

Comparative Studies on Antioxidant Properties of *Cissus quadrangularis* Solvent Extracts

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ABSTRACT

Cissus quadrangularis L. is one of the important medicinal plants, belonging to the family Vitaceae and common name is 'Hadjod'. It is a succulent plant found in warm tropical areas. The plant is well known in Ayurveda for its bone fracture healing properties. In the present study the antioxidant property of different plant samples eluted with solvents like methanol, ethanol and petroleum ether were checked by different assays like total antioxidant capacity, FRAP and ABTS assay. The samples showed increase in antioxidant property with the increase in the concentration indicating that they have increased antioxidant property. The stem and leaves sample extracted with all the three solvents like methanol, ethanol and petroleum ether showed higher antioxidant property than the fruit and root samples when analysed by FRAP assay. The fruit extract from all the three solvent showed higher total antioxidant capacity and leaf extract showed the least antioxidant capacity. ABTS assay was carried out for all the samples and the root extract from different solvents showed a better capacity of antioxidants when compared to other samples like stem, leaf and fruit. The antioxidant property of the fruit sample was found to be minimum when checked with all the three assays.

Key words: Antioxidant, FRAP, ABTS, Total antioxidant capacity, *Cissus quadrangularis*.

INTRODUCTION

Medicinal plants are one of the main sources for new pharmaceutical and health care products as most of the plants contain phytochemicals which have curative/protective properties against various diseases. Most phytochemicals, especially phenolics have been proved to benefit health of the human beings by scavenging free radicals or quenching reactive oxygen species (1).

Phenolics are, at least in part, plants responsible for antioxidant activity, and their contents in plants were associated with antioxidant activity (2). Ascorbic acid also has antioxidant activity and is essential for the maintenance of normal function of living cells (3). It has been reported that the majority of drugs come from natural resources and that approximately 60 to 80% of the world's population still believe in folk/traditional medicine (4).

According to World Health Organization (WHO), nearly 80 per cent of the people in developing countries consume traditional medicines for nourishing health and gaining strength. Trivedi *et al.*, (2006) have reported that in entire world around 20,000 to 35,000 species of plants are used in pharmaceuticals, nutraceuticals, cosmetics and as medicines. Many traditional herbal therapies have originated from the medicinal properties of different plant species. In pharmaceuticals, the essential component of both research and development are medicinal plants. *Cissus quadrangularis* is commonly known as "Hadjod", found on the lower slopes of the Western Ghats and dry areas of Arabia, Africa, India, Sri Lanka, Malaysia and Thailand (5) and commonly used as a food item in Southern India (6) and Sri Lanka (7). In Ayurvedic System of medicine, stem of *C. quadrangularis* Linn is very important part of the

plant and used in piles, bone fracture, pain in joints, swelling, scurvy, gout, asthma, scurvy, disease of ear and nose bleeding (8).

MATERIALS AND METHODS

Sample Collection

Fresh & healthy plant parts of *Cissus quadrangularis* like stem, leaf, flower, fruit & root were collected in a separate sterile polythene bags from the area in and around Arogyavaram, Madanapalli (tq), Chittoor (dist), Andhra Pradesh. Collected plant parts were examined and identified with the help of regional floras. Specimens were further confirmed with reference to Herbarium sheets available in the department of Botany, Tumkur University, Tumkur, Karnataka, India.

Preparation of Solvent Extracts

The cleaned, healthy plant materials are cut in to small sections and dried under shade for three to four weeks. The dried material was ground into fine powder in an electric grinder. Powder so obtained was stored in desiccators' setup and used for extraction.

Extraction was carried out using 5gm of each sample coarsely powdered plant material with 50 ml of solvent and kept for 48 hrs with slight shaking. Here, ethanol, petroleum ether and methanol (HPLC grade) were used as a solvent for extraction; different solvents elute different compounds from the sample. The extraction was done at room temperature. All the extracts were filtered through Whatmann No.1 paper to get filtrate as extracts and were dried to concentrate the samples. The residual powder was weighed and was re dissolved in the respective solvents to get a final concentration 1mg/ml. The powder was stored in airtight containers under refrigeration condition.

