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Study Of Fatty Acids Composition And Antioxidant Potential Of Two Corchorus Species Seed Oils Fatty Acids Methyl Esters From Rajasthan

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Abstract-

Background- Corchorus olitorius and Corchorus trilocularis are the medicinal plants belonging to family Tiliaceae found in western Rajasthan.

Aims-The Physico-chemical characteristics, fatty acids composition, antioxidant potential and total phenolic content of the two unexplored Corchorus species (C.olitorius and C.trilocularis) fatty acids methyl esters (FAMEs) seed oils from Rajasthan were analyzed.

Methods and materials-The seed oils of both the Corchorus species were extracted by using soxhlet apparatus. The fatty acid compositions were determined by using GC-MS technique. The antioxidant potential were analyzed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and Ferric reducing ability of plasma (FRAP) methods.

Results and conclusion-The C.olitorius seed oil was reported with higher amount of oleic acid as compared to C.trilocularis. The results confirmed that both the Corchorus species have significant antioxidant properties. C.olitorius has the higher trolox equivalent antioxidant capacity than in C. trilocularis. Similarly DPPH activity reported in C.olitorius FAMEs seed oil is quite higher than in C.trilocularis. The total phenolic content in C.olitorius and C.trilocularis were 32.58±2.1 mg GAE/g oil and 25.98±1.5 mg GAE/g respectively. These results suggested that both these Corchorus species could be employed as a possible source of monounsaturated fatty acids and also phenolic components with antioxidant properties in the food industry, and pharma sectors.

Keywords- Corchorus, fatty acids composition, Antioxidant activity and Total phenolic content

Introduction

Western Rajasthan's arid zone has its significance and distinct characteristics in terms of indigenous and a huge proportion of economically and medicinally important plants. Rajasthan's arid zone is an excellent example of where medicinal plants are commonly employed in ordinary life as part of traditional medical cures. However, very limited literature is available about the

antioxidant capacity of arid-zone plants.[1] Seed oils obtained from plants are rich in antioxidants. Antioxidants are significant health-protective agents. According to scientific research, antioxidants minimize the chances of chronic illnesses such as cancer and cardiovascular disease. Whole grains, seeds, fruits, and vegetables are excellent sources of natural antioxidants. The distinguishing feature of antioxidant agents is to trap free radicals.[2] Free radicals can damage nucleic acids, proteins, lipids, and even DNA, as well as cause the initiation of degenerative illnesses.[3] The human body contains several defensive systems, both enzymatic and non-enzymatic, that combat the detrimental damage caused by free radicals and some other oxidants. Therapeutic plants have been employed for their medicinal powers since prehistoric times. The plants' crude extract is now being employed in the development and production of traditional medicines.[4,5] Plants have an abundance of compounds with varied pharmacological and biological activity, and they are recognized to be a useful source of biochemical or phytochemical substances for the treatment of many ailments. Several techniques were developed to assess the antioxidant content of different plant parts. The ferric reducing antioxidant power (FRAP) and DPPH(1,1-diphenyl-2-picrylhydrazyl) tests, in particular, have been frequently utilized. [6] Fatty acids (FAs) are vital chemical elements of cells that serve as fuel for various biological and metabolic functions, including muscle activity, and have both nutritive and medicinal benefits. They are mainly three types of fatty acids Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) found in medicinal plants (PUFA).[7] Antioxidants operate as free radical scavengers, preventing and rectifying the damage caused by free radicals. Recently, medicinal plants mainly from Rajasthan have been the target of pharmaceutical companies and research organizations for exploring the novel drugs.

