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Research Article

QUANTITATIVE PHYTOCHEMICAL AND GC-MS ANALYSIS OF LEAF AND BARK EXTRACT OF *DOLICHANDRONE ATROVIRENS*

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ABSTRACT

The present study deals with the quantitative phytochemical and GC-MS analysis of aqueous methanolic bark and leaf extract of *Dolichandrone atrovirens*. This study concerns the quantitative screening of total glycosides, saponins, total phenol content, total flavonoid content and vitamin C content. Further assessed the *Dolichandrone atrovirens* leaf and bark extracts by using GC-MS. The preliminary screening test results in the detection of bioactive principles and GC-MS analysis revealed the presence of 12 compounds in leaf extract and 11 compounds in bark extract. This analytical technique identifies the presence of pharmacologically active constituents present in the metabolic aqueous bark and leaf extract of *Dolichandrone atrovirens.* So that it can be recommended as a plant of phytopharmaceutical importance

Keywords: Aqueous methanolic, GC-MS, bark and leaf extract, *Dolichandrone atrovirens.*

INTRODUCTION

From the ancient time the plant has been main sources of drugs, which are used to treat the patient. Still today the herbal materials continue to perform a major role in primary health care as therapeutic remedies in many developing countries and may serve as the source for the development of more effective drugs[1,2]. These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access and less side effects[3-5]. Medicinal plants as commonly used for the primary care treatment and it is well known in rural areas for various developing countries[6,7]. The medicinal value of plants due to the some chemical substances that produce a definite physiological action on the human body and most notable bioactive compounds of plants are alkaloids, tannins, flavonoids and phenolic compounds[8]. Plants represent a reservoir of effective bioactive compounds and play a dominant role in the maintenance of human health and these are non-phytotoxic, more systemic and easily biodegradable[9,10]. Knowledge of the chemical constituents of plants is used to discover the therapeutic agents of the plants and also discovering the actual significance of folkloric remedies[11]. Chromatography is the basic analytical technique for quality control and standardization of phytotherapeuticals[12]. James and Martin is first described the Gas-Liquid Chromatography (GLC) on the year 1952 and had been one of the most important tools for the separation of chemical compound[13]. There are a number of different kinds of chromatography used for analysis of chemical compounds, but the recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants[14]. The combination of speed, sensitivity and a high resolving power in gas chromatography provides a very adequate technique for the separation of complex samples Mass spectrometry coupled with chromatographic separations such as gas-chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants for identify the presence of bioactive compounds and phytochemical constituent. The combination of speed, sensitivity and a high resolving power in gas-chromatography provides a very adequate technique for the separation of complex samples. *Dolichandrone atrovirens* leaf and bark are an important medicinal plant used widely in Indian folk medicine and yet have the antioxidant and antidiabetic activity[15,16]. This plant is owned by the family Bignoniaceae. It is a deciduous tree, up to 6 meter tall, leaflets velvety, pinnate leaves and wavy. The flowers are a white, borne in cymes in leaf axils. Seeds are rectangular with broad wings on each side. Fruit capsule is up to a foot long, brown, ribbed, seed winged. Hence the objective of the present study is to identify the phytochemical constituents of *Dolichandrone atrovirens* leaf and bark with the aid of GC-MS technique.

MATERIALS AND METHODS

Plant materials and Preparation of extract

Dolichandrone atrovirens barks and leaves were collected from Chitheri hills at Salem in the month of November 2009 and used for the current study. Herbarium voucher number for plants identification- No. BSI/SC/6/26/08-09/Tech.1382. The shade dried coarse powders of the plant material (1.5 kg) were extracted with 80 %v/v aqueous methanol by maceration at room temperature for 72 h and the extract was filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50°C).

Estimation of Glycosides

2.5 g of *Dolichandrone atrovirens* barks and leaves extracts were taken into the flask and added to the 15 ml of hot distilled water until dissolved. 25 ml of 80 percent alcohol was added to the flask and 50 ml of 95 percent alcohol also added and shake well. These resulting solutions are allowed to settle down and washed the filtrate by using 80 percent alcohol then evaporate. The evaporate taken in to the 50 ml cylinder with stopper and make the volume 30 ml by using water and add 10 percent volume/volume of H_2SO_4 and

constant shake and provide an opportunity to stan 24 hours at room temperature. Supernatant liquid was decanted and washed by cold water through the filter paper then the filter paper was dissolved in 45 percent alcohols and adds 2 to 3 drops of 10 percent ammonia to neutralize the acid. The content was evaporating to make dryness in the tarred beaker and increase weight is the represents the total glycosides[17].

