GC-MS ANALYSIS OF AQUEOUS EXTRACT OF *SCOPARIA DULCIS* L. LEAVES

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ABSTRACT

Scoparia dulcis L. is a plant with various medicinal roles. The present study deals to analyse the phytochemical components in the aqueous extract of *S.dulcis* leaves through GC-MS. This plant is reported to have medicinal activities such as anti-diabetic, anti-bacterial, anti-fungal and anti-oxidants etc. The aqueous extract of the leaves was subjected to column chromatography and eluted with chloroform and methanol. The eluted fractions were tested with thin layer chromatography. The GC-MS analysis of fractions indicated the presence of some important compounds like 3-Buten-2-one-4 (2,5,6,6, tetramethyl 2-cyclohexane 1-yl), Tetra cyclo pentadeca-5,12 dien-3 one, Coumarine,3 acetylamino-6-phenyl, 4,5,7,Trihydroxyl isoflavone, n-Hexadecanoic acid, Octadecanoic acid, methyl ester α-Ketosteric acid, Myricetin, D-astycarpidan-1-methanol, acetate(ester), 1,2,Benzenedicarboxylic acid mono (2-ethylhexyl)ester and lycophene which have known medicinal values similar to the medicinal roles as found in the plant. The result of this study offered a platform of using aqueous extract of *S.dulcis* leaves as herbal alternative for various diseases.

Keywords: *Scoparia dulcis* L, GC-MS, phytochemical compounds, Medicinal plants.

1. INTRODUCTION

Plants have been used as medicinal agents in organized (Ayurveda, Sidhha, and Unani) and unorganized (folk, tribal, and native) forms of traditional medicine since time immemorial. The screening of medicinal plants for active chemicals has become increasingly intriguing to understand the innovative mechanism of action as herbal medicine becomes more accepted as an alternative type of health treatment [1]. Plants used in traditional and modern medicine provide inspiration and serve as models for the development of new medications with improved medicinal, chemical, and physical qualities than the original molecules [2]. Many publications exist on GC-MS analytical investigations on various plants and plant parts and researches were carried out to found if there were any active bio molecules with medicinal properties [3, 4]. Sweet broomweed, *Scoparia dulcis* L. (Scrophulariaceae), is a perennial and ubiquitous herb in tropical and subtropical regions. It is well-known in these places as a folk-medicinal plant having medicinal and magical properties [5]. It is spreading like a weed in many places of India.

It has tikta, laku, seta, and katu ayurvedic qualities. The whole plant is used in traditional remedies such as ayurveda and siddha formulations to treat renal and vesical canali culi, fever, wounds, ulcers, skin disorders, diarrhoea, antidote, stomach problems, hypertension, diabetes, inflammation, bronchitis, haemorrhoids, and hepatosis, as well as an analgesic in dysmenorrhea and leucorrhea situations [6]. Keeping in mind, the current work is carried out to determine the bioactive constituents in the leaves aqueous extract of *S.dulcis* through GC-MS analysis.

2. MATERIAL AND METHODS

2.1. Collection and identification of plants

The medicinal plant *S. dulcis* were collected from Kumarcoil, Thuckalay, Kanyakumari District, Tamil Nadu, and the plant was authenticated by a specialist. The collected leaves were washed with fresh water to remove the soil and adhered matters. The leaves were then ground separately into coarse powder. The dried powder was then stored in air-tight containers separately and used for further analysis.

2.2. Preparation of aqueous extracts

Ten g of dried leaves were subjected to extraction with water. The extract was kept in rotary shaker at 190-200 rpm for 24 hrs. It was then filtered with the help of muslin cloth and centrifuged at 10,000 rpm for 5 minutes and the supernatant was collected, dried and used for the further analysis.

2.3. Characterization of the crude extract of leaves of *S. dulcis* L

2.3.1. Column chromatography

A clean and dry 100 ml capacity of column was taken. The column was placed initially with glass wool with the help of glass rod. The slurry of silica gel of mesh size (60-120 μm mesh size) was filled to two third of the column. About 2.0 g of the aqueous extract of *S. dulcis* leaves was adsorbed to 10 g silica gel in methanol and was layered on the top of the column. The column was eluted with solvent system of chloroform and methanol 9:1. The fractions were collected in bottles.

2.3.2. Thin layer chromatography

The collected fractions were subjected to thin layer chromatography, to find out the probable number of compounds present in them. The solvent system used is methanol and chloroform.

