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In-vitro Antioxidant Studies of *Cissus quadrangularis (L) extracts*

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ABSTRACT

Cissus quadrangularis Linn, the plants have been used in traditional medicines for treatment of different ailments from ancient times. Medicinal plants have been one of the richest bio resources for traditional and folk medicines till date. In the present investigation the medicinal property of Cissus quadrangularis Linn was done by determining the antioxidant properties of different parts of the plant when eluted. belongs to the family Vitaceae. It is a wild indigenous medicinal plant found in India, locally known as Hadjod. The antioxidant property was determined by DPPH assay, DMPD assay and reducing power assay for all the samples. The DPPH reduced with the increase in the concentration of the sample and methanolic extract of root and leaf showed least IC-50 value with 600 µg/ml. The methanolic extract of the samples proved to have a higher potential to reduce DMPD with a standard concentration of sample. The methanolic leaf extracts showed higher reducing power activity. The methanolic extracts showed a better antioxidant property for all the assays when compared to the ethanolic and petroleum extract of the samples.

Key words: Cissus *quadrangularis*, Antioxidant, DPPH, DMPD, Reducing power.

INTRODUCTION

Medicinal plants and plant extracts represent the oldest and most widespread form of medication. At least 25% of the active compounds in currently prescribed synthetic drugs were first identified in plant sources [1]. According to WHO more than 80% of the world's population relies on traditional herbal medicine for their primary health care [2].

In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period. However, due to over population, urbanization and continuous exploitation of these herbal reserves, the natural resources along with their related traditional knowledge are depleting day by day [3]. WHO has even estimated that in each year about one fourth of the 500 million prescriptions given in US mention plant extracts or active ingredients obtained from plants.

Cissus quadrangularis (Linn) is a common perennial climber, which is distributed throughout India particularly in tropical regions. It belongs to family Vitaceae and is also called as *Vitis quadrangularis.* The plant is commonly known as Vajravalli in Sanskrit, Hadjod in Hindi, Kandvel in Marathi, Haddjor in Punjabi, Hadbhanga in Oria, Vedhari in Gujrati, Perandi in Tamil and Nalleru in Telugu.The photochemical analysis of plant has proved to contain potassium, calcium, zinc, sodium, iron, lead, cadmium, copper, calcium oxalate and magnesium. Other constituents of the plant are resveratrol, piceatannol, pallidol, parthenocissus, 31 methyl triacontanoic acid, taraxeryl

acetate, taraxerol, iso‑pentadecanoic acid, phenol, tannin, carotene and vitamin.Toxicology studies have showed that the *Cissus quadrangularis* extract does not produce any toxic effect [4, 5]. Antioxidant are the substances which scavenge free radicals and they play an important role in the prevention of free radical-induced diseases by donating hydrogen radicals to the primary radicals which gets reduced to non-radical chemical compounds and then gets converted to oxidize antioxidant radicals [6; 7]. The chemical constituents of *Cissus quadrangularis* posses novel flavonoids and indanes, as well as phytosterols and keto-steroids which have shown to be powerful and efficient antioxidants [8, 9]. They have even been efficient in inhibiting lipase and amylase, thereby providing a mechanism for weight loss via reduced oxidative stress, dietary fat, and carbohydrate blocking.

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), Chittor (dist), and 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, petrolium 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 power 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

DPPH Radical Scavenging Activity

The radical scavenging activity of DPPH was determined using the method reported by Brand–Williams *et al*. [1995]. Briefly, 10 mg of each plant fraction was dissolved in 10 ml of methanol. The stock solution was prepared by dissolving 24 mg of DPPH in 100 ml of methanol and kept in a refrigerator until used. The working solution was obtained by diluting the DPPH stock solution with methanol to obtain an absorbance of about 0.98 (±0.02) at 517 nm. In a glass vial, 3 ml of the working solution was mixed with 100 µl of the plant extract or the standard solution and its absorbance was measured at 517 nm for a period of 30 min. The percent scavenging activity was calculated.
Where % DPPH is the percent of unreacted DPPH, [DPPH] T=0 is the concentration of DPPH before reaction with antioxidant sample. [DPPH]T=t is the concentration of DPPH after reaction with antioxidant sample at time t. Ascorbic acid was used as a standard. EC50 value was also determined which is the effective concentration that has the potential to scavenge 50% of the DPPH radicals. TEC50 or the time taken by the sample to scavenge 50% of the DPPH radicals was also determined.