Estimation of Saponins

5 g of *Dolichandrone atrovirens* barks and leaves extracts were dissolved in 50 ml of 90 percent alcohols and water bath for half hour and filtered and the residue was washed. Then the residue is treated with 50 ml of petroleum ether and reflexed half an hour and the petroleum ether portion was discarded and the ramming part filters through the thimble. The thimble keeps in to the soxhlet apparatus for extraction. Do the above same treatment using the carbon tetrachloride and ethyl acetate. Then the residues dissolved in 10 ml methanol and poured slowly into 50 ml of acetone and collect the precipitant then dried and weighed[17].

Estimation of Total Phenol Content

0.1 ml of *Dolichandrone atrovirens* barks and leaves extracts (0.1 mg/ml) was combined with 0.5 ml of Folin-Ciocalteu reagent and 1.5 ml of sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml with distilled water and allowed to stand for 2 h. The total phenols of barks and leaves extracts was measured at 765 nm by folin ciocalteu reagent. The Dilute methanolic extract (0.5 ml of 1:10 g/ml) or Gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with Distilled water) and aqueous sodium carbonate (4 ml, 1 m). The mixture was allowed to stand for 15 min and the total phenols were determined by Spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/ ml Solutions of gallic acid in methanol: water (50:50) and the total phenol values were expressed in terms of gallic Acid (equivalent mg/g of dry mass)[18].

Estimation of Total Flavonoids

500 µl of *Dolichandrone atrovirens* barks and leaves extract was mixed with mixed with 0.1 ml of 10 % aluminium chloride and 0.1 ml of potassium acetate (1M). In this mixture, 4.3 ml of 80 percent methanol was in addition to make 5 mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415 nm. The total flavonoids content was expressed as rutin equivalent in mg/g or %w/w of the extract[19,20].

Estimation of Ascorbic Acid

5 mL of standard ascorbic acid (100 g/mL) was taken in a conical flask containing 10 mL 4 percent oxalic acid and was titrated against the 2, 6-dichlorophenol indophenols dye. The appearance and persistence of pink color were taken as the end point. The amount of dye consumed (V 1 mL) is equal to the amount of ascorbic acid. 5 mL of sample (prepared by 0.5 g of leaf and bark extract of *Dolichandrone atrovirens* was dissolved separately in 4 percent oxalic acid, filtered and made up to 100 ml and centrifuged the 5 ml of the supernatant was pipetted out [21] was taken in a conical flask having 10 mL of 4 percent oxalic acid and titrated against the dye (V 2 mL). The amount of ascorbic acid was calculated using the following formula;

$$\text{Ascorbic acid (mg/100 g)} = (0.5 \text{ mg}/V_1 \text{ mL}) \times (V_2/15 \text{ mL}) \times (100 \text{ mL}/\text{Wt. of sample}) \times 100$$

GC-MS analysis

Instrument and Identification of compounds

The Gas-liquid chromatography, sample vaporized and injected onto the head of the chromatographic column. Then the sample transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid. The principle of gas chromatography is adsorption and partition. Within the family of chromatography-based methods gas chromatography (GC) is part of the most widely used techniques. In this study the GC-MS analysis of *Dolichandrone atrovirens* leaf and bark extract with in aqueous methanol, was performed using GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler 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 1 ml/min and an injection volume of 0.5 EI was employed. The identification of each compound was carried out by comparison of relative retention time and mass spectral data obtained with literature and a computerized MS data bank from National Institute Standard and Technology (NIST) having more than 62,000 patterns. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0.

RESULT AND DISCUSSION

The results of quantitative phytochemical screening shown in Table 1 which estimate the total amount of total glycosides, saponins, phenol, flavonoid content and vitamin C presence in the *aqueous* methanolic bark and leaf extract of *Dolichandrone atrovirens* Thus, the preliminary qualitative test may be useful in the detection of bioactive principles. Followed by the GC-MS analysis was employed to define the chemical compound present in the methanolic bark and leaf extract of *Dolichandrone atrovirens*. GC-MS analysis of leaf extracts *Dolichandrone atrovirens* indicating the presents of phytochemical constituents namely, methyl-α-D-Glucopyranoside, 4-C-methyl-Myo-Inositol, n-Hexadecanoic acid, Phytol which contribute to the medicinal activity of the extract and list of major phytochemical constituents identified by GC-MS are presented in Table 2. Similarly bark extracts *Dolichandrone atrovirens* indicating the presents of (1-tert-Butyl-3-(3-methoxyphenyl)-bicyclo [1.1.1]pentan, Limonene dioxide, 8,11,14-Eicosatrienoic acid and Squalene which contribute to the medicinal activity of the extract and list of major phytochemical constituents identified by GC-MS are presented in Table 3. GC-MS methods were carried out early for various compounds for the prediction of the biological activities[22-24].