The TLC was performed on precoated 20 x 20 cm and 0.25 mm thick plates using silica gel G for TLC which were left overnight for air drying. These plates were activated by hot air oven at 100°C for 1 hr. The extract was spotted on TLC plates. The plates were dried and developed in suitable solvents for rapid screening by methanol and chloroform in a ratio of 5:1. The plates were run in the above solvent system and dried at room temperature. Derivatisation of TLC plates was done by UV light at 254 mm. Different spots were observed in the 4th fraction, and the corresponding R_f values were determined. R_f value of each spot was calculated as follows:

R_f = Distance travelled by the solute/Distance travelled by the solvent.

2.3.3. GC-MS analysis of the active fraction

GC-MS analysis of the fraction 4 was analyzed using Agilent GC-MS 5975 Inert XL MSD (United States) gas chromatography equipped with J & W 122-5532 G DB-5 ms × 0.25mm × 0.25μm and mass detector (EM with replaceable horn), operated in EMV mode. Helium was used as carrier gas with the flow rate of 1.0 ml/min. The injection port temperature was operated at 250°C.

The column oven temperature was held at 80°C for 2 min then programmed at 10°C to 250°C, which was held for 0 min, and then at 5°C to 280°C which was held for 9 min. Electron impact spectra in positive ionization mode were identified.

3. RESULTS AND DISCUSSION

3.1. Characterization of the crude extract of leaves of *S. dulcis* L

Totally 15 fractions were collected and tested for TLC and only the 4th fraction alone showed TLC spots. Therefore, this spot alone was further subjected for GC-MS analysis.

3.2. GC-MS analysis of the aqueous extract of *S. duclis* leaves

GC-MS is a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures. Volatile and semi-volatile substances can be separated with remarkable precision using GC, however they cannot be identified. MS can provide detailed structural information on most compounds, allowing them to be precisely identified and quantified while also allowing them to be easily separated. In many ways, gas chromatography and mass spectrometry are extremely compatible techniques that were used in this investigation to identify the biomolecules present in the *S.dulcis* aqueous extract [7, 8]. Many researchers have worked on the GC-MS analysis of the medicinal plants [9, 10, 11].

The results of GC-MS chromatogram of aqueous extract *S.dulcis* leaves are indicated in fig. 1. The probable bioactive compounds present in the extract with their Retention Time (RT), molecular weight and molecular formula was indicated in Table 1. There are number of reports on the phytochemical constituents of *S.dulcis* [12, 13].

The bioactive compounds identified were 3-buten-2-one-4 (2,5,6,6, tetramethyl 2-cyclohexane 1-yl), Tetracyclo pentadeca-5,12 dien-3 one, Coumarine,3 acetylamino-6-phenyl, 4,5,7,Trihydroxyl isoflavone, n-Hexadecanoic acid, α-Ketosteric acid, Myricetin, D-astycarpidan-1-methanol,acetate(ester), 1, 2, Benzene-dicarboxylic acid mono (2-ethylhexyl)ester and Lycophene. In the present study aqueous extract of *S. dulcis* displayed a considerable antioxidant activity which is justified by the content of flavonoids. As reported by Mahakunakorn et al., [14] these 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.
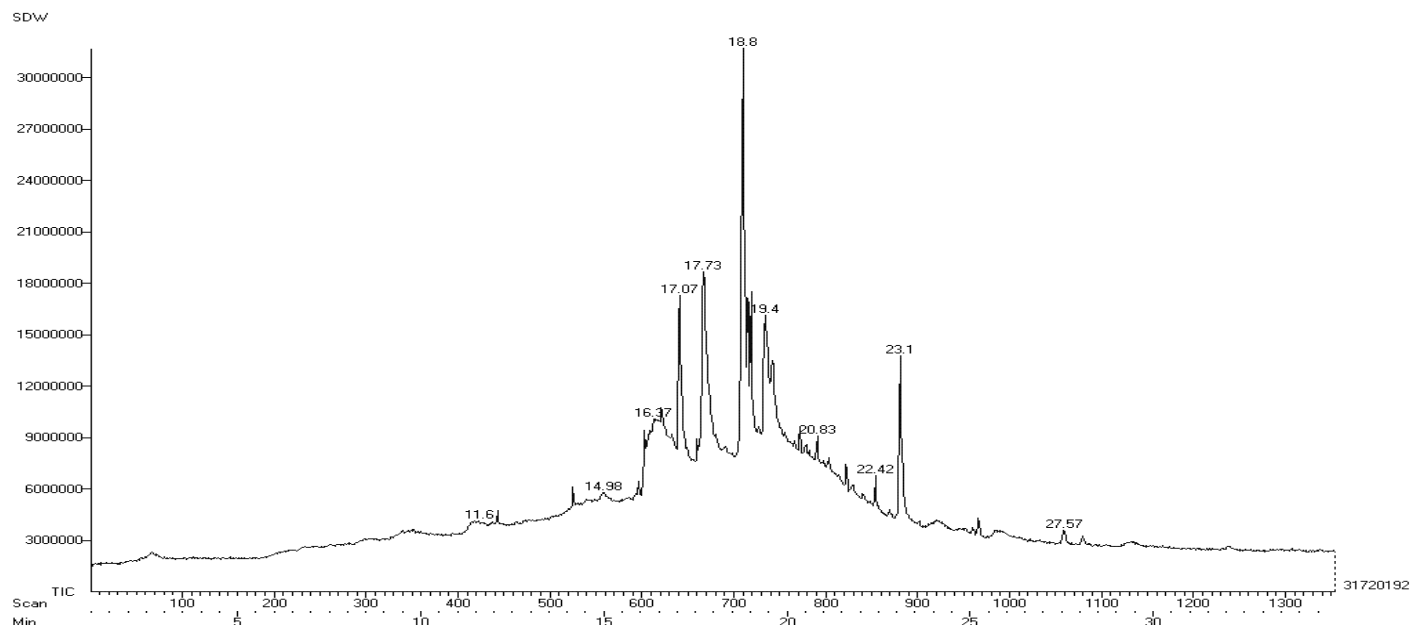