Phosphomolybdate Antioxidant Assay

The phosphomolybdate antioxidant assay was carried out according to the procedure reported by Umamaheswari and Chatterjee [2008]. Briefly, 25 mg of each plant fraction was dissolved in 10 ml of methanol. Phosphomolybdate reagent was prepared by mixing 0.6 M sulphuric acid (100 ml), 4 mM ammonium molybdate (100 ml) and 28 mM sodium phosphate (100 ml) solution. In a test tube, 3 ml of phosphomolybdate reagent, 300 µl of the plant extract or standard solution or methanol was taken and mixed. The test tubes were capped with silver foil and incubated in water bath at 95^0 c for 90 minutes. After the contents of the test tubes were cooled down, the absorbance of the test tube contents were measured at 765 nm against a blank. Ascorbic acid was used as a standard. The antioxidant activities of sample fractions were expressed as micrograms per millilitre of Ascorbic Acid Equivalents (µg/ml of AAE).

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 37^0 c for 15 minutes before use. The FRAP reagent (2.85 ml) was mixed with 150

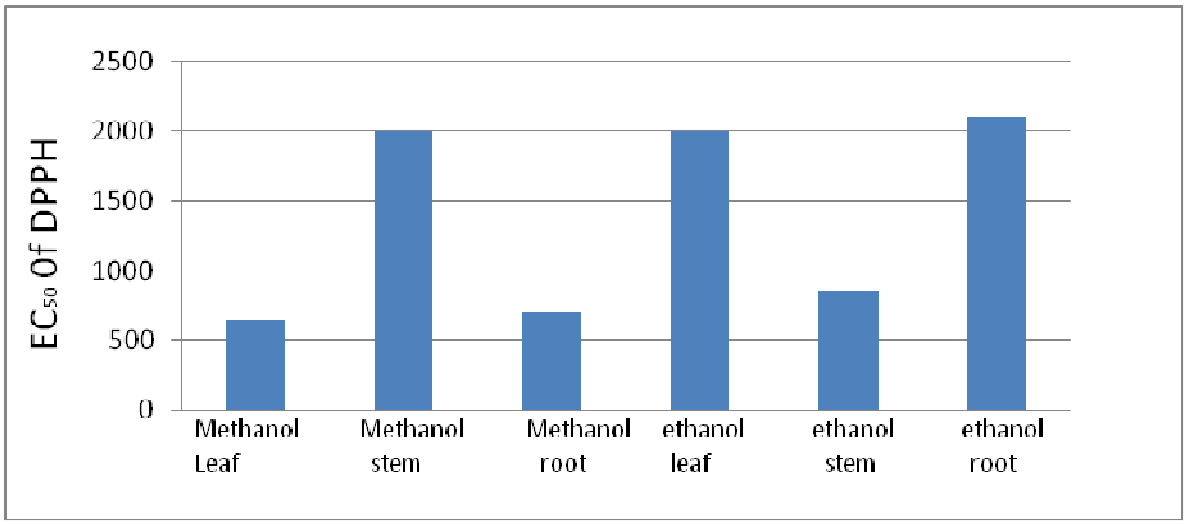
µl of a plant extract or standard. The mixture was incubated for 30 minutes in dark. The absorbance of the mixture was then noted at 593 nm. The FRAP values of samples were expressed as micrograms per millilitre of Ascorbic Acid Equivalents (µg/ml of AAE).

RESULTS

DPPH Radical Scavenging

The DPPH assay was carried out for the extracts and the IC-50 value was determined where in the DPPH was reduced to 50% from its initial concentration. The methanol root and leaf extract showed IC-50 value with 600µg/ml. The DPPH reduced with the increase in the concentration of the sample (Figure 1).

Figure 1: EC-50 value for DPPH assay



DMPD Assay

The methanolic extract of the samples proved to have a higher potential to reduce DMPD with a standard concentration of sample. The leaf methanolic extract showed highest OD value determining that it has high capacity of antioxidant property (Figure 2-4).

