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Research Paper

PHYTOCHEMICALS AND GC-MS PROFILING IN THE BULB OF *Scilla indica* BAKER.

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Abstract

The preliminary phytochemical screening in the bulb of *Scilla indica* revealed the presence of protein, carbohydrate, alkaloids, flavonoids, glycosides, phenols, tannins, saponins, steroids and sterols, terpenoids, anthraquinones and volatile oil in ethanolic extract. The aqueous extract showed negative response for saponins, steroids and sterols and volatile oil and screened protein, carbohydrate, alkaloids, flavonoids, glycosides, phenols, tannins, terpenoids, and anthraquinones. GC/ MS chromatogram of ethanolic bulb extract revealed the presence of 18 components. Hexatriacontane (CAS), Sulfurous acid, butyl decyl ester, 3-(4-methoxy-phenyl)-5-(2-nitro-benzylsulfanyl)-4-phenyl-4h-[1, 2, 4]triazole, 6-Ethyl-2-methyl-3-hydroxypyridine, Octadecane (CAS) are the five major components identified in the ethanol extract of *Scilla indica* bulb.

Key words: Phytochemical, GC-MS, *Scilla indica*, bulb.

INTRODUCTION

Natural products have played an important role in the development of drug for various diseases. Until 1990's scientists thought that most of the compounds produced by plants were useless waste products. These waste compounds are called as secondary metabolites. But later found that these perform a huge array of functions. The secondary metabolites have complex stereo structure with many chiral centers which may be essential for biological activity. Many of these cannot be synthesized economically on a commercial basis [3]. The secondary metabolites from natural sources are good candidates for drug development because being elaborated within the living systems, they are perceived to exhibit more similarities to drugs and show more biological friendliness than totally synthetic drugs [11].The families such as Berberidaceae, Leguminosae, Boraginaceae, Apocynaceae, Asclepiadaceae, Liliaceae, Gnetaceae, Ranunculaceae, Rubiaceae, Solanaceae and Rutaceae members contain rich alkaloid and other compounds in their plant parts. Hence in the present study *Scilla indica* belongs to Liliaceae used in heart diseases was selected to screen the phytochemicals and to analyse the bulb by GC-MS method.

MATERIALS AND METHODS

Scilla indica was collected from Kanjamalai and identified with Flora of the Presidency of Madras and Flora of Tamilnadu, India,The bulb was cleaned and separated from the leaf and dried in the oven and powdered [4,6].

Qualitative Phytochemical Screening

The ethanol and water solvent extracts of powdered sample using soxhlet for phytochemicals were screened using the following procedure. Carbohydrates, proteins and aminoacids, alkaloids, anthraquinones, flavonoids, glycosides, phenols and tannins, saponins, steroids and sterols, triterpenoids and volatile oil were qualitatively analyzed [5,7].

Test for carbohydrates

a) Fehling's test: Five ml of Fehling's solution was added to 2 ml of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars.

Tests for proteins and amino acids

a) Biuret test: To 1 ml of extract, equal volume of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate were added. The appearance of violet colour indicates the presence of proteins.

Tests for alkaloids

a) Wagner's reagent: To 1 ml of the extract, a few drops of Wagner's reagent was added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

Borntragers test for anthraquinones

a) 5 ml of extract was added with 10 ml of benzene. The mixture was shaken and the appearance of a pink, red or violet colour in the lower phase indicates the presence of free anthraquinones.
b) For combined anthraquinones, 5ml of extract was boiled with 10 ml of aqueous sulphuric acid and filtered while hot. The filtrate was shaked with 5 ml of benzene and the organic layer was separated. To half of its own volume, 10 per cent ammonia solution was added. A pink, red or violet colour in the ammonia phase (lower layer) indicates the presence of anthraquinone derivatives in the extract.

Test for flavonoids

a) Shinoda test: To 1 ml of the extract, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of pink colour indicates the presence of flavonoids.

Test for glycosides

a) Keller killani test: The extract was added with acetic acid containing traces of ferric chloride and transferred to a test tube containing sulphuric acid. Formation of a reddish brown colour, at the junction, which gradually turned to blue, confirms the presence of glycosides.

Test for tannins and phenolic compounds

To 1 ml of the extract, few ml of 5% neutral ferric chloride was added. The development of a dark bluish black colour indicates the presence of tannins.

a) To 1 ml of the extract, few ml of gelatin solution was added. The formation of a white precipitate reveals the presence of tannins and phenolic compounds.