Ferric Reducing Antioxidant Potential – FRAP

The total antioxidant activity of each plant extract was measured by ferric reducing antioxidant power assay of Benzie and Strain (1999). Fresh FRAP reagent was prepared by mixing 25 ml of 300 mM of acetate buffer pH 3.6, 2.5 ml of 10 mM TPTZ solution made in 40 mM of hydrochloric acid and 2.5 ml of 20 mM ferric chloride solution. The mixture was then warmed at 37 °C for 15 minutes before use. The FRAP reagent (2.85 ml) was mixed with 150 µl of a plant extract or standard. The mixture was incubated for 30 minutes in dark. The absorbance of the mixture was then noted at 593 nm. The FRAP values of samples were expressed as micrograms per millilitre of Ascorbic Acid Equivalents (µg/ml of AAE).

Total Antioxidant Activity

Total antioxidant activity is a quantitative assay, since the antioxidant activity is expressed as the number of equivalents of Ascorbic acid. The assay is based on the reduction of Mo (VI) to Mo(V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH with the maximal absorption at 695nm (5µg/ml) as standard as at this concentration, absorbance becomes almost constant (9). The basic principle is the scavenging ability of fruit juices on the phosphomolybdenum reagent. Different concentrations of juices were prepared in distilled water. 4.5 ml of phosphomolybdate reagent was added. After incubation at 95°C temperature for 90 minutes, optical density was measured at 695nm. All the samples were prepared in triplicate and the average of the three reading obtained was taken as the final absorbance.

Total antioxidant capacity was calculated by the following formula.

$$\text{Total antioxidant capacity (\%)} = [(As-Ac) / (Aaa - Ac)] \times 100.$$

Where, Ac = control absorbance, As = sample absorbance, Aaa = amino acid absorbance. Total antioxidant activity was also determined in terms of ascorbic acid (in mg/ml).

ABTS Assay

ABTS•+ Assay protocol has developed by Re et al. was followed. ABTS was dissolved in water to a mM concentration. ABTS radical cation (ABTS•+) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the study of antioxidant activity of citrus juices, the ABTS stock solution was diluted with methanol to an absorbance of 0.70 (+0.02) at 745 nm and equilibrated at 30 °C. After addition of 3.4 ml of diluted ABTS•+ solution (A734nm = 0.700 + 0.020) to 100 µl of diluted sample solutions, the absorbance reading was taken at 25 °C exactly 1 min after initial mixing up to 6 minutes. Appropriate solvent blanks were run in each assay. All the determinations were carried out at least three times, and in triplicate, on each occasion and at each separate

concentration of the standards and samples. The percentage inhibition of absorbance at 734 nm is calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data.

RESULTS AND DISCUSSION

The antioxidant property for different parts of the plant like stem, root, leaves and fruit was investigated with different solvents. The antioxidant property of the sample determines the capacity to reduce the nasent oxygen and reducing the diseases.

FRAP Assay

When extracted with petroleum ether showed higher OD value for stem sample and the fruit sample showed the lowest The FRAP assay was carried out for ethanolic extract and the stem samples showed higher OD value followed by the leaf samples. The least was observed in root samples. The OD value increased with increase in the concentration of the sample (Figure 1). The methanolic extract of leaf showed better results for FRAP assay whereas root showed the least (Figure 2). The stem samples values (Figure 3).