Corchorus is a genus belonging to the Tiliaceae family mostly found in tropical and temperate climates. Pharmacological qualities abound throughout this genus. Spasmolytic action,antihistaminic activity, hepatobiliary, renal, and hematological activity, antibacterial activity antiestrogenic, anticonvulsant, and antimalarial activity were also demonstrated by s. Corchorus olitorius.[8-16] This genus's several species have been employed in traditional medicine. Corchorus is a traditional medication that is used to treat pains, diarrhea, enteritis, fever, and tumors. [17] The leaves extract has demulcent, laxative, carminative, stimulating, appetizer, and

tonic properties. [18] Corchorus seeds are used to treat purgative, tonic, stomachic, fever, and blockages of gastrointestinal disorders.[19] C. olitorius is used to make jute fiber. It is used to cure respiratory infections as well as as a tonic and nervine; components include volatile oil, flavonoids, and phenolic compounds. [20]

C. trilocularis methanolic extract contains triterpenoid triloculatol-A and triloculatol-A3 glucoside, which have been demonstrated to have glucuronisidase inhibiting and enzyme inhibitory activity. [21] The herb has been claimed to have anti-inflammatory and demulcent properties.[22-24] C. trilocularis seeds are used to treat fevers. C. trilocularis is also utilized in traditional Indian medicine to treat syphilis.[25] Due to the worldwide trend of using trash and agribusiness wastes, oil extraction might provide financial value to a huge number of seeds that are otherwise abandoned. For millennia, the extraction and utilization of vegetable oils have been crucial in the creation of a wide range of industrial and dietary products. Thus, the purpose of this study was to determine the physicochemical properties, fatty acid composition, DPPH· and FRAP antioxidant capacities of Fatty acids methyls esters (FAMEs) of unexplored seed oils extracted from two Corchorus species to contribute to research into the prospective use of these seed oil as nutrient supplements. To the best of our knowledge, the study of the antioxidant potential of FAMEs of C. trilocularis and C. olitorius seed oils is a novel approach to exploring the additional nutritional potency of these medicinal plants from western Rajasthan

Methods and Materials-

Collection of Seeds and sample preparation- The seeds of C. trilocularis and C. olitorius were collected from different places in the Jodhpur, Jaisalmer, and Barmer districts of Rajasthan. The seeds were cleaned with water, dried in shade, and finally crushed and grounded with mortar.

Chemicals and standards-Folin- Ciocalteu phenol reagent, gallic acid,

, DPPH·, 6-hydroxy-2,5,7,8- tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), and methanol were purchased from Sigma Aldrich Chemical All solvents and chemicals were of analytical grade.

Extraction of oils- The C. trilocularis and C. olitorius seed oils were extracted using a soxhlet apparatus and n-hexane (40^0-60^0 C) was used as a solvent. For about 1 hour, the seed oils were refluxed with methanolic NaOH. The aqueous phase was first acidified with HCl before being

extracted with diethyl ether. The organic phase was dissolved in hexane solvent and then refluxed further for 1 hr with a 2% H_2SO_4 methanolic solution. FAMEs were obtained after extraction and solvent removal.[25] The oils were analyzed for moisture content and nitrogen content by using standard methods.

Analysis of fatty acids composition - Shimadzu with QP2010S type GC-MS spectrophotometer was used to assess the fatty acid content of seed oils. A capillary column made of Rtx-5MS column (BPX 70 TM; length: 30 m; diameter: 0.25 μm; film thickness: 0.25 μm) was utilized. The carrier gas was helium, with a flow velocity of 1 ml/min. The injector temperature was 240°C, while the detector temperature was 250°C. The oven temperature was set to 60°C and subsequently increased to 150°C at a speed of 6°C/min for 15 minutes before reaching 200°C at a pace of 8°C/min for 15 minutes. For comparison, FAMEs with retention indices were employed. The measurements were taken thrice, and the average result was utilized to calculate the findings.

Analysis of Antioxidant activity-

The antioxidant activities of C. trilocularis and C. olitorius were evaluated by two different test methods:

Ferric reducing antioxidant power (FRAP)- The FRAP test was carried out in the manner reported earlier by Benzie and Strain.[25] The FRAP reagent was made by combining a 10: 1: 1 solution of acetate buffer, TPTZ, and $FeCl_3$. An aliquot of the FAMEs (100 μ L) and 200 μL of distilled water was added to 2mL of the FRAP reagent and incubated at 35°C for half an hour in the dark. At 593 nm, the absorbance was measured using a spectrophotometer against a blank experiment. The results were compared with the standard curve prepared using different concentrations of Trolox.