Tests for saponins (Foam test)

a) About 1 ml of alcoholic extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 min. The formation of 1 cm layer of foam indicates the presence of saponins.
b) 5 ml of the extract was taken in a test tube and few drops of 5% sodium bicarbonate solution were added. The mixture was shaken vigorously and kept for 3 min. Formation of honey comb like froth shows the presence of saponins.

Test for steroids and sterols

a) Salkowski's test: The extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer - turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterol compounds, in the extract.

Test for triterpenoids

a) Liebermann - Burchard's test: The extracts were dissolved in 2 ml of chloroform and 10 drops of acetic anhydride and 5 drops of concentrated sulphuric acid were added. Appearance of red to violet colour indicates the presence of triterpenoids.

Test for volatile oil

a) 2 ml aliquot of extract was evaporated on a porcelain crucible. If the residue has an aromatic smell it indicates the presence of volatile oil.

GC –MS analysis

Preparation of extract

2 µl of the soxhlet ethanol extract of *Scilla indica* bulb was employed for GC/MS analysis [9].

Instruments and chromatographic conditions

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

RESULTS

The ethanol and aqueous extract of *Scilla indica* bulb was screened for phytochemicals qualitatively and presented in Table 1. The preliminary screening revealed the presence of carbohydrate content, protein content, alkaloid, flavonoid, glycosides, phenols, tannin, saponin, steroids and sterol, terpenoid content and volatile oil in ethanol extract. The aqueous extract showed negative response for saponin, steroids and sterol and volatile oil.

Table- 1 Qualitative phytochemical screening of bulb sample

S.No	**Constituents**	**Ethanol**	**Water**
1	Carbohydrate	+	+
2	Proteins and aminoacids	+	+
3	Alkaloids	+	+
4	Flavonoids	+	+
5	Glycosides	+	+
6	Phenols and Tannins	+	+
7	Saponins	+	-
8	Steroids and sterol	+	-
9	Anthraquinones	+	+
10	Triterpenoids	+	+
11	Volatile oil	+	-

GC/MS Analysis

The components identified by GC/MS study in the bulb sample of *Scilla indica* are presented in the Table-2 with their retention time molecular formula, molecular weight and peak area percentage and represented infigure-1. The bulb sample contained 18 components.

Hexatriacontane (CAS) (peak area %17.43), Sulfurous acid, butyl decyl ester (peak area % 17.43), 3-(4-methoxy-phenyl)-5-(2-nitro-benzylsulfanyl)-4-phenyl-4h-[1, 2, 4]triazole (peak area %1 6.37), 6-Ethyl-2-methyl-3-hydroxypyridine(peak area %7.62), Octadecane (CAS) (peak area % 6.24)are the five major components identified in the ethanol extract of *Scilla indica* bulb. The peak area percentage of the remaining components ranged between 5.83 and 1.06. These five major active components were found at the retention time of 37.34, 37.34, 33.01, 35.33 and 30.68 respectively.

