Phytochemical evaluation, antioxidant assay and GC MS Profiling of Bioactive components present in *Camelina sativa* L. leaves

Rakshit Pathak[1,2*], Shweta Goyal[1,2], Anjali Kumari[2], Mohammed Mohsin[2], Ganga Bisht[1]

[1]DSB Campus, Kumaun University, Nainital
[2]Defence Institute of Bio-Energy Research (DRDO)
Haldwani, Uttarakhand, 263139, India
rakshitpathak325@gmail.com

ABSTRACT - *Camelina sativa* L. is a well-known oil seed crop and gaining attraction of various researchers as a source of biofuel. It is not only just an oilseed crop but its leaves have many bioactive components present which has been not investigated and analyzed so far. The present study was aimed to evaluate the phytochemical constituents and characterization of various bioactive components present in Camelina leaves. Spectrophotometric method was used to analyze phytochemical components as well as FRSA assay; GC MS spectrometry was studied to identify chemical constituents of methanolic extract of leaves. Result revealed the presence of phenolic content in various polar extract of *Camelina sativa* leaves, FRSA activity was also reported in each solvent extract. GC MS report shows the presence of almost sixty-five compounds in the methanolic extract but only fourteen compounds were in noticeable amount. From the results, it can be concluded that the leaves of Camelina contains many phytoconstituents which are neutraceutically important and leaves can be recommended as pharmacological applications.

Keywords: - *Camelina sativa*; Methanol; GC MS; Phytochemicals; Polar solvents.

Abbreviations

IPA	Iso Propyl Alcohol
ROS	Reactive Oxygen Species
ANOVA	Analysis of Variance
GA	Gallic Acid
F-C	Folin - Ciocalteu
F-D	Folin - Denis
GC-MS	Gas Chromatography – mass spectrometry

1. INTRODUCTION

Oxidation process is very basic reaction of the organism for its survival, all the cells of the body shows oxidative metabolism, but the main adverse effect of this oxidative metabolism is production of free radicals and other ROS that damage the body cells and causes various diseases in the organism. Day by day the evidences are coming throughout the world about the involvement of excess free radicals in number of degenerative diseases such as cancer, inflammatory joint disease, atherosclerosis, diabetes, senile dementia, asthma and degenerative eye disease[1]. Oxidation process also rancid the food material as a result the food quality, nutritional values, texture and flavor deteriorate in very rapid manner. A report estimate that almost half of the vegetable and food crops lost due to these free radical reactions[2, 3]. Plants not always need outer support to grow because the internal chemical properties of the plants make them efficient to fight with these free radicals and other pathogenic attacks. Plants and their parts have its own defence mechanism which supports it to grow and to act in a condition when foreign bioactive agents attack on it. This defence mechanism consists of number of bioactive components which are generally termed as primary and secondary metabolites, these bioactive substances also show great efficacy against various pathogenic diseases. Primary metabolites generally contain proteins, sugar, amino acids and chlorophyll whereas a large range of polyphenols, terpenoids and alkaloids comes under secondary metabolites.

Camelina sativa L. is a plant belonging to the brassicaceae family and known for its quality to grow in any climatic condition and in very harsh environment; this quality shows that plant has some extra beneficial phytochemicals which supports the plant during its whole life cycle[4, 5]. The main useful part of this plant is its seed which contains very much amount of oil used for biofuel purpose; however other plants parts are also contains very nutritional bioactive components. In the present investigation our main objective was to estimate some important phytoconstituents present in the green leaves of the *Camelina sativa* and also to check its ability to trap the free radicals generated from the chemical oxidation.

2. MATERIAL & METHOD:

2.1. Plant material and chemical

The leaves of *Camelina sativa L.* were harvested in the month of December 2018 at DIBER Hq. field, Haldwani (District: Nainital), UK- India, where the plants were cultivated under natural conditions. The leaves collected were washed thoroughly with tap water and dried in hot air oven (at 35 ± 2 ° C) for 72 hours. Dried leaves were grounded in mixer grinder to form a homogeneous powder. (REMI, India).