The DPPH free radical scavenging activity was calculated using Cheung and coworkers' technique.[27] In brief, each extract received 0.2 mM DPPH at methanol (DPPH reagent) in a volume ratio of 4:1. After 10 minutes of exposure to low-light conditions, the absorbance was measured at 520nm using a spectrophotometer. Ascorbic acid is used as standard. The antioxidant activity (AOA) was measured by the formula given below:-

$$\textbf{DPPH free radical scavenging activity} = \frac{\textbf{Abs of control- Abs of sample}}{\textbf{Abs of control}} \times 100$$

The data were presented as IC_{50} (inhibitor concentration)values, which are defined as the sample concentration that reduces the absorbance of the DPPH reagent by 50%.

All the antioxidant activity tests were carried out in triplicate and data was expressed by mean ± S.D.

Analysis of Total Phenolic Content

Total phenolic content was determined using Folin-reagent, Ciocalteu's reagent.[28] In brief, 500 µL of each FAMEs was combined with Folin-reagent, Ciocalteu's then 1.5 mL of sodium carbonate was added, followed by 2.75 mL of dH2O, and the resulting solutions were centrifuged for 5 minutes. The absorbance of supernatants was measured at 725 nm. The total phenolic content was measured in mg of gallic acid equivalents (GAE)/g of FAMEs. All evaluations were performed thrice, and data were reported as mean ± S.D.

Results and Discussion

Physico-chemical characteristics of Corchorus species:-

The Physico-chemical properties of C. olitorius and C. trilocularis seeds oils were shown in table-I. The C. olitorius and C. trilocularis seeds oils were found to contain 38% and 32% of oil. The moisture contents were 3.05% and 5.06% in C. olitorius and C. trilocularis respectively Several Physico-chemical characteristics, including iodine value (I.V), and saponification value (S.V), the moisture content is important in determining the quality and hence the economic worth of edible seed oils. C.olitorius has a reduced iodine value (I.V-66), which may have contributed to its higher oxidative storage stability whereas the C. trilocularis is reported to have quite a higher iodine value (I.V-80). The refractive index obtained for the c. trilocularis is 1.437 and for the C. olitorius is 1.457. The saponification value (152.40 mg $KOH.g^{-1}$) found in this study for C. olitorius was similar to those reported for conventional vegetable oils.[29] The saponification value for the C. trilocularis was found to be 112 mg $KOH.g^{-1}$.

Fatty acids composition of Corchorus species:-

The fatty acid profile of C. trilocularis and C. olitorius seed oils was studied using the GC-MS method. Figures 1&2 showed the GC-MS spectra and Table- 2 showed the fatty acid components of C. olitorius and C. trilocularis seed oils. The current research reported that oleic acid i.e, monounsaturated fatty acid (MUFA) was the most prevalent fatty acid in both the Corchorus

species. The percentage of oleic acid reported in C. olitorius and C. trilocularis were 55.1 % and 45.5 % respectively. MUFA resists oxidation better than polyunsaturated fatty acids (PUFA). According to the data found, seed oil from Corchorus species may have greater oxidative stability qualities due to the availability of a higher amount of MUFA as compared to PUFA in their seed oils. Moisturizing skin care products, bathing oils, hair conditioners, and cosmetics all include lipids with a high monounsaturated fatty acid concentration.[30]

Furthermore, the other fatty acids reported in both the Corchorus species were myristic, palmitic, stearic, linoleic, and linolenic acids. The results are given in table-2 also revealed that both Corchorus species seed oil had a lower ratio of saturated to unsaturated fatty acid (SFA/UFA), suggesting that the oil may have higher nutritional characteristics. As a result, the richness of unsaturated fatty acids in the Corchorus species is extremely valuable for their positive health effects.[31] In addition to this, both the Corchorus species seed oils reported lower content of polyene fatty acids as compared to the monoene fatty acids. The seed oils with polyene fatty acid content are not highly resistant to external forces; for example, α- -linolenic acid (18:3, ω- 3) or (punicic acid 18:3, ω -5).[32,33] Long-term use of significant levels of linoleic acid is suspected to raise the risk of developing cancer and ulcerative colitis. [34,35]