Figure 2: DMPD Assay for methanolic extract

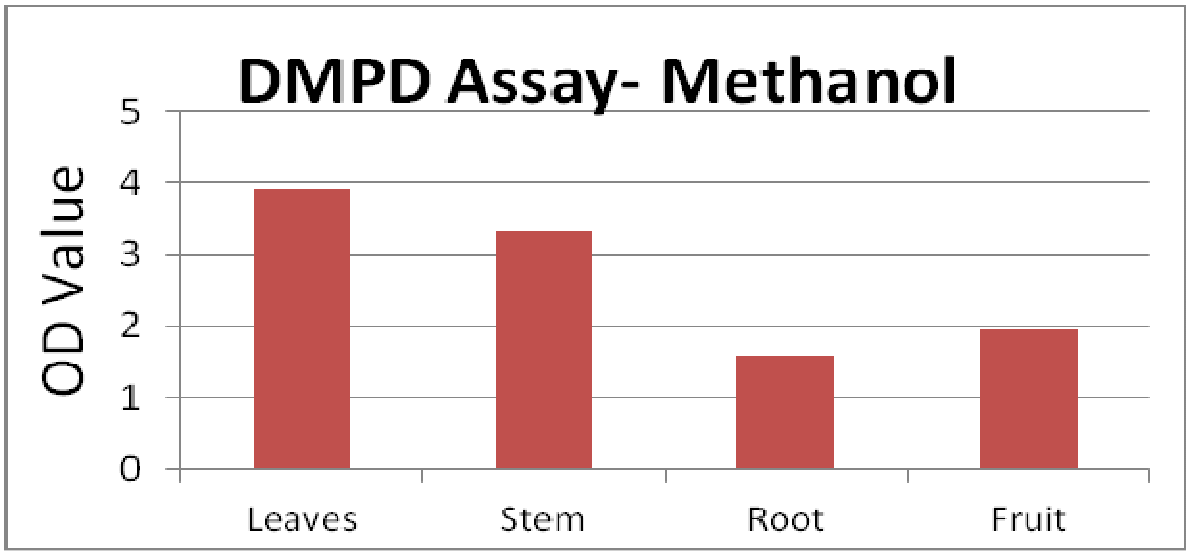


Figure 3: DMPD Assay for Ethanolic extract

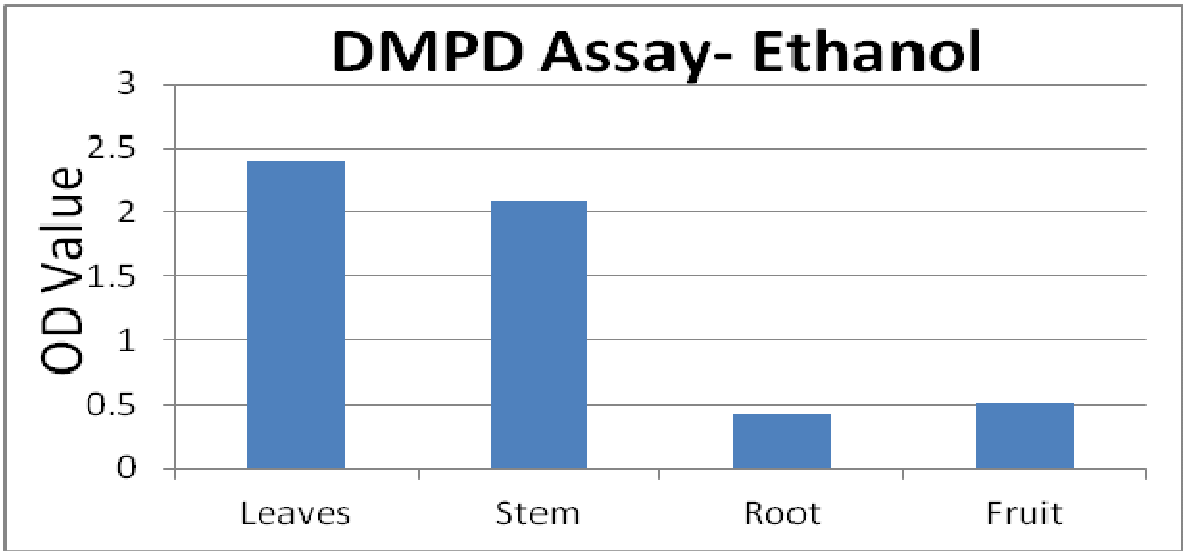
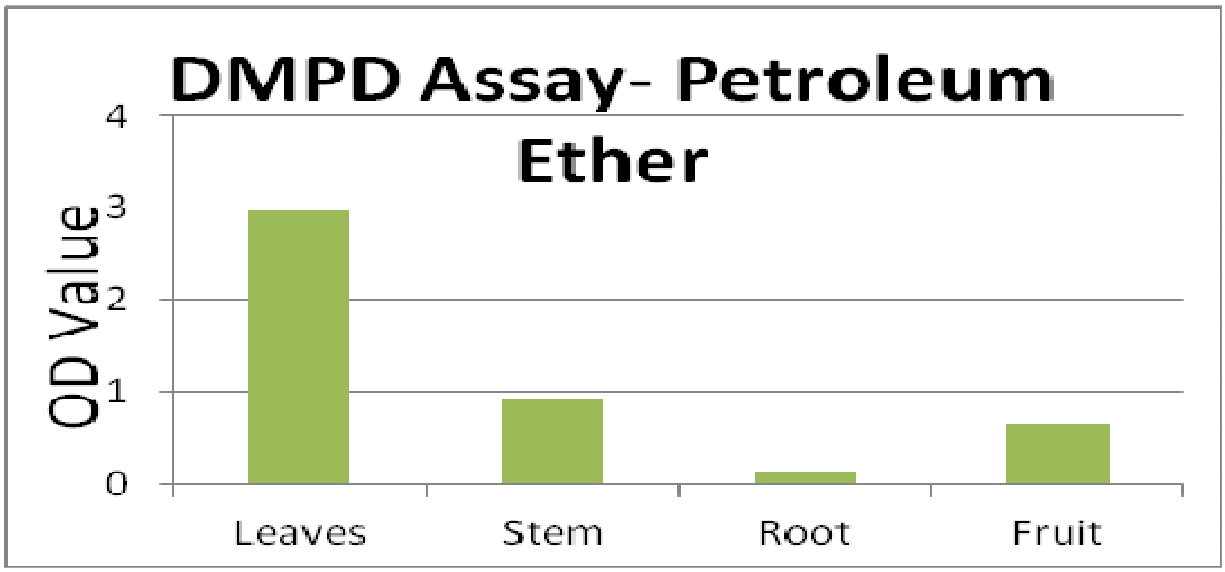


Figure 4: DMPD Assay for Petroleum ether extract



Reducing Power Assay

Methanolic leaf extracts show higher reducing power activity than the ethanolic and petroleum leaf extracts. The ethanolic sample shows lower power to reduce ferric. The methanolic and ethanolic extract of stem shows similar reduction ability, but the petroleum extract shows a lower potential to reduce ferrous containing substances. The methanolic root extract has a comparable higher potential than the other two solvents (Figure 5-7).

