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Evaluating *in vitro* antioxidant activity and GC-MS analysis of *Scoparia dulcis* Linn (Scrophulariacae)

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ABSTRACT

Free radical scavenging activity was observed in both the methanol extract (M.E) and aqueous extract (A.E) of *Scoparia dulcis* respectively. In this study significant free radical scavenging activity was determined by evaluating the inhibition concentration (IC_{50}) in each test. In 2, 2-diphenyl-1-picrylhydrazyl (DPPH) model the extract displayed potential free radicals scavenging activity with IC50 of M.E is 311.13µg/mL and A.E is 441.96µg/mL. Nitric oxide model displayed IC50 of 293.77µg/mL in M.E and 434.93µg/mL in A.E. While superoxide ion model showed IC50 of 281.02 µg/mL and 440.14µg/mL respectively for both methanol and aqueous extract when compared to standard ascorbic acid. The presence of phenol, flavonoid and total antioxidant in both the extract justifies the antioxidant potential of the plant which brings about its free radicals scavenging potential. GC-MS analysis showed the presence of 6 different phytochemicals with (Z)-7-Hexadecenyl acetate found to be the compound with maximum peak percentage 51.51% in M.E and β-Cyclocitral with 43.90% in A.E respectively. Thus we conclude that the antioxidant activities may be due to the cumulative effect of the phytochemicals present in the plant which genuinely designate them as free radical scavenger.

INTRODUCTION

Free radicals play a dual role as they can be either harmful or helpful to the body (Pham-Huy *et al.,* 2008). So, it will be appropriate to examine the possible role of free radicals in disease and most importantly, harnessing the therapeutic phytochemicals from *Scoparia dulcis* against these pro-oxidants. It is well documented that a number of physiological processes in human body lead to the generation of a series of oxygen-centered free radicals namely reactive oxygen species (ROS) and reactive nitrogen species (RNS) as by-products. However, imbalance in their production impairs the innate antioxidant defense system of the cell, resulting in peroxidation of unsaturated fatty acids, membrane protein damage (proteins, carbohydrates denaturation) and DNA mutation (nucleic acids denaturation) causing oxidative/nitrosative stress (Maes *et al.,* 2011) which ultimately initiate the genesis of many multifactorial diseases. While the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Propylgallate (PG) and butylated hydroquinone have often been implicated to achieve immediate result, recent data indicates that these synthetic antioxidants could have carcinogenic effects thus fueling an intense search for newer and efficient antioxidants (Yevgenia *et al.,* 2013). *S. dulcis* commonly called as sweet broom weed is a perennial herb, widely distributed in tropical and subtropical regions. Traditionally it has been used as remedies for varieties of ailments like stomach troubles, hypertension, diabetes, inflammation, bronchitis, hemorrhoids, hepatosis, analgesic, diuretic, antipyretic and cytotoxicity (Freire *et al.,* 1993; Hayashi *et al.,* 1993; Ahsan *et al.,* 2003). Phytochemical screening revealed that *S. dulcis* contains a number of active principles such as diterpenoids, flavonoids, tannins, alkaloids, triterpenes, hexacosonol, β-sitosterol, ketone-dulcitone, ammelin, glutinol and scoparinol (Satyanarayana, 1969; Edeoga *et al.,* 2005; Chow *et al.,* 1974; Freire *et al.,* 1993; Ahmed *et al.,* 2001). In addition, other active principles such as scoparic acid A&B, scopadulcic acid A&B, scopadulciol and scopadulin (Hayashi *et al.,* 1990) have also been identified and all these isolated phytochemical have shown to contribute to the medicinal properties of the plant.

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Thus, showing the potential medicinal efficacy of each extracted compounds present in the plant, permits not only the demonstration of their physiological activity but also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity (Manna and Abalaka, 2000). Hence, it is important to determine the antioxidant efficacy of *S. dulcis* and also for its phytochemicals constituents.

MATERIALS AND METHOD

Drugs and chemicals

Standards like gallic acid, quercetin, ascorbic acid, sodium nitroprusside, Phenazonium methosulphate, NADH (Nicotinamide adenine dinucleotide) and DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) were purchased from Sigma-Aldrich Co., St. Louis, USA. All the other chemicals were of analytical grade obtained from Sisco research laboratory, Mumbai, India. All the quantitative determinations of antioxidant properties were performed spectrophotometrically using UV-1800, UV-spectrophotometer, Shimadzu.

