochemistry 20: 6006 - 6012.

Loo AY, Jain K, Darah I. 2008. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. Food Chem. 107: 1151 - 1160.

Nenadis N., Zafiropoulou I., Tsimidou M., 2003. Commonly used food antioxidants: Acomparative studyin dispersed systems. Food Chemistry 82, 403 - 407.

Nishikimi M, Rao NA, Yagi K. 1992. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 46:849 - 854.

Osawa T, Namiki M A. 1981. A novel type of antioxidants isolated from leaf wax of eucalyptus leaves. *Agric. Biol. Chem.* 45, 725 - 739.

Prieto P, Pineda M, Aguilar M. 1999. Spectophotometric quantitative of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 269: 337 - 341.

Shimada KK, Fujikawa KY, Nakamura T. 1992. Antioxidative properties of xanthone on autoxidation of soybean oil in cyclodextrin. J. Agric. Food Chem. 40: 945 - 948.

Siddhuraju P and Becker, K. 2007. The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts. Food Chemistry, 101, 10 - 19.

Singh B, Bhat TK, Singh B. 2003. Potential therapeutic applications of some antinutritional plant secondary metabolites. J. Agr. Food Chem. 51: 5579 - 5597.

Soares JR, Dinis TCP, Cunha AP, Almedia LM. 1997. Antioxidant activities of some extract of *Thumus zygis*. Free. Radic. Res. 26: 469 - 478.

Yang J, Guo J, Yuan J. 2008. *In vitro* antioxidant properties of rutin. Lebensm. Wiss. Technol. 41: 1060 - 1066.

Yen GC, Duh PD, Tsai HL. 2002. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chem. 79: 307 - 313.

Karimi E, Oskoueian E, Hendra R, Jaafar H ZE, 2010. Evaluation of *Crocus sativus* L. Stigma Phenolic and Flavanoid compounds and its antioxidant activity. Mole.J., 15: 6244 - 6256.

Norhaiza M, Maziah M, Hakiman M, 2009. Antioxidative properties of leaf extracts of a popular Malaysian herb, Labisia pumila. J. Med. Plants Res., 3 (4): 217 - 223.

Rice – Evans CA, Miller NJ, Pagangaa G, 1997. Antioxidant properties of phenolic compounds. Trends plant Sci., 2: 152 - 159.

Sun J, Chu YF, Wu X, Liu R, 2002. Antioxidant and antiproliferative activities of common fruits J. Agric. Food. Chem.., 50: 7449 -7454.

Velioglu YS, Mazza G, Gao YL, Oomah BD, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food chem., 46: 4113 - 4117.