Figure 5: Reducing power Assay for methanolic extract

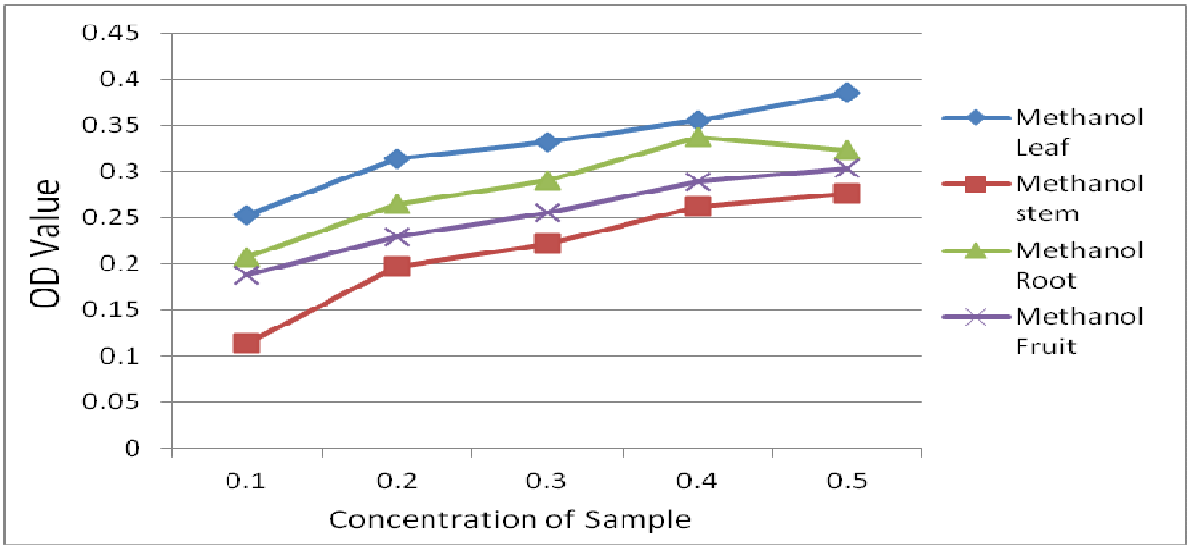


Figure 6: Reducing power Assay for Ethanolic extract

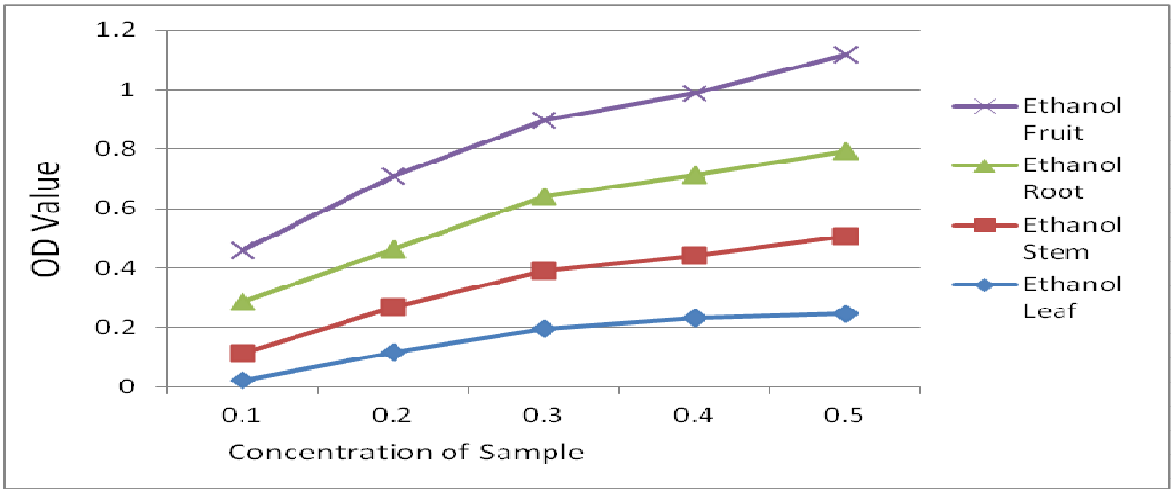
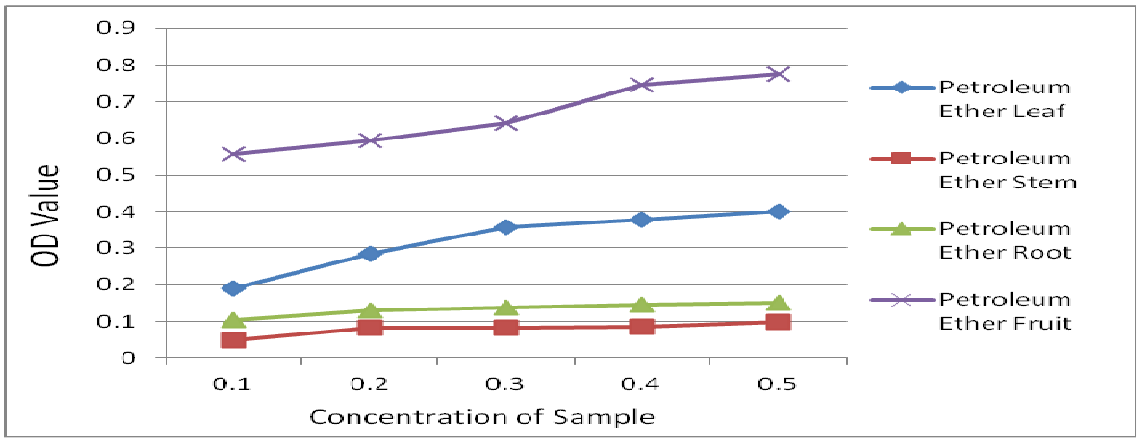