Collection and identification

The plant *S. dulcis* purchased from IMPCOPS, Chennai and was authenticated by Dr. D Aravind, National institute of Siddha, Department of Medicinal Botany. Voucher specimens have been deposited at the Herbarium of the institute, Reg no NIS/MB/62/2012. 30 g of *S. dulcis* leaves were extracted with 250 mL of sterile distilled water and 70% methanol respectively using the Soxhlet apparatus at 60˚C. The extract was then filtered with Whatman No 1 filter paper and then freeze dried stored at 4˚C for further investigation. The extraction efficiency was quantified by determining the weight of each of the extracts and the percentage yield was calculated.

Total antioxidant capacities

Total phenolic and flavonoids of the contents of the extracts were determined by method described by Demiray *et al.,* (2009) and Wang *et al.,* (2000) respectively. Total antioxidant capacities of both extracts were evaluated as per the method described by Prieto *et al.,* (1999)

Free radical scavenging activities

The free radical scavenging capacities of the extracts were determined using DPPH model according to Blois (1958). The nitric oxide and superoxide anion scavenging assays were carried out according to the method of Sreejayan *et al.,* (1997) and Liu *et al.,* (1997) Reducing power was carried out by the method of Yildirim *et al.,* (2001).

Gas chromatography

Agilent 6890 gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35^{o}C, hold for 2 minutes, then ramp at 20^{o}C per minute to 260^{o}C and hold for 5 minutes. The helium carrier gas was set to 2 ml/minute flow rate (constant flow mode).

Mass Spectrometry

JEOL GC mate II bench top double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000 software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Mass spectrometry library search

Identification of components from purified compound was match with their recorded spectra and compared with the data bank mass spectra of NIST library V 11 provided by the instruments software.

RESULTS AND DISCUSSION

Extraction yield, total phenolic, flavonoid contents and total antioxidant activity

The recent growth in the knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution which promises a new age of health and disease management. The most common ROS/RNS includes superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO^-) nitric oxide (NO) could be produced in most cells when subjected to stress. So, naturally there is a need for dynamic balance between the amount of free radicals and antioxidants generated to help regulate proper homeostasis at the cellular level.

Table 1: Yield of methanol and aqueous extract of *Scoparia dulcis.*

S. No	Plant extract of *Scoparia dulcis*	Weight of dry extracts in grams	Initial dry plant extracts in grams	Extraction Yield % After freeze drying
1.	**Methanol extract**	5.6	30	18.66%
2.	**Aqueous extract**	4.9	30	16.33%

The interest in the physiological role of bioactive compounds present in plants has increased dramatically over the last decade, particularly in relation to human health. The pharmacological effects exerted by polyphenols on the human body are thought to be strongly related with their high antioxidant capacity (Hainal *et al.,* 2011). In our study both methanol and aqueous extract of *S. dulcis* displayed a considerable antioxidant activity which is justified by the content of polyphenol like phenol and flavonoids (table-2).

As reported by Mahakunakorn *et al.,* (2004) these phenolics and flavonoids compounds present in extracts are believed to intercept the free-radical chain of oxidation and donate

hydrogen from the phenolic hydroxyl groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation.

Table 2: Total antioxidant, phenol and flavonoid content of *S. dulcis* plant extracts.

Samples	Total phenol content (mg/g Gallic acid equivalent)	Total flavonoid content (mg/g quercetin Equivalent)	Total antioxidant capacity (mg/g ascorbic acid equivalent)
S. dulcis methanol extract	149.57±1.17	104.03±1.32	430.47±3.98
S. dulcis aqueous extract	115.09± 4.47	84.84±1.03	237.69±2.05

Values are the mean of duplicate experiments and represented as mean ± SD.

Free-radical scavenging activity

DPPH, superoxide and nitric oxide radical scavenging activity stays one of the most widely used method for screening the antioxidant activity of plant extract. The extract displayed a creditable DPPH scavenging (Fig-1) nitric oxide radical scavenging (Fig-2) and superoxide ion scavenging activity (Fig-3) for their respective extract. However, in the entire three tests methanol extract displayed better scavenging activity than aqueous extract for this study. These finding suggest that the plant extract could have contain phytochemicals that is capable of donating hydrogen to a free radical in order to remove the odd electron which is responsible for the radical's reactivity.