Figure 1: FRAP Assay- Ethanolic extract

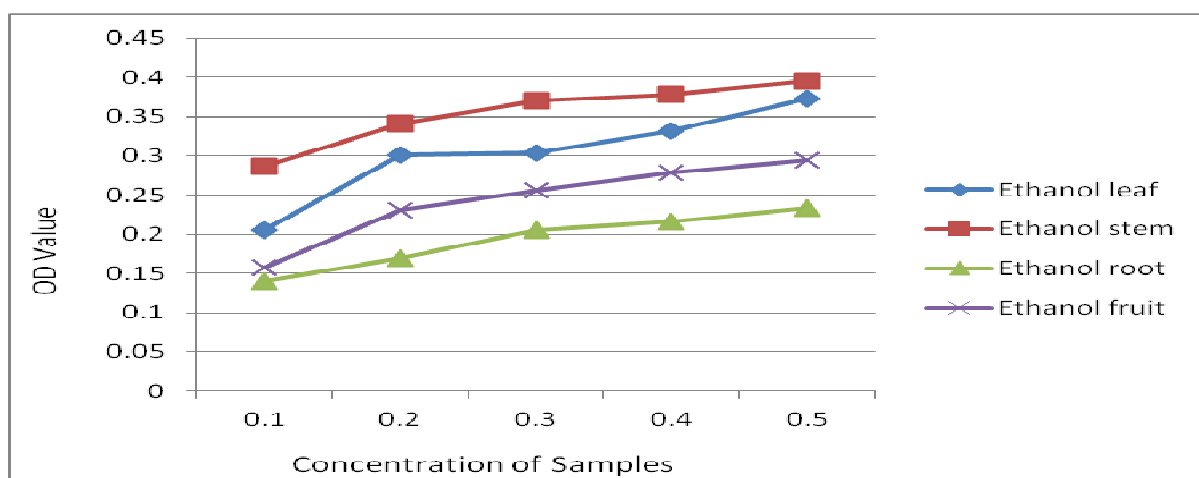
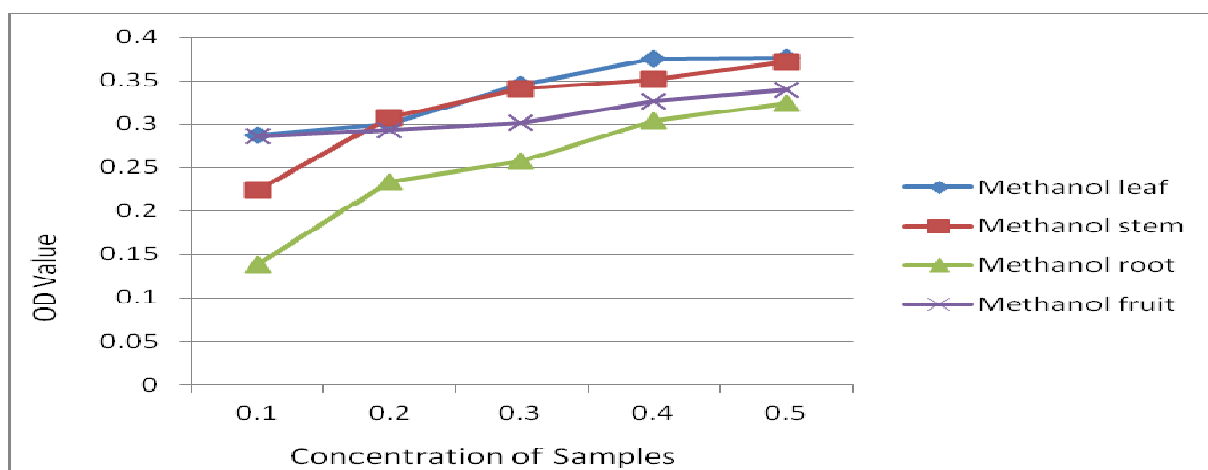


Figure 2: FRAP Assay- Methanolic extract



Total antioxidant capacity

The fruit extract of methanolic solvent showed higher total antioxidant capacity and leaf extract showed the least antioxidant capacity (Figure 4). The ethanolic extract of the root sample showed better antioxidant capacity when compared to other samples extracted from ethanol (Figure 5). The root sample when extracted with petroleum ether showed a higher increase in the antioxidant capacity. Where as the stem sample showed an increase in the antioxidant capacity for few concentrations and then reduced (Figure 6).