Table 1: Quantitative Phytochemical screening aqueous methanolic bark and leaf extract of *Dolichandrone atrovirens*

Quantity/Amount Present* (Per g of extract)			
S. No.	**Phytochemical Constituents**	***Dolichandrone atrovirens* Leaf Extract**	***Dolichandrone atrovirens* Bark Extract**
1	Total Glycosides	123.73 ± 2.63 mg	141.3 ± 4.51 mg
2	Saponins	15.99 ± 1.62 mg	17.36 ± 1.22 mg
3	Total Phenol Content	71.95 ± 0.82 mg of Gallic acid equivalent	93.51 ± 0.61 mg of Gallic acid equivalent
4	Total Flavonoid Content	44.11 ± 3.18 mg of Rutin equivalent	56.16 ± 2.04 mg of Rutin equivalent
5	Vitamin C Content	11.35 ± 1.36 mg	14.5 ± 0.84 mg

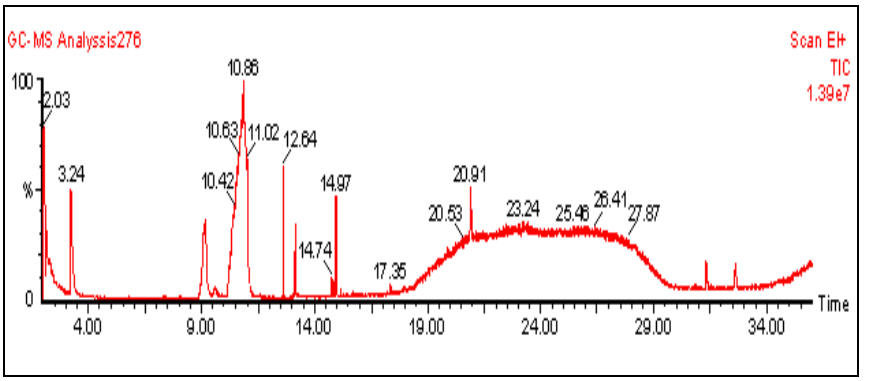

Figure 1: *Dolichandrone atrovirens* leaf extract (GC-MS)

Table 2: Compounds identified by GC-MS analysis from *Dolichandrone atrovirens* leaf extract

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	3.24	1-Butanol, 3-methyl-, formate	$C_6H_{12}O_2$	116	4.77
2.	9.17	α-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	94	8.56
3.	10.86	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	94	59.66
4.	12.64	9-Octadecenoic acid, 12-(acetyloxy)-, methyl ester, [R-(Z)]-	$C_{21}H_{38}O_4$	54	1.68
5.	13.14	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.77
6.	14.74	1-Tridecyne	$C_{13}H_{24}$	180	0.26
7.	14.97	Phytol	$C_{20}H_{40}O$	296	1.59
8.	15.18	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268	0.09
9.	17.35	Ethanol, 2-(2-propenyloxy)-	$C_5H_{10}O_2$	102	0.18
10.	20.91	Vinyl 10-undecenoate	$C_{13}H_{22}O_2$	210	19.42
11.	31.33	11,12-Dihydroxyseychellane	$C_{15}H_{26}O_2$	238	0.97
12.	32.62	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	$C_{25}H_{36}O_2$	368	1.06

Table 3: Compounds identified by GC-MS analysis from *Dolichandrone atrovirens* Bark extract

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	7.72	1-tert-Butyl-3-(3-methoxyphenyl)-bicyclo[1.1.1]pentan	$C_{16}H_{22}O$	230	0.32
2	10.88	Limonene dioxide	$C_{10}H_{16}O_2$	168	1.10
3	12.70	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	17.55
4	14.69	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	3.91
5	14.95	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	36.47
6	15.29	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	14.69
7	15.90	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	2.10
8	16.99	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	2.61
9	17.10	5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-	$C_{22}H_{36}O_2$	332	15.08
10	19.66	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	$C_{15}H_{26}$	206	1.38
11	23.76	Squalene	$C_{30}H_{50}$	410	4.78

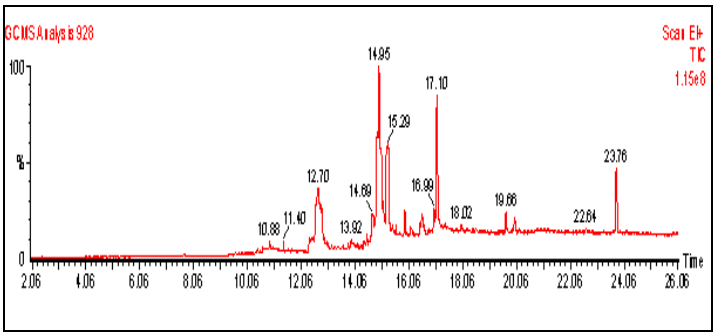

Figure 2: *Dolichandrone atrovirens* bark extract (GC-MS)

CONCLUSION

GC-MS method is a direct and fast analytical approach to identifying the phytochemical constituents of plant extract. In the present study twenty one chemical constituents have been identified from aqueous methanolic leaf and bark extract of *Dolichandrone atroviren.* The present study, which reveals that the plant has the antidiabetic and antioxidant property may due to the above mentioned phytochemicals.

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