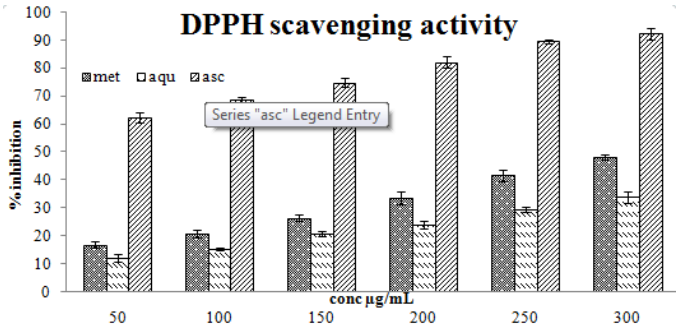

Fig. 1: Comparative DPPH scavenging activities of *Scoparia dulcis* extract and ascorbic acid. Values are the mean of duplicate experiments and represented as mean ± SD.

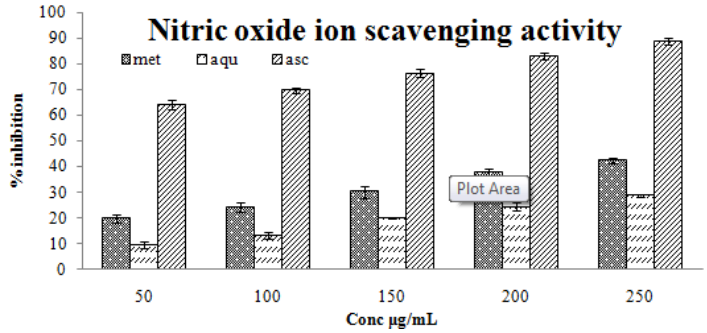

Fig. 2: Nitric oxide scavenging activities of *Scoparia dulcis* extract and ascorbic acid. Values are the mean of duplicate experiments and represented as mean ± SD.

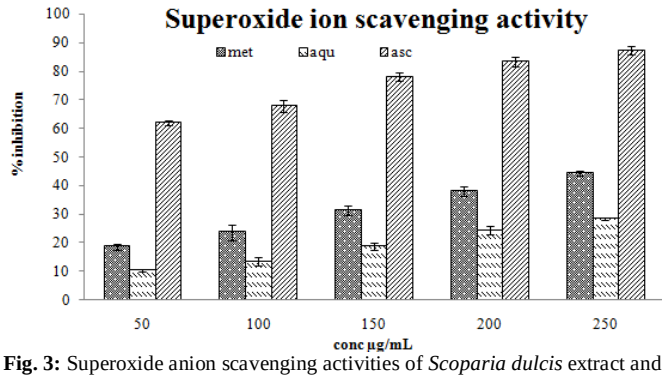

Fig. 3: Superoxide anion scavenging activities of *Scoparia dulcis* extract and ascorbic acid.Values are the mean of duplicate experiments and represented as mean ± SD.

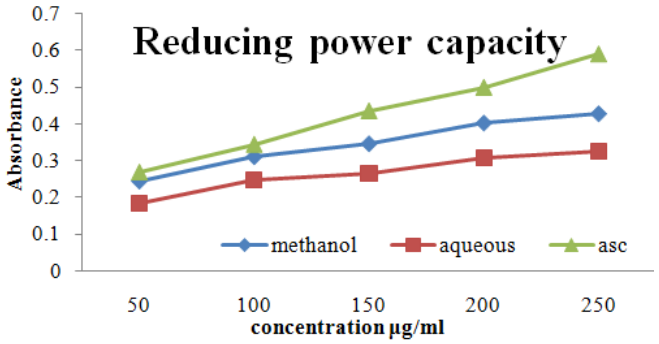

Fig. 4: Comparative reducing power capacity of *Scoparia dulcis* extract and ascorbic acid. Reducing power compared between methanol, aqueous extract of *S. dulcis* and ascorbic acid respectively.

Table 3: IC_{50} values of different extracts of *S. dulcis* in DPPH, Nitric oxide (NO) and superoxide ion scavenging assay.

Extract	IC_{50} µg/ml		
	DPPH	NO	O_2^-
Methanol extracts	311.13	293.77	281.02
Aqueous extracts	441.96	434.93	440.14
Ascorbic acid	40.12	38.99	40.29

Comparision of IC_{50} between methanol, aqueous extract of *S. dulcis* and ascorbic acid respectively.