Figure 3: FRAP Assay- Petroleum ether extract

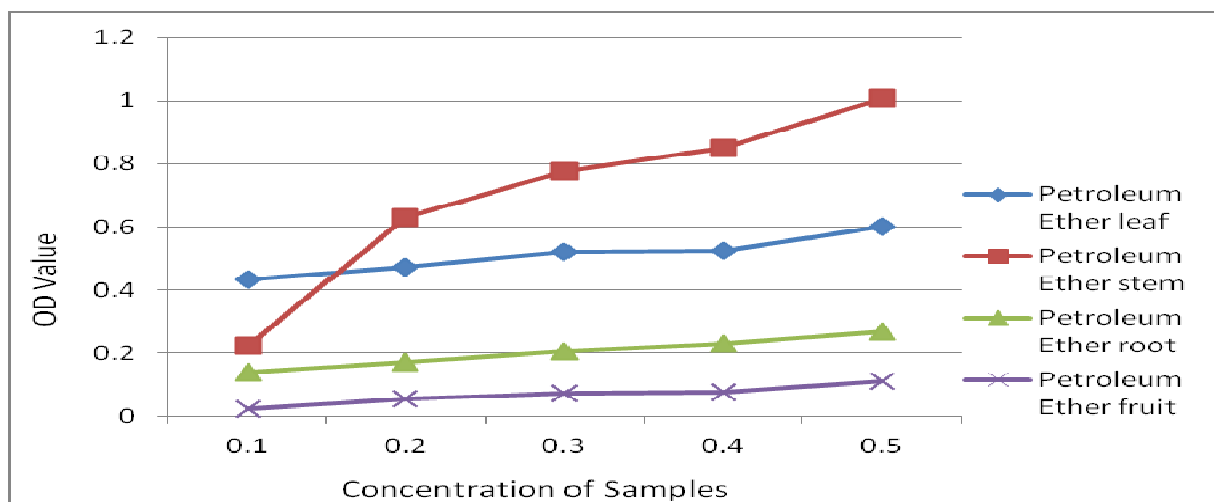


Figure 4: TAC Assay- Methanolic extract

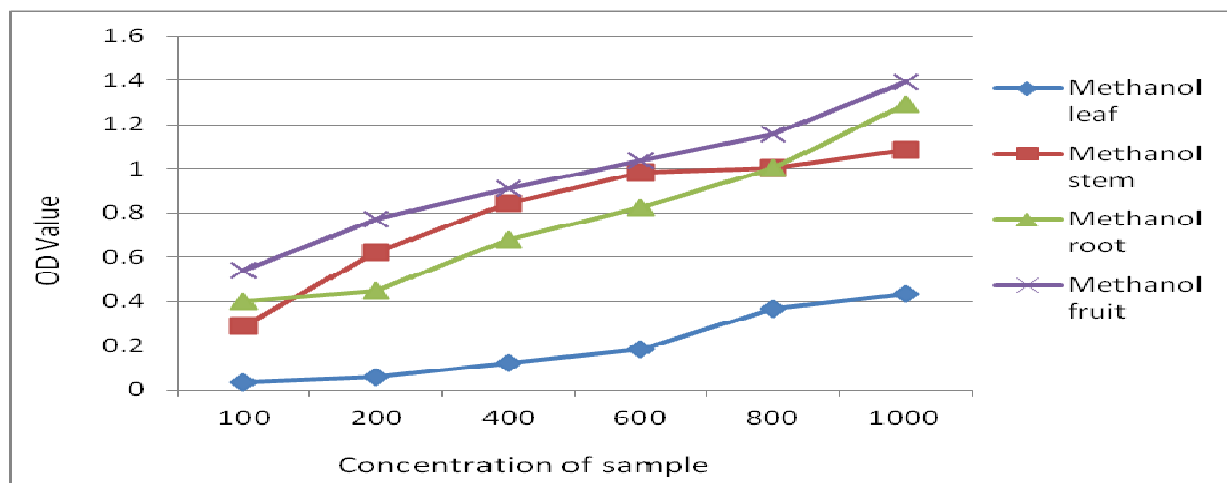
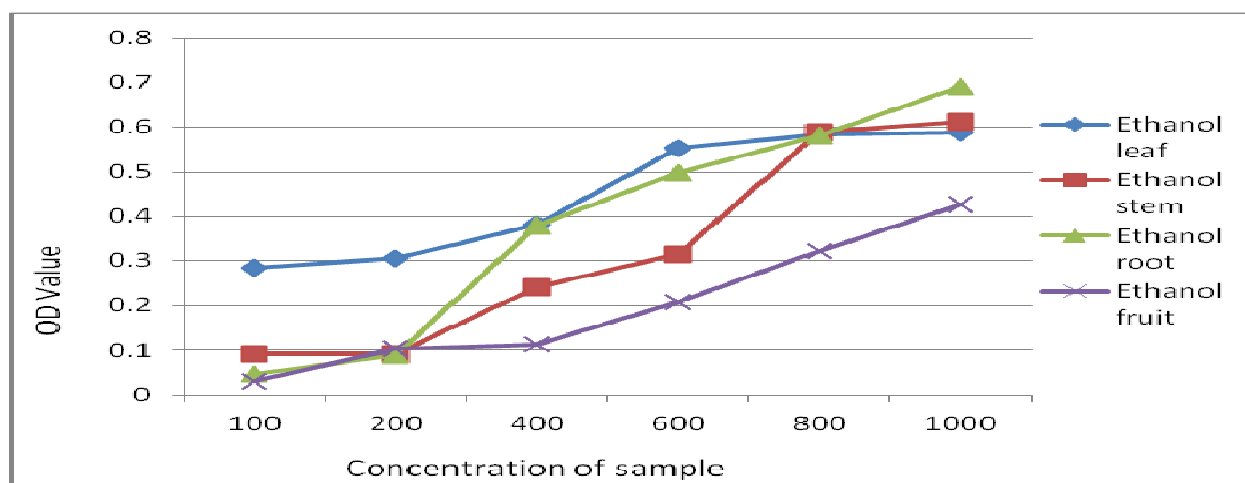


Figure 5: TAC Assay- Ethanolic extract



ABTS Assay

The ethanolic root extract showed a higher OD value for ABTS assay in the form of linear scale. Whereas the other three samples showed very less antioxidant capacity when checked with ABTS assay (Figure 7). The ABTS assay was carried out for methanolic extract and was determine that the root samples showed better OD value for the assay and the other samples showed lower OD values for the assay (Figure 8). The ABTS assay of the petroleum ether

extract from all the samples showed similar kind of OD value for ABTS assay. The increase in the concentration of the sample showed increase in the antioxidant property when checked with ABTS assay (Figure 9).