2.2. Preparation of Camelina leaves extract

50 g of powdered leaves were extracted in 250 ml of different solvents {viz. Methanol, Ethanol, IPA, Aq. Ethanol (70:30 v/v) and water} by soxhlet method[6]. The extracts were subsequently evaporated using rotary evaporator (IKA RV10, Germany) to obtain viscous leaf extract. Extracts was reduced to dryness using water bath at 60°C for 1 hour. The plant extracts were then stored at 4°C that was used for further phytochemical analyses.

2.3. Phytochemical Analyses

2.3.1. Total Phenolic Content

Phenolic content in the plant extract was estimated by Folin-Ciocalteu reagent using a reported method (Ainsworth and Gillespie; 2007)[7] with certain modifications. Dried plant extract (100mg) was accurately weighed, transferred to volumetric flask and made up to the volume 10 ml with methanol. The solution was centrifuged (HanilBioMed Inc. Korea) at 13,000 rpm for 5 min. The supernatant (100 µl) was collected and reacted with 200 µl of 10 % (v/v) Folin–Ciocalteu reagent. The assay tubes were vortexed for 5 minutes and then 800 µl of 70 mM Na_2CO_3 as added to it. Finally, the assay tubes were incubated at room temperature for 2 h. The absorbance was measured at 765 nm and total phenolic content (µg g^{-1} dry wt catechol equivalent) was calculated from a standard curve using catechol (Sigma-Aldrich, USA).