The IC_{50} value for both the extract in DPPH, nitric oxide and superoxide ion scavenging model were tabulated in table-3. It is observed that *S. dulcis* extracted with methanol significantly has the enhanced ability to scavenge free radicals, and this could be attributed to the fact that methanol being a polar solvent proved better than the non-polar aqueous solvent for extraction. The inverse correlation between total phenol and flavonoid content and IC_{50} values in methanol and aqueous were also justified, because with higher total phenolic and flavonoid content in the plant, the lower the amount of extract is required to reduce the DPPH, nitric oxide and superoxide ion scavenging activity. These results were also found to be concomitant with the total antioxidant activity observed in this study for both the extract (table-2). This is in agreement with Rice *et al.*, (1997) who reported that phenolic compounds and flavonoids have been associated with anti-oxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals. To further supports our finding recent studies carried out by Pratap *et al.*,

(2014) and Arasan and Abdul, (2012) reported that various extracts of *S. dulcis* have showed potent free radical scavengers. Although the scavenging abilities of the extracts were lower than those of ascorbic acid standard, it was evident that the both methanol and aqueous extracts did show some proton-donating ability and this could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Reducing properties of the antioxidants are generally associated with the quality of antioxidants activity of the plant. In this study a steadily increased in reducing capacity with direct proportion to the increasing concentration of the extract (fig-4) where methanol, aqueous and ascorbic extract at 250µg/mL was found to be 0.426, 0.324 and 0.589 µg/mL (fig-4). Reducing properties of the antioxidant are general associated with the presences of reductones and they exert anti-oxidant action by breaking the free radical chain and donating hydrogen atoms, thus preventing peroxides formation. As already discuss earlier, methanol being a potent extraction solvent could have extracted enhanced reductones content which again supports its potent reducing capacity when compared to that of aqueous solvent.

Our studies presented a positive relationship between the free radical scavenging activity the effective antioxidant capacity displayed by total phenol, flavonoids and antioxidant content in both methanol and aqueous extract which clearly justifies the therapeutic efficacy of *S. dulcis* extracts as an alternative to synthetic antioxidant.

GC-MS analysis of bioactive constituents

Knowledge of the chemical constituents of plants is desirable not only for the discovery of new therapeutic agents, but also disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and determining the actual significance of folkloric remedies (Milne et al.,1993). GC-MS analysis was carried out for both the extracts and results pertaining to these compounds (fig: 5 & 6) were identified through mass spectrometry attached with GC. Molecular weight, molecular formula and structure of the isolated compounds were ascertained.

GC-MS analysis shows the presence of different phytocompounds in methanol extract (Table-4) namely 2-hexyldecanoic acid methyl ester (6.10%), dextromoramide (31.88%), (Z)-7-Hexadecenyl acetate (51.51%), Cis-5-eicosenoic acid methyl ester (3.98%), 2-heptadecyl imidazoline (3.85%) and methyl eicosanoate (2.64%). The two most dominant compounds identified as dextromoramide with 31.88% belonging to opioid family are known to have analgesic activity this is in agreement with Ratnasooriya *et al.*, (2003) who reported significant analgesic and the anti-hyperalgesic activity for *S. dulcis* decoction. The other most dominant compound (Z)-7-hexadecenyl acetate with 51.51% with no anti-oxidant activity reported.

Result tabulated in (Table-5) for aqueous extract also shows the presence of different phytocompounds namely 1-Methyl-2-pyrrolidone- (4.83%), N1-Acetylspermine- (15.59%), L-(+)-ascorbic acid 2,6-dihexadecanoate- (22.71%), 3- Procaine-(6.74%), Cyclohexylamine-(6.20%) and beta-cyclocitral-(43.90%). The concentrated aqueous extract contains a variety of phytochemicals, among which the most dominant component of this plant with 22.71% is L-(+)-Ascorbic acid 2,6-dihexadecanoate and it has been reported to have antioxidant, anti-inflammatory and anti-nociceptive properties (Akinmoladun *et al.,* 2007; Okwu and Emenike, 2006). Acetylspermine belonging to polyamines family, play essential role in the proliferation and development of mammalian cells. In addition, polyamines have been shown to exert antioxidant activity (Ha *et al.,* 1998; Fujisawa, 2005). Phytochemical investigation of *S. dulcis* carried out so far, has reported terpenes such as triterpenes (Tsai *et al.*, 2011), benzoylated diterpenes (Ahsan *et al*, 2003) to be the most commonly isolated compound but never a monoterpenes has been reported isolated from this plant. The terpene identified in this

Table. 4: Bioactive components identified in Methanol extract of *S. dulcis*.