Fig. 1: GC–MS spectrum of aqueous extract of *S. dulcis* leaves

Table 1: GC–MS pattern of different fractions of aqueous extract of *S. dulcis* leaves

S. No	Compound Name	RT	Molecular Weight	Molecular Formula
1	3-Buten-2-one-4 (2,5,6,6, tetramethyl 2-cyclohexane 1-yl)	11.6	206.4 g/mol	$C_{14}H_{22}O$
2	Tetracyclo pentadeca-5,12 dien-3 one	14.98	264 g/mol	$C_{20}H_{24}$
3	Coumarine,3 acetylamino-6-phenyl	16.4	229.23 g/mol	$C_{13}H_{11}NO3$
4	4,5,7,Trihydroxyl isoflavone (Genistein)	17.07	270.24 g/mol	$C_{15}H_{10}O_5$
5	n-Hexadecanoic acid	17.73	256.42 g/mol	$C_{16}H_{32}O_2$
6	Octadecanoic acid, methyl ester	18.8	298.5 g/mol	$C_{19}H_{38}O_2$
7	α-Ketosteric acid	19.4	326.5 g/mol	$C_{20}H_{38}O_3$
8	Myricetin	20.83	318.23 g/mol	$C_{15}H_{10}O_8$
9	D-astycarpidan-1-methanol,acetate(ester)	22.42	326.4 g/mol	$C_{20}H_{26}N_2O_2$
10	1,2,Benzenedicarboxylic acid mono (2-ethylhexyl)ester	23.1	278.34 g/mol	$C_{16}H_{22}O_4$
11	Lycophene	27.62	536.9 g/mol	$C_{40}H_{56}$

Flavonoids (Myristein and Genistein) were the most abundant component in the *S.dulcis* aqueous extract, followed by 3-Buten-2-one-4 (2,5,6,6, tetramethyl 2-cyclohexane 1-yl), Tetra cyclo pentadeca-5,12 dien-3 one, Coumarine,3 acetylamino-6-phenyl, 4,5,7, Trihydroxyl isoflavone (Genistein), n-Hexadecanoic acid, Octadecanoic acid ,methyl ester, α-Ketosteric acid, Myricetin, D-astycarpidan-1-methanol, acetate (ester), 1,2,Benzenedicarboxylic acid mono (2-ethylhexyl)ester and Lycophene. The identified biochemical constituents in this study such as Myristein and Genistein, have

potent activities. Plant flavonoids, especially genistein, have shown promising pharmacological properties to ameliorate diseases including cancer [15, 16], anti-oxidant properties, numerous clinical implications in the treatment and prevention of diseases like diabetes, cardiovascular diseases, cancer, and osteoporosis [17], anti-angiogenesis [18]. Myristein is shown to have antioxidant effects, anti-diabetic effects, effect against Alzheimer's disease, anti-cancer effects and anti-infectious activity [19].

4. CONCLUSION

The present GC-MS analysis of the aqueous extract of *S. dulcis* leaves indicated the presence of phytochemicals especially flavonoids which have important medicinal activities which correspond well with the reports of the medicinal activities of this plant. Further research need to be performed to explore the bioactive constituents in this plant.

Conflict of interest

None declared

5. REFERENCES

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