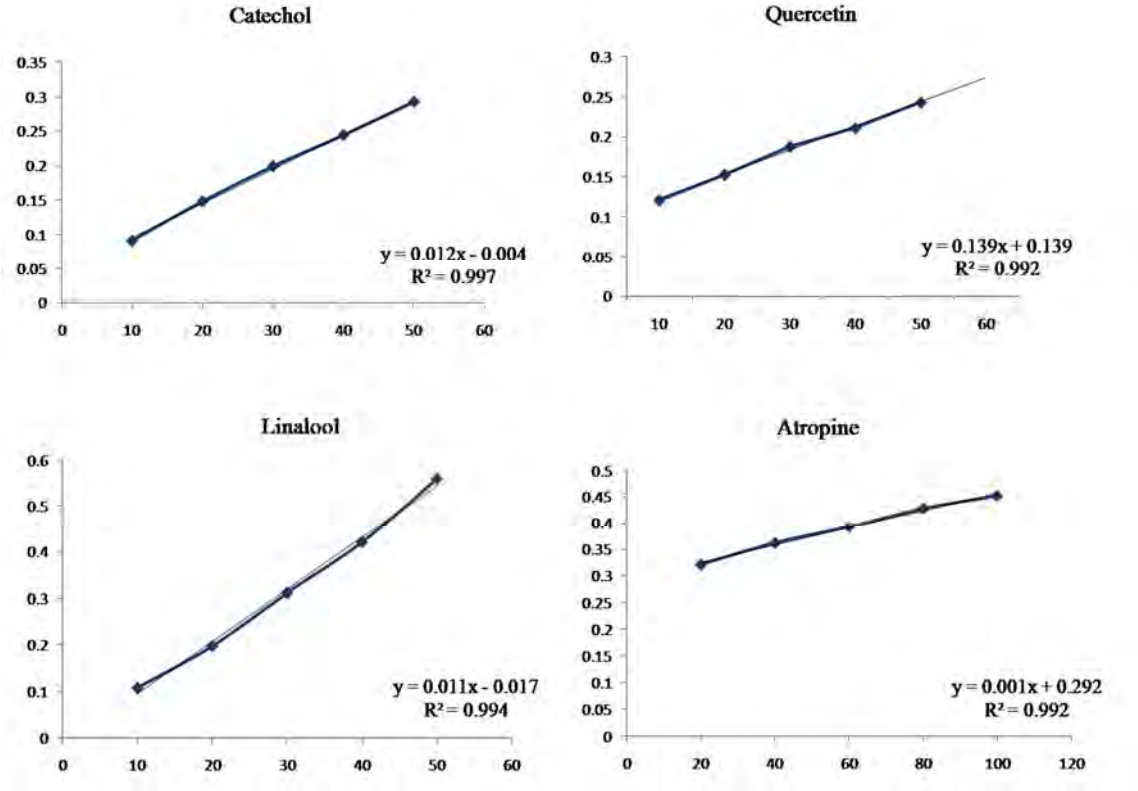

Fig 1: Showing Standard curve for Phenolics (Catechol as Standard), Flavonoids (Quercetin as Standard), Terpenes (Linalool as Standard) and Alkaloids (Atropine as Standard)

2.3.2. Total Flavonoid Content: -

Flavonoid content was estimated according to the method (Lee and Safinar; 2012)[8], dried plant extract (100 mg) was accurately weighed and transferred into volumetric flask and made up to the volume 10mL with methanol. The solution was centrifuged (HanilBioMed Inc. Korea) at 13,000 rpm for 5 min, 0.5 ml of supernatant was taken in assay tube and then 1.5 ml methanol was added to it. Then, 0.1mL 10% $AlCl_3$, 0.1ml of 1M potassium acetate , 2.8mL distilled water was added to the tube. The resulting sample tubes were incubated for 30 minutes and finally absorbance was taken at 415 nm and total flavonoid content (µg g-1 dry wt quercetin equivalent) was calculated using quercetin as standard.

2.3.3. Total Terpenoid Content: -

Terpenes are the most common secondary metabolite which found almost in every plant species. It has 5 carbon containing isoprene units. Quantification of Terpenoids was done using the method of Ghorai[9]. The absorbance for test and standard was measured at 538 nm and terpenoid content was calculated using linalool (Lil) as standard and expressed as µg linalool equivalent g-1 dry wt.

2.3.4. Estimation of Alkaloids Content: -

Each sample extract (1 ml) was mixed with 1 ml of 2 N HCl and filtered. 1 ml of this solution was then transferred to the tube which already contains 5 ml of bromocresol green and 5 ml of 0.1 M phosphate buffer (pH 7.4) this reaction mixture was mixed well with 4 ml of chloroform incubated at room temperature for 30 minutes. The absorbance was measured at 470 nm and total alkaloids content was calculated using atropine (AE) as standard, and expressed as µg/g AE dry wt.

2.4. Chemical constituent estimation using Gas Chromatography Mass Spectrometry

GC MS is such an analytical technique which coupled the separation method of gas chromatography and then fragmentation of the separated compound using mass spectrometry. Main applicability of this instrumentation is to identify multi component mixtures such as essential oils, fatty acids, terpenes, hydrocarbons and solvents etc. For this purpose, lyophilized methanolic sample was taken and then mg/ml solution was prepared for injecting in the instrument. GC MS was done at Advanced Instrumentation Research Facility, JNU, New Delhi. Sample was placed in GC–MS QP-2010 model (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a CombiPAL AOC-20i+s] auto sampler (CTC Analytics, Zwingen, Switzerland). The compounds were separated on a Rxi®-5Sil-MS capillary column (30 x 0.25 mm ID and 0.25 µm film thickness). The carrier gas was helium, split ratio 10, injector temperature 260.00°C. The column oven temperature program used was: 50.0°C (hold for 4 min) to 280.0°C at 10°C/min hold for 23 min. The ion source temperature and interface temperature were set at 230.00 °C and 270.00 °C respectively and the MS mode was electron impact (EI). The compounds were separated by GC and further fragmented by Mass spectrometer and identified by comparing the mass spectra obtained with NIST14 and WILEY8.LIB from the US National Institute of Technology and Standards (NIST) mass spectra libraries. For calculating the total area percentage of peak and relative percentage, we considered the peaks which were repeatedly present in at least two TIC.