Conventional vegetable oils such as canola and safflower were reported to have a higher amount of oleic acids. These seed oils are stable enough to be used for food frying. Furthermore, high oleic oil consumption may provide a nutritional benefit by lowering oxidative stress in vivo.[36,37]

DPPH free radical scavenging activity of Corchorus species-

In terms of antioxidant activity, both Corchorus species' FAMEs interacted with and inhibited radical scavenging shown in Table-3. FAMEs derived from C. olitorius had stronger antioxidant activity than FAMEs obtained from C. trilocularis at all concentrations. FAMEs derived from C. olitorius had the higher DPPH scavenging activity of the two studied FAMEs. Further the C. olitorius FAMEs also had a lower value of IC_{50} (6.54± 1.19mg/mL). According to Wang et al., fatty acids from Camellia sinensis L. edible seed oil extract have DPPH radical scavenging action with an IC_{50} value of 35.8 mg/mL.[38] A lower IC_{50} value favors a higher antioxidant potency. [39] The radical scavenging activity of C. olitorius FAMEs was shown to be higher than that of Camellia sinensis L., which plays a key role in human diets as a health-promoting agent.

FRAP activity of Corchorus species-

The FRAP antioxidant activity reported for the FAMEs of C.olitorius and C.trilocularis were 71.52±1.35 μmolTE and 52.71±1.45 μmolTE. Both DPPH and FRAP methods indicated that FAMEs of both the Corchorus species have a higher antioxidant capacity, probably due to having a higher total phenolic content. The antioxidant activity analyzed for the Corchorus species was higher than that shown by the apricot, peach, cherry, black cherry, and plum seed oils reported by Fratianni F, et.al. [40]The FRAP analyses confirmed the significant antioxidant potential of the Corchorus species

Total Phenolic content of Corchorus species-

In terms of phenolic chemicals, the quantities detected in C.olitorius FAMEs (32.58±2.1 mg GAE/g) and C.trilocularis FAMEs (25.98±1.5mg EAG/kg) are equivalent to those found in other vegetable oils.[41,42] Due to their potential health benefits, phenolic substances have sparked great research. Antiviral, anti-allergic, antiplatelet, anti-inflammatory, anticancer, and antioxidant properties have been found for phenolic content.[43] C.olitorius and C.trilocularis have quite high phenolic content. In seed oils, phenolic chemicals have a key influence on flavor, storage stability, and oxidation resistance. Gallic acid levels in both the corchorus species FAMEs were much higher than those discovered in walnut (3.39mg/100 g) by Donno. D et al. [44] As previously noted, phenolic substances such as gallic acid have powerful antioxidant, anti-diabetic, and antihyperlipidemic properties. [45]

Conclusions- The fatty acid content of C. olitorius and C. trilocularis seed oils was studied, and it was found that these oils had a higher proportion of MUFA in their lipid profile, which contributes to their high oxidative stability. The considerable antioxidant activity found both in C. olitorius and C.trilocularis FAMEs seed oils, could contribute as a nutritional potent antioxidant, aiding in disease control, or as a nutritional supplement, boosting the stability, longevity, and food quality. The high oil content of both the Corchorus species suggested a potential supply of oil rich in MUFA (oleic fatty acid). Because of the large concentration of unsaturated fatty acids, these oils would be an appropriate alternative for many other conventional unsaturated oils. However, before these Corchorus seed oils can be declared acceptable for dietary purposes, toxicological tests must be conducted to determine their safety.

The physicochemical parameters of these seed oil found under investigation are consistent with those of standard seed oils. FAMEs of Corchorus species seed oil demonstrated relatively good oxidative stability with both DPPH and FRAP methods. The current study's findings are critical for future chemical studies of both these Corchorus species seed oils and their commercial applications as feedstock in foodstuffs and industries.