Figure 6: TAC Assay- Petroleum ether extract

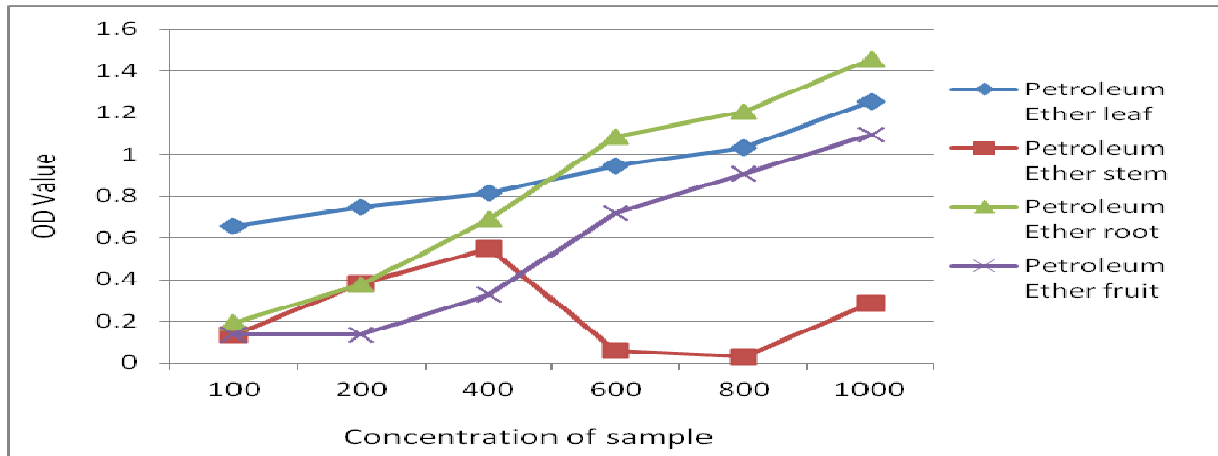


Figure 7: ABTS Assay- Ethanolic extract

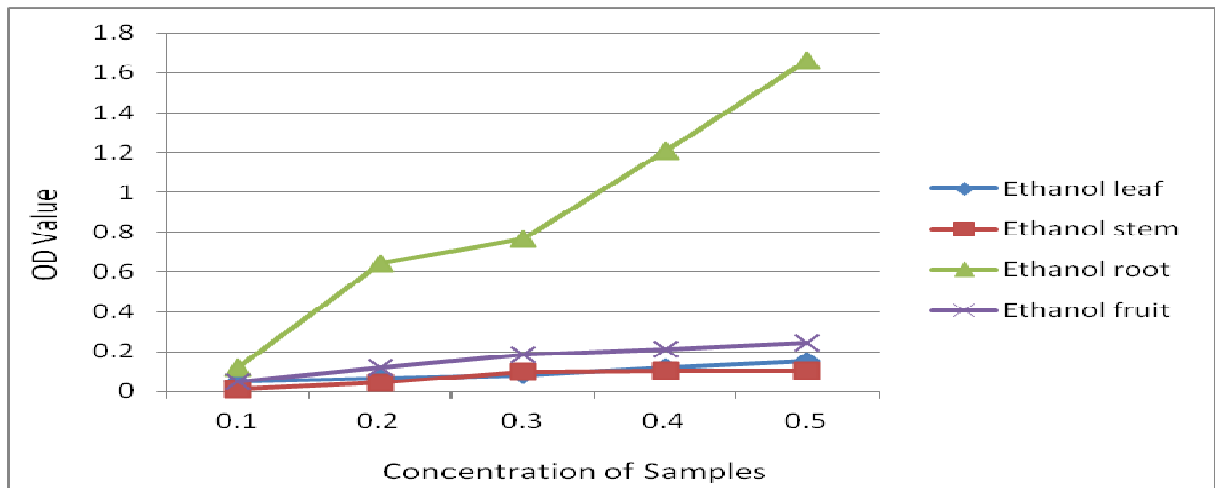


Figure 8: ABTS Assay- Methanolic extract

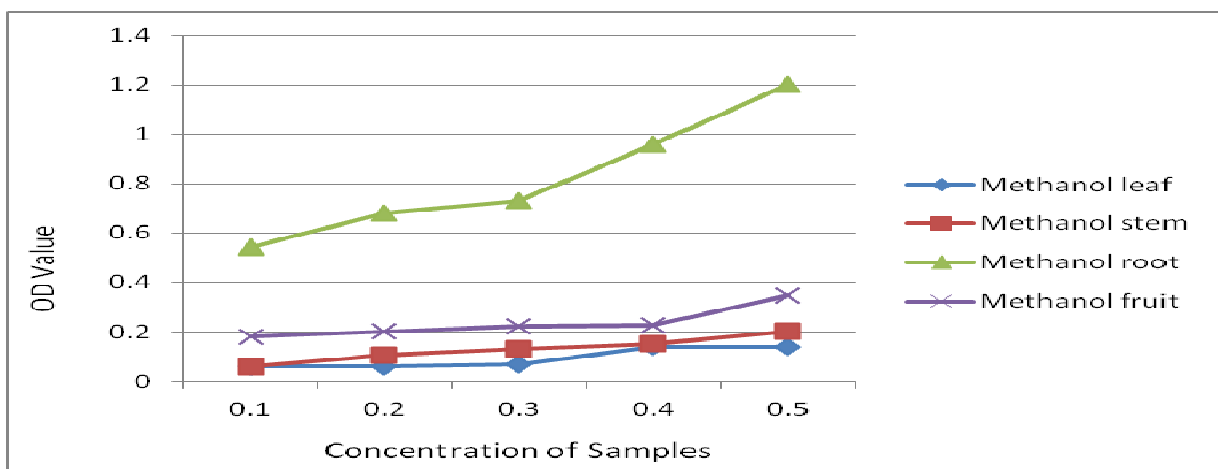
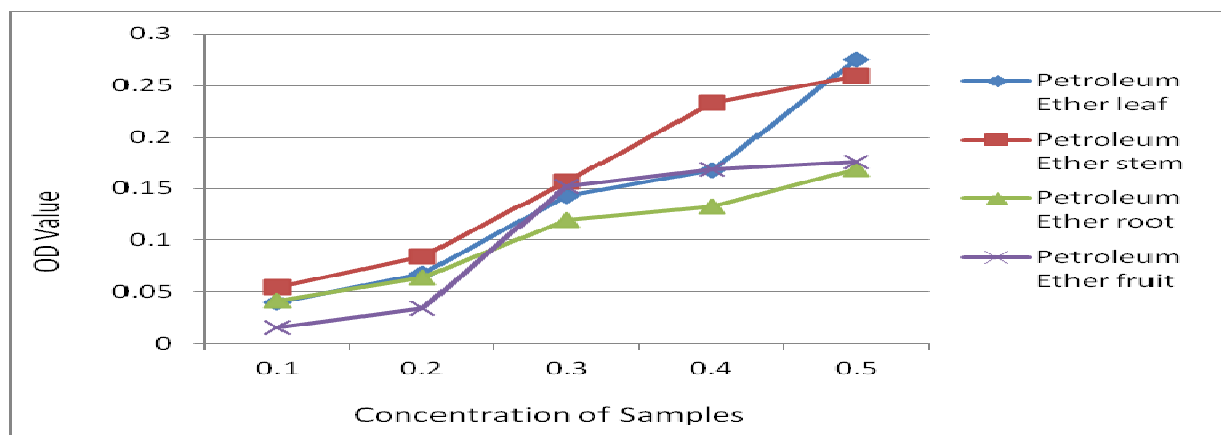


Figure 9: ABTS Assay- Petroleum ether extract



DISCUSSION

The plant is very much known in Ayurveda for the treatment of rheumatoid arthritis, osteoporosis, osteoarthritis (10) scurvy, menstrual disorders and epistaxis (11). In East Africa it is used with tamarind for the treatment of gonorrhoea (12). A stem paste is useful in burns, wounds, bites of poisonous insects and for saddle sores of camels and horses (13). The stem of *C. quadrangularis* is used for the treatment of gastritis, constipation, eye diseases, piles and anemia. The alcoholic extract of *C. quadrangularis* stem has been found to be useful in enhancing bone fracture healing, ossification of fetal bone, and increasing the thickness of trabecular bone (14, 15, 16, 17)

The ethanolic extract of *C. Quadrangularis* seems to have estrogenic properties by increasing the serum estrogen rather than directly acting on estrogens' receptors (18).

The extract of *C. quadrangularis* can stimulate osteoblastic proliferation and differentiation and may effect the promotion of mesenchymal stem cells to osteoblasts. The production of osteoblasts is related to 6'- O-trans-cinnamoyl-catalpol which is present in *C. quadrangularis* (19).

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