2.5. Antioxidant assay:

2.5.1. By DPPH method:

2 ml of DPPH solution (0.1 mM) was added in different sample (0 to 50 µlit) and prepared the volume up to 1ml with adding the respective solvents. Finally, kept all the mixtures in dark place for 40 minutes, after this absorbance were taken at 517 nm.

2.4.2. By ABTS method:

ABTS cation radical was produced by the reaction of 7mM ABTS in water and 2.45mM Potassium persulfate in equal proportion, mixture kept in dark for overnight. ABTS·+ solution was then diluted with methanol to obtain an absorbance of 0.700 (±0.002) at 734 nm. After this, 1 ml of this solution added to the 1 ml sample (0 to 50 µlit) and kept the mixture in dark for 5 to 7 minutes. Absorbance were taken at 734 nm.

FRSA (%) calculated with the formula: -

$$\textbf{FRSA\%} = \frac{\textbf{Abs (control) - Abs (test) x 100}}{\textbf{Abs (control)}}$$

2.5. Statistical analyses

All phytochemical evaluations were performed in triplicates. The Statistical package for Social Sciences (SPSS 16.0.0 module) software was used for statistical analysis (ANOVA) for all experiments. The treatments and controls means values were compared by least significant difference (LSD) test at a significance level of $P \leq 0.05$. Duncan's multiple range test (DMRT) was also performed to check the significance of the differences between mean values.

3. Result and Discussion

3.1. Phytochemical analyses: -

For phytochemical evaluation, five different polar solvents were used; all the five solvents were of different polarity. All solvents exhibit a decent amount of the secondary metabolites, but in all parameters methanol was showed the highest amount of all the four phytoconstituents. Phenolics (contains Phenolic ring in their structure) are the major component of any plant organism and acts as a strong defence mechanism against the various pathogens also highly responsible for trapping the free radicals. Phenolic components are highly involved in the growth mechanism of plant such as pigmentation, reproduction and many more other activities inside the plant. Highest Phenolic content was found in the methanolic extract (291.87µg/g) followed by EtOH, IPA, Aq Ethanol and aqueous extracts (220.23, 175.12, 156.41 and 67.13 µg/g) respectively. Flavonoids (having Flavone ring in their basic skeleton) are also important plant pigments which plays a vital role in the bioactivity of any plant species; highly responsible for the colour and aroma of the plant. Flavonoids were also highest in case of methanolic extract (133.94 µg/g) followed by IPA, EtOH, AqEtOH and Aqueous extracts (120.39, 74.59, 38.39 and 17.6 µg/g) respectively.

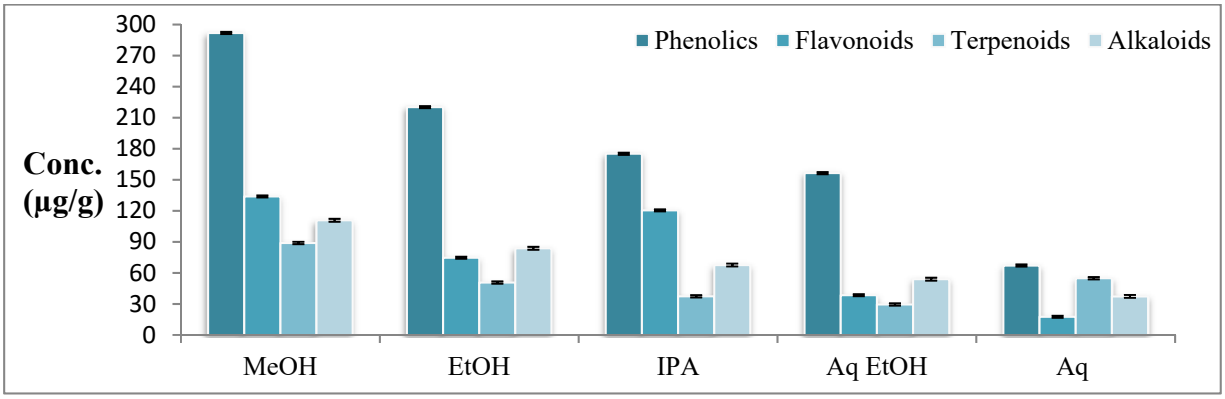

Fig 2: Phytoconstituents p/r in different polar extracts of Camelina leaves

(* Each value expressed as mean, ± Standard Error (n=3)
* The mean difference is significant at the 0.05 level)