Conflict of Interest- There is no conflict of interest.

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Table.1-Physico - Chemical Properties of Seeds

S.No.	Plant Species	Oil %	Protein %	Moisture %	SV	IV	RI
1.	**Corchorus olitorius**	38	23	3.05	152.40	66	1.457
2.	**Corchorus trilocularis**	32	18	5.06	112	80	1.437

Table-2 Component fatty acid (uncorrected weight percentage) of seed oils determined by GC-MS

S.No.	Plant species	Myristic acid C14:0	Palmitic acid C16:0	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3	Others
1	**Corchorus olitorius**	4.5	8.1	5.1	55.1	18.1	6.1	3.0

2	**Corchorus trilocularis**	2.5	8.5	7.1	45.5	21.1	10.1	5.2

Table-3 - Total Saturated and unsaturated, MUFA and PUFA in Corchorus olitorius, Corchorus trilocularis seed oils

S.No.	Plant species	Total saturated Fatty acids	Total unsaturated Fatty acids	MUFA (Oleic acid)	PUFA (Linoleic + Linolenic acid)	Others
1	Corchorus olitorius	17.7	79.3	55.1	24.2	3.0
2	Corchorus trilocularis	18.1	76.7	45.5	31.2	5.2

Table 4- DPPH Antioxidant at different concentrations, free radical scavenging activity in terms of IC_{50}, FRAP activity and total phenolic content of Corchorus species and gallic acid.

Plant species	DPPH activity Concentration ± SD (mg/mL)							FRAP activity(µmoL TE/g)	Total phenolic content (mg GAE/g oil)
	0.25	0.5	.75	1.0	2.0	4.0	IC_{50} ± SD (mg/mL)		
Corchorus olitorius	25±1.34	45±0.98	58±1.31	86±0.56	92±1.57	99±0.79	6.54± 1.19	71.52±1.35	32.58±2.1
Corchorus trilocularis	22.34±1.45	35±1.24	47±0.75	68±1.12	75±1.36	80±1.23	9.24± 1.25	52.71±1.45	25.98±1.5
Ascorbic acid	55.74±1.19	64±1.23	75±0,45	89±1.32	92±1.12	98±1.14	3.45±1.13	ND	ND
Gallic acid	ND	ND	ND	ND	ND	ND	ND	ND	78.56±1.4

* Values are given as Mean±SD (n=3)
GAE- Galic acid equivalent
TE- Trolox equivalent
ND-Not detected