Figure 7: Reducing power Assay for Petroleum Ether extract



DISCUSSION

The stem pulp of *Cissus quadrangularis* L. (Vitaceae), a commonly consumed diet in India has been used for fracture healing, eye diseases, chronic ulcers, tumours, asthma and piles [10]. The tender shoots and young leaves are used in various food preparations. The juice of the plant is said to be beneficial in scurvy [11]. The medicinal

properties have been described in literature as early as 1970 [12]. Ethnobotanical uses of the plant have been reported by [13, 14] investigated that the stem and root extract of this plant possess antioxidant and antimicrobial activity.

The Phytochemical study has been investigated for *Cissus quadrangularis* and it has been proved to be a natural source for ascorbic acid, carotene, anabolic steroidal substances, and calcium in high content. The stem contains two asymmetric tetracyclic triterpenoids, and two steroidal principles. The presence of β-sitosterol, δ-amyrin, δ-amyrone, and quercetin has also been reported in the recent years [15].

Antioxidant and free radical scavenging potential from methanol extract of *Cissus quadrangularis* (CQE) were studied using the model of hepatotoxicity induced by carbon tetrachloride in rats and concluded that the free radical scavenging activity of the plant extract may be responsible for the therapeutic action against tissue damage[16].

REFERENCES

[1] Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH, *Natural plant chemicals: sources of industrial and medicinal materials. Science (New York, NY)*, **1985**, *228:1154–60*.
[2] Hashim H, Kamali EL, Mohammed Y, *Curr Res J Biol Sci*, **2010,** 2:143‑6.
[3] Pande PC, Tiwari L, Pande HC, *Indian J Trad Knowl* ,**2007**, *6:444‑58*.
[4] Attawish A, Chavalttumrong D, Chivapat S, Chuthaputi S, Rattarajarasroj S, Punyamong S, *J Sci Technol* , **2002**, *24: 39‑51*.
[5] Prasad GC, Udupa KN, *Indian J Med Res*, **1963**, *51:667*.
[6] Jadhav SJ, Nimbalkar SS, Kulkarni AD & Madhavi DL, *Lipid Oxidation In Biological and Food Systems. In: Food Antioxidants, New York*. **1995.**
[7] Yamaguchi T, Takamura H, Matoba T & Terao J, *Biochem*, **1998**, *62 : 1201-1204.*
[8] Oben JE, Enyegue DM, Fomekong GI, Soukontoua YB, Agbor GA, *Lipids Health Dis*, **2007**, *6:4*.
[9] Sharp H, Hollinshead J, Bartholimew BB, Oben J, Watson A, Nash RJ, *National Product Communications*, **2007**, *2:817-22*.
[10] Asolkar, L.V, Kakkar, K.K, Chakre, O.J, *Glossary of Indian Medicinal Plants with active principles, CSIR Publication, New Delhi*, **1997**, *145-150*.
[11] Kirtikar K.R, Basu B.D, *Indian Medicinal Plants, 3rd revised and enlarged ed., By Basu, l.M.*,**2000**, *841-843*.
[12] Udupa KN, Chaturvedi GN, Tripathi SN, *Advances in research in Indian medicine*, **1970**, 12: 165-196.
[13] Kumbhojkar MS, Kulkarni DK, Upadhye AS, *Ethnobotany***, 1991**, 3:21-25.
[14] Murthy KNC, Vanitha A, Swami MM, Ravi shankar G, *J. Med.Food* , **2003**, 6:99-105
[15] Jakikasem S, Limsiriwong P, Kajsongkarm T, Sontorntanasart T, *Thai J Pharm Sci*., **2000**, *24:25*.
[16] Jainu and C.S. Devi**,** *African Journal of Biomedical Research*, **2005,** *8(2), pp.95-99.*