Terpenes are the simple hydrocarbons and contain the five carbon isoprene unit in their structure; they are the biggest class of phytoconstituents and most commonly found in the essential oils. Depending on the carbon unit they are of different types. In case of Camelina leaves highest Terpenes were reported in Methanolic extract (88.89 µg/g) followed by Aqueous extract, EtOH, IPA and Aq Ethanol (54.90, 50.72, 37.4 & 54.09) respectively. Alkaloids are another important class of secondary metabolites contains at least one nitrogen atom in their structure, they have numerous physiological effects on the humans and other animals, for plants they work as a defence line against various pathogens and herbivorous. Camelina leaves exhibit highest alkaloids when extracting solvent was methanol (110.83 µg/g) followed by ethanol, IPA, Aq Ethanol and water (83.75, 67.58, 53.92 and 37.27) respectively.

3.2. Identification of important chemical constituent p/r in Camelina leaves using GC - MS:

The result of GC MS analysis of the Camelina methanolic leaves extract showed number of important phytoconstituents. Almost sixty-five compounds were identified through GC MS, in which fourteen compounds were in noticeable amount (Table 1). Linolenic acid (17.69 %) and palmitic acid (15.51%) were major phytoconstituents comes in the GC MS spectrum. Linolenic acid is an essential fatty acid belongs to the omega-3 fatty acids family, present in fish oil and plant oils and highly involve in the inhibition of the synthesis of prostaglandin resulting in reduced inflammation and prevention of certain chronic diseases[10].

Table 1: Important Phytoconstituents p/r in Camelina leaves

No.	R.T.	Peak Area (%)	Compound	MF	MW	Nature of Compound
1.	35.29	17.69	Linolenic Acid	$C_{18}H_{30}O_2$	278	PUFA
2.	32.03	15.51	Palmitic Acid	$C_{16}H_{32}O_2$	256	SFA
3.	12.29	7.12	1,5-Anhydro-6-Deoxyhexo-2,3-Diulose	$C_6H_8O_4$	144	Glycoside
4.	47.45	6.75	Hexacosane	$C_{26}H_{54}$	366	Acyclic Alkane
5.	50.08	6.22	beta-Sitosterol	$C_{29}H_{50}O$	414	Steroid
6.	53.45	4.56	Montanic acid	$C_{29}H_{58}O_2$	438	Saturated Fatty Acid
7.	34.46	3.96	Octadecatrienoic acid	$C_{19}H_{32}O_2$	292	PUFA
8.	45.73	2.69	Tritetracontane	$C_{43}H_{88}$	604	Alkanes
9.	45.44	1.90	α - tocospiro B	$C_{29}H_{50}O_4$	462	Oxa Spiro ketone
10.	45.27	1.38	α - tocospiro A	$C_{29}H_{50}O_4$	462	Oxa Spiro ketone
11.	34.98	1.64	Stearic acid	$C_{19}H_{38}O_2$	298	SFA
12.	10.45	1.24	Molinate	$C_9H_{17}NOS$	187	Heterocyclic
13.	53.70	1.79	Phytyldecanoate	$C_{30}H_{58}O_2$	450	Ester
14.	58.28	1.35	Melissic acid	$C_{31}H_{62}O_2$	466	Saturated fatty acid.
15.	Others	**26.2**				
Total		**100**				