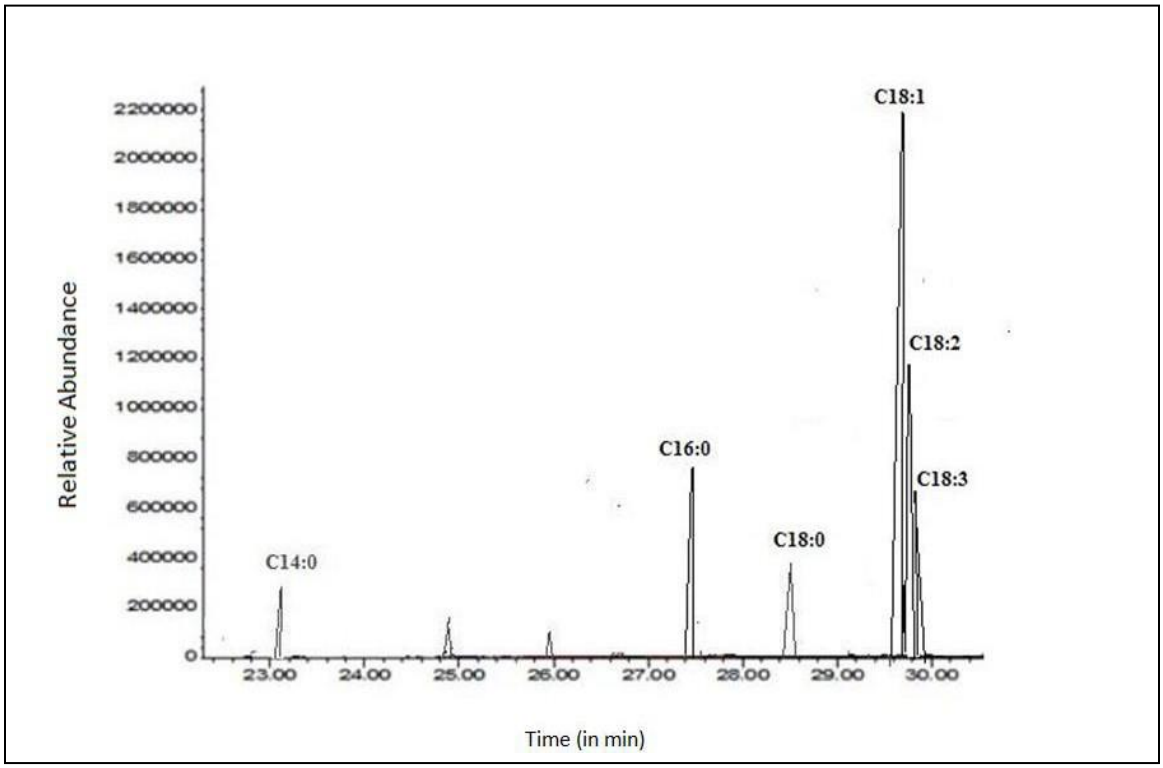

Figure.1- GC-MS spectra of C.olitorius FAMEs

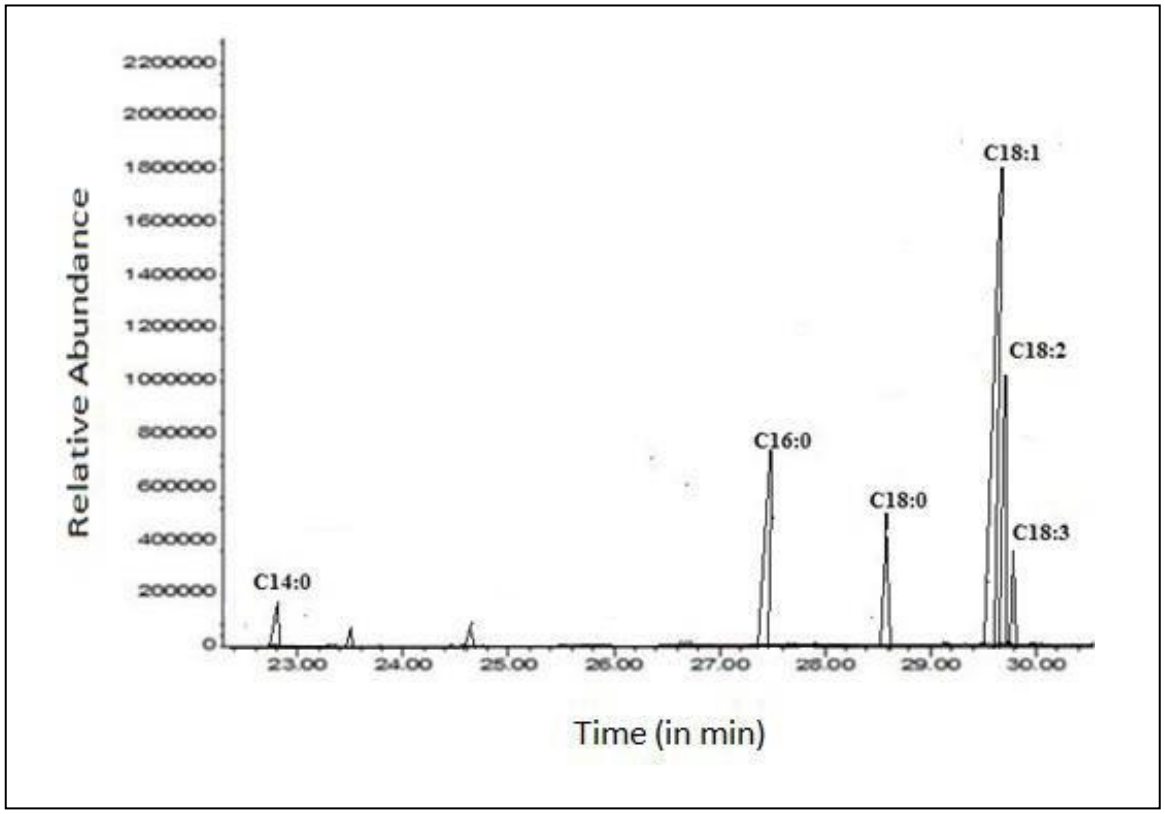

Figure.2 - GC-MS spectra of C.trilocularis FAMEs