Peak No.	RT (Min)	Compound Name	Compound Nature	Peak area (%)
1	**15.05**	2-Hexyldecanoic Acid Methyl Ester MW- 270.25 MF- $C_{17}H_{34}O_2$	Methyl palmitate	**6.10**
2	**17.07**	Dextromoramide MW- 542.62 MF- $C_{29}H_{38}N_2O_8$	Opioid	**31.88**
3	**19.05**	(Z)-7-Hexadecenyl Acetate MW- 282.4614 MF- $C_{18}H_{34}O_2$	Oleic acid	**51.51**
4	**19.85**	2-Heptadecyl Imidazoline MW- 354.70 MF- $C_{20}H_{40}N_2$	Imidazoline	**3.98**
5	**20.83**	Cis-5-Eicosenoic Acid Methyl Ester MW- 324.54 MF- $C_{21}H_{40}O_2$	fatty acid methyl ester	**3.85**
6	**21.05**	Methyl Eicosanoate MW- 326.55 MF- $C_{21}H_{42}O_2$	Arachidic acid methyl ester	**2.64**

Table 5: Bioactive components identified in aqueous extract of *S. dulcis*.

Peak No.	RT (Min)	Compound Name	Compound Nature	Peak area (%)
1	15.75	1-Methyl-2-pyrrolidone MW-99.1311 MF- C_5H_9NO	Pyrrolidine.	**4.83**
2	16.98	N1-Acetylspermine MW- 187.2825 MF- $C_9H_{21}N_3O$	polyamine	**15.59**
3	17.22	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate MW- 414.53 MF- $C_{22}H_{38}O_7$	Vitamin	**22.71**
4	17.87	Procaine MW- 236.31 MF- $C_{10}H_{20}N_2O_2$	Amino ester	**6.74**
5	18.97	β-cyclocitral MW- 152.23 MF- $C_{10}H_{16}O$	Monoterpenes	**43.90**
6	19.80	Cyclohexylamine MW- 99.10 MF- $C_6H_{11}NH_2$	Aliphatic amine	**6.20**