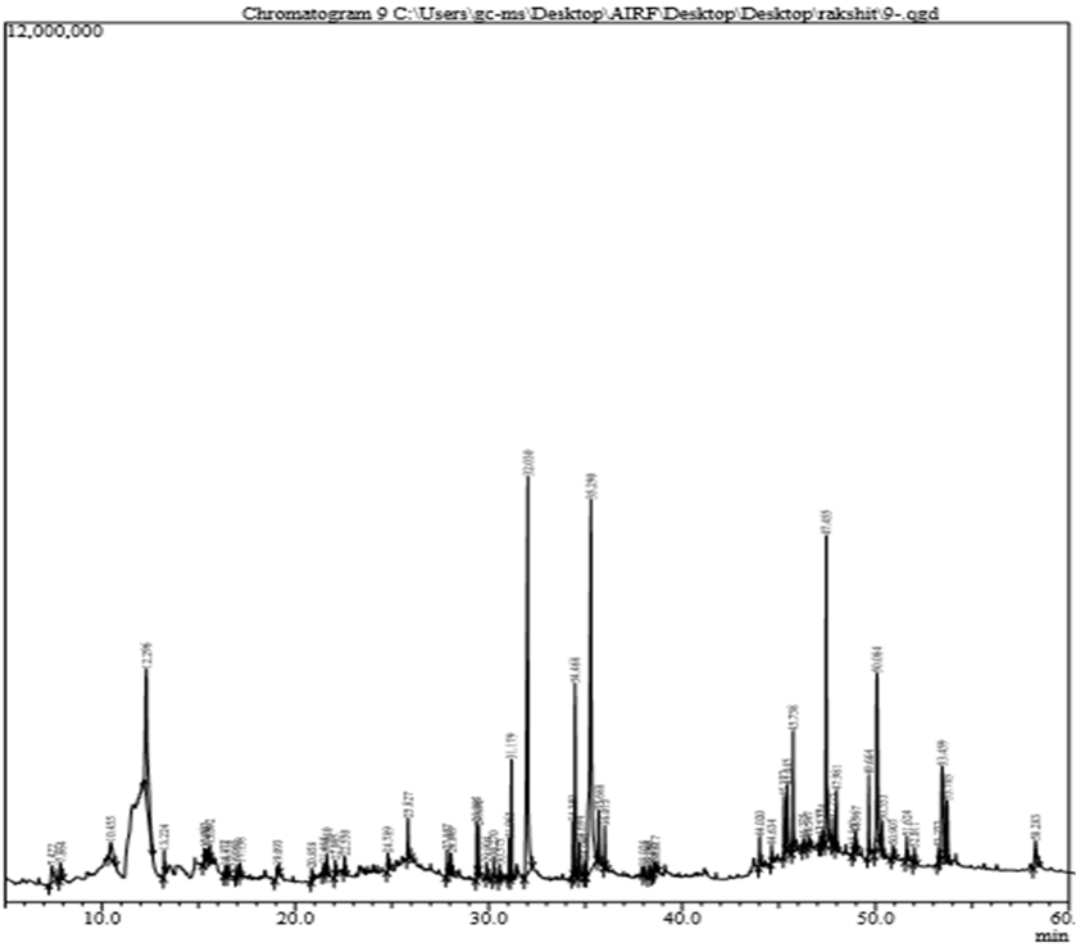

Fig 2: GC MS Chromatogram of methanolic extract of CS leaves

Palmitic acid is the most common saturated fatty acid found in many animals, plants and microorganisms. It is widely applicable in a variety of personal care products and cosmetics[11]. 1, 5-Anhydro-6-Deoxyhexo-2, 3-Diulose (7.12 %) was also one important phytochemical comes in the report, it is a type of glycoside and used as a preservative. Hexacosane (6.75 %) was also analyzed in the report, it is a straight-chain alkane comprising of 26 carbon atoms. It has a role as a volatile oil component and a plant metabolite[12]. ß-Sitosterol (6.22 %) generally called as "plant sterol ester". It is very much valuable for the formation of various types of drugs, dietary sterol and has the potential to prevent the human cancer. It contains a double bond in its structure and susceptible to oxidation. It has distinct properties of anti – carcinogenic and anti-atherogenicproperties[13]. Montanic acid (4.56 %) was also reported in GCMS, it is a saturated fatty acid works as wax generally used for the coating of the fruits.