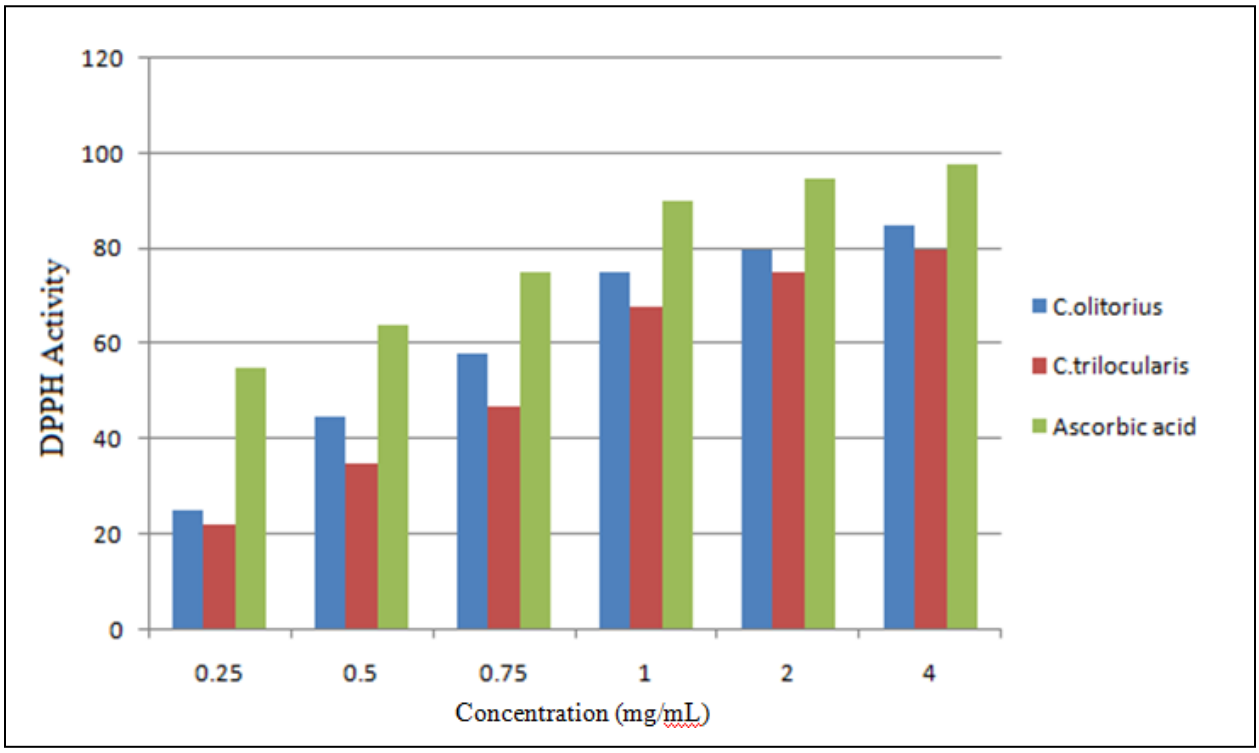

Fig.3- DPPH free radical scavenging activity of C.olitorius, C.trilocularis and ascorbic acid