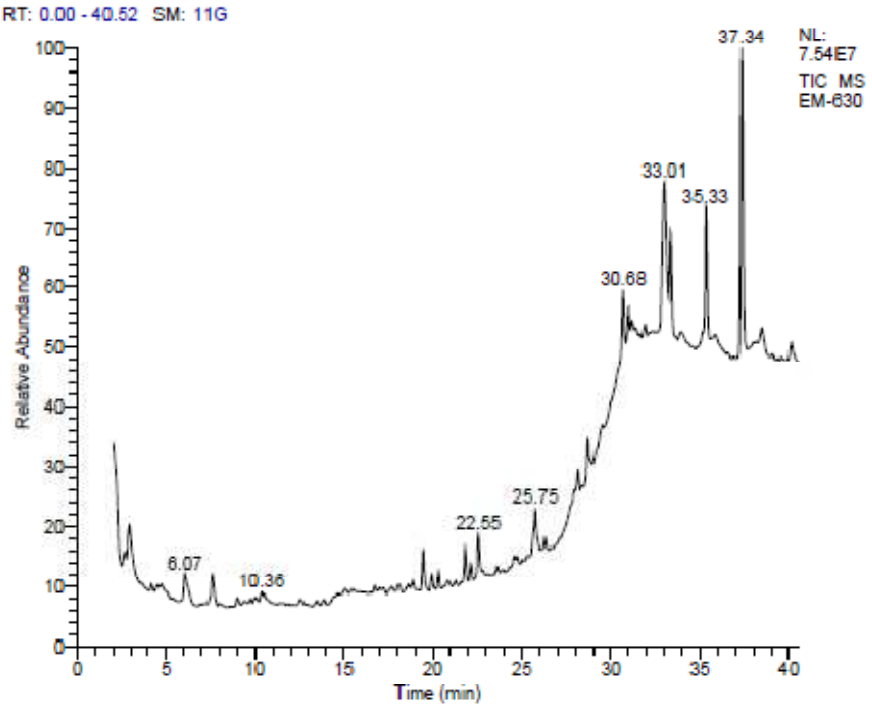

Figure1: GC- MS Chromatogram of ethanol extract of *Scilla indica* bulb

Table-2 Phytocomponents identified in the ethanol extract of *Scilla indica* bulb by GC-MS

S. No.	RT	NAME OF THE COMPOUND	MOLECULAR FORMULA	MW	PEAK AREA %
1	4.73	DELTA.3-Carene	C10H16	136	1.60
2	6.07	Dodecane, 5,8-diethyl- (CAS)	C16H34	226	3.00
3	7.59	Docosane (CAS)	C22H46	310	2.62
4	18.86	(1'S,5S)-5-Aminomethyl-3-(1'-phenylethyl)-1,3-oxazolidin -2-one	C12H16N2O2	220	1.06
5	19.47	3,7,11,15-tetramethyl-2-Hexadecen-1-ol	C20H40O	296	2.49
6	19.47	Phytol, acetate	C22H42O2	338	2.49
7	21.80	1-(Benzyloxy)-2-fluoro-2-phenyl-3-(p-toluenesulsulfonyloxy)propane	C23H23FO4S	414	2.02
8	22.55	10-Methoxy-9-phenylphenanthrene	C21H16O	284	2.90
9	25.75	9-Octadecenoic acid (Z)-, ethyl ester (CAS)	C20H38O2	310	3.50
10	28.71	3-Buten-2-ol, 4-phenyl- (CAS)	C10H12O	148	2.40
11	29.44	(5à,6à)-4,5-Epoxy-6-acetoxy-17b.hydroxy-17-methyl-3á-	C27H26N2O6	474	1.11

		phthalimidomorphinan			
12	30.68	Octadecane (CAS)	C18H38	254	6.24
13	30.99	Benzene, 1,1'-(1,3-butadiene-1,4-diyl)bis- (CAS)	C16H14	206	5.83
14	33.01	3-(4-methoxy-phenyl)-5-(2-nitro-benzylsulfanyl)-4-phenyl-4h-[1,2,4]triazole	C22H18N4O3S	418	16.37
15	33.35	Heptacosane (CAS)	C27H56	380	5.33
16	35.33	6-Ethyl-2-methyl-3-hydroxypyridine	C8H11NO	137	7.62
17	37.34	Hexatriacontane (CAS)	C36H74	506	17.43
18	37.34	Sulfurous acid, butyl decyl ester	C14H30O3S	278	17.43

DISCUSSION

The results showed that the ethanol extract was more efficient than the aqueous extract. The positive response of the above mentioned compounds to the ethanolic extract may be due to the dissolution capacity of phytochemicals in the organic solvents [1]. Phytochemical screening is an essential step towards discovery of new drugs as it provides the information regarding the presence of a particular primary and secondary metabolites in the plant extract of clinical significance. The medicinal plants have some chemical substance called phytochemicals that produce a physiological action on the human body. GC-MS is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC-MS chromatogram of the ethanolic extract of leaf of *Scilla indica* showed 18 peaks indicating the presence of 18 phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library, 18 phytoconstituents were characterized and identified. These compounds are grouped in to alcohol, esters, ketones & fatty acid. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosive investigation and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage [8].

The various active compounds present in the bulb may have many biological activities such as antimicrobial activity, wound healing, rheumatism, sprain and fractures [2].This indicates the phytopharmaceutical importance of *Scilla indica*. The presence of various bioactive compounds justifies the use of plant bulb for heart disease by traditional practitioners. However the isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give good results.

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