3.3. Antioxidant assay by DPPH and ABTS Method:

Antioxidants are essential for checking the oxidation reaction and also inhibits the reactivity of the free radicals in any cellular organism. Higher the amount of secondary metabolite, higher will be the capacity to act as an antioxidant. For *in vitro* antioxidant assay DPPH and ABTS are most widely used methods.

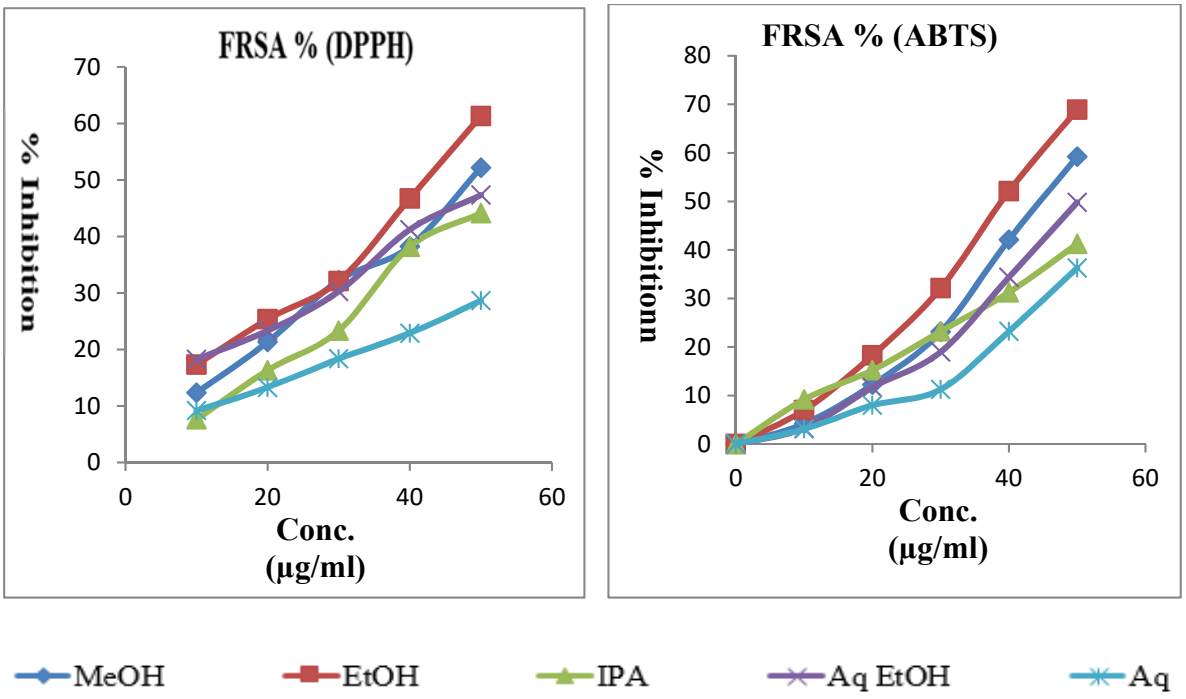

Fig 3: FRSA Activity percentage inhibition of different CS Leaves extracts

In both DPPH and ABTS activity highest inhibition concentration was exhibited by ethanolic extract (50 µlit.) i.e. 61.32 and 68.91 % followed by Methanol, Aq Ethanol IPA and water extracts respectively.

4. Conclusion

Camelina sativa is a well-known oil seed crop used for the production of the biofuel, present investigation exhibits the properties of their leaves as a potent source of secondary metabolites. These secondary metabolites are helps to reduce the oxidation reaction. Due to these properties of the leaves this plant is also need less amount of pesticides or fungicides to grow. From the results of the present investigation, it can be concluded that the leaves of Camelina contains many phytoconstituents which are neutraceutically important and leaves can be recommended as pharmacological applications.

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