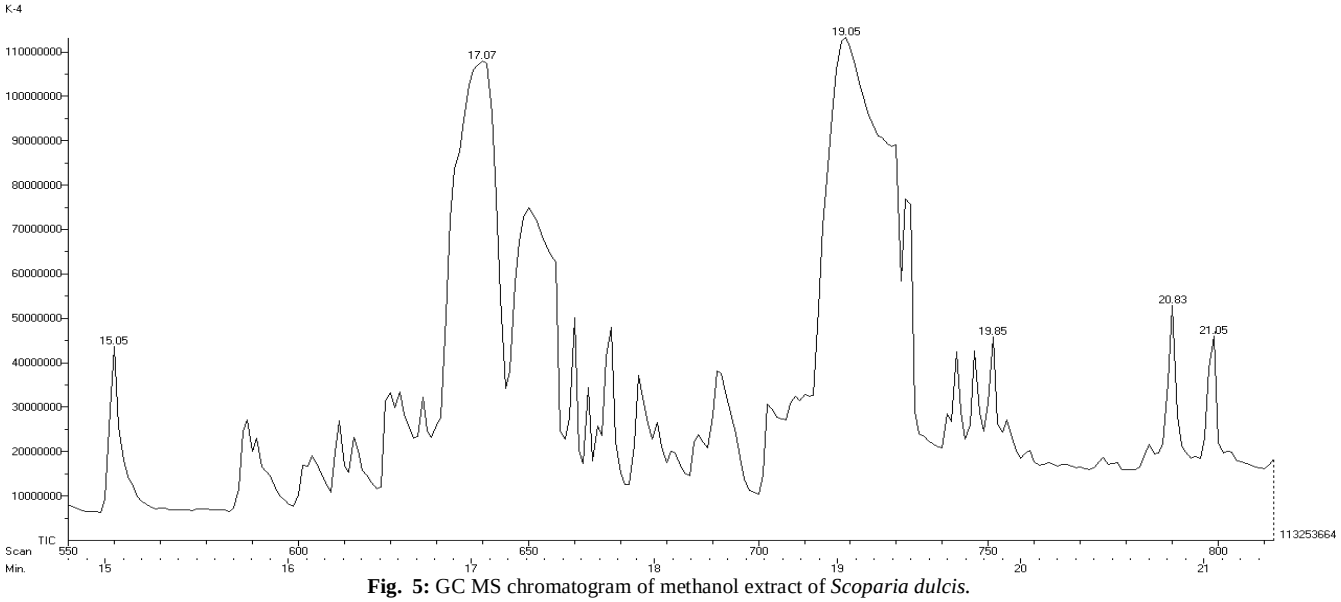

Fig. 5: GC MS chromatogram of methanol extract of *Scoparia dulcis.*

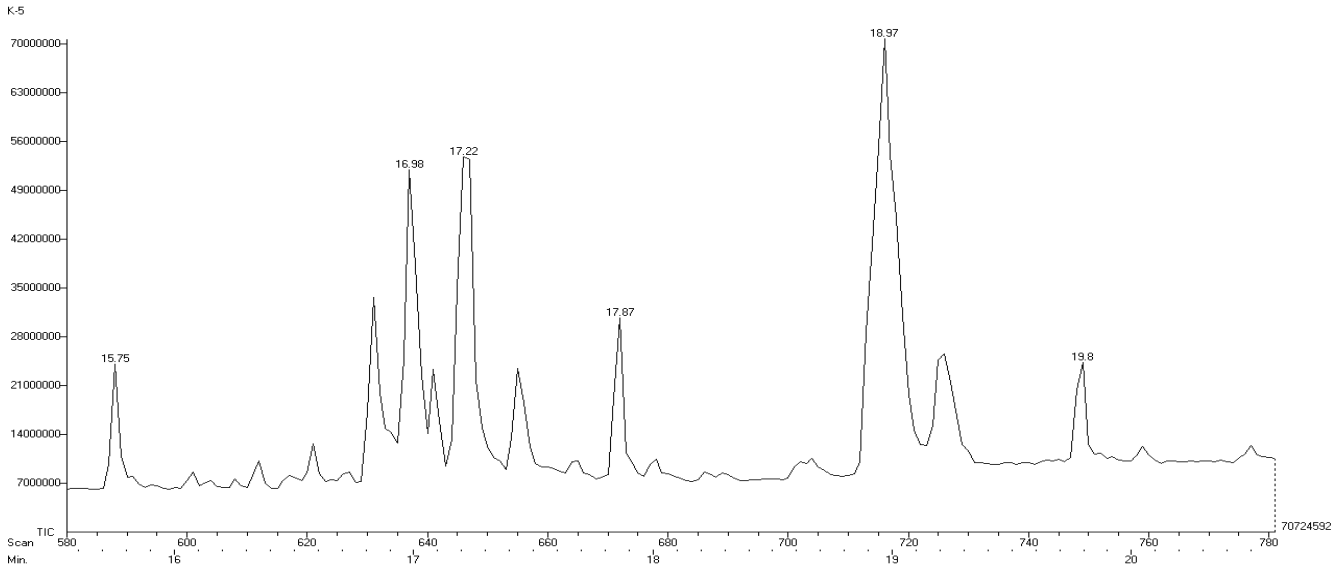

Fig. 6: GC MS chromatogram of aqueous extract of *Scoparia dulcis.*

particular study is a monoterpenes β-cyclocitral with the highest peak percentage of 43.90%. As such there has been no antioxidant activity recorded by β-cyclocitral however, it has been reported to have dissolution effect on cyanobacterial cell walls and membranes (Ozaki *et al.,* 2009). Recent publication on *S. dulcis* aqueous extract via HPTLC analysis established a class of compounds including terpenoids, flavonoids, stilbenes, phenolic compounds and proanthocyanidins (Wankhar *et al.,* 2015)

CONCLUSION

Thus summarizing these results, "it is evident that methanol extract of *S. dulcis* proved to have superior antioxidant capacity when compared to aqueous extract in this particular study and this may have resulted due to the greater extraction capacity of methanol when used as solvent. Hence, the possibility of using a crude extract as an antioxidant would greatly reduce the need to obtain pure compounds via expensive industrial purification techniques. Further in depth toxicity and dosage may reveal its efficacy of the plant as an alternative to anti-oxidant therapy.

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