Int. J. Chem. Sci.: 13(2), 2015, 955-962
ISSN 0972-768X
www.sadgurupublications.com

COMPARATIVE STUDIES ON THE FATTY ACID COMPOSITION OF PETROLEUM ETHER EXTRACT OF LEAVES AND FLOWERS OF *CATHARANTHUS ROSEUS* AVAILABLE IN BANGLADESH BY GC-MS ANALYSIS

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ABSTRACT

Catharanthus roseus is one of the medicinally important plants belonging to the family of Appocyanaceae. It has been used extensively by ayurvedic practitioner to treat dermatitis, abscesses, eczema, psoriasis, sores, corns, ringworm, scabies, epilapsy, malaria, heart tonics and tumor. It has several commercially valuable secondary metabolites including the anticancer agent, vincristine, vinblastine and the hypertension drugs ajmalicine and serpentine. The fatty acid compositions of the petroleum ether extract of leaves and flowers of *Catharanthus roseus* grown in Bangaladesh were determined by gas chromatography-mass spectrophotometer. 5 compounds were identified from the extract of leaves (24.18%) and 5 compounds were identified from extract of flowers (55.53%) of *Catharanthus roseus.* In the case of leaves the main fatty acid was methyl elaidate (8.57%) and in the case of flowers, it was methyl palmitate (13.13%), respectively.

Key words: *Catharanthus roseus,* GC-MS, Fatty acid compositions.

INTRODUCTION

Plant products have been part of phytomedicine since time immemorial. These can be derived from any part of the plant like leaves, flowers, bark roots, fruits and seeds etc[1].

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Any part of the plant may contain active components. Herbal medicines have become more popular in the treatment of any diseases due to popular belief that green medicine is safe, easily available and with less side effects. Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use[2]. The medicinal properties of some plants have been documented by some researchres[3-5]. Medicinal plant constitutes are the main source of new pharmaceuticals and healthcare products[6]. Extraction and characterization of several phytocompounds of these green factories have given birth to some high activity profile drugs[7]. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or less of genetic diversity[8]. Knowledge of the chemical constituents of the plant is desirable because such information will valuable for the synthesis of complex chemical substances.

Catharanthus roseus (Common name-Periwinkle, Vinca; Bengali-Nayantara, Synonymes-Vinca rosea; Family-Apocyanaceae) popularly known as madagascar periwinkle is a potential source for anti-leukemic alkaloids. It is cultivated mainly for its alkaloids, which are having anticancer activities[9]. It is an evergreen subshrub or harbeceous plant growing up to 1 m tall[10].

Catharanthus roseus is administered as a cooling medicine. It is used for the treatment of diabets, fever, malaria, throat infection and chest complaints. It is also used for the regulation of menstrul cycles, and as a euphoriant[11]. The plant is an important source of indole alkaloids, which are present in all plant parts. The physically important and antineoplastic alkaloids namely Vincristine and Vinblastine are mainly present in the leaves whereas antihypertensive alkaloids such as ajmalicine, serpentine and reserpine are reported to be present in the roots[12]. Vincristine and Vinblastine alkaloids are used in the treatment of various types of lymphoma and leukemia[13,14]. These Catharanthus alkaloids are also used for the treatment of both malignant and nonmalignant diseases and in platelet and platelet associated disorder.

However many researches have been carried out on *Catharanthus roseus*, but no systematic research on fatty acid composition of leaves and flowers of the plant by GC-MS analysis has been reported. Therefore, the present study was undertaken with an objective to carry out a complete investigation of the compositions of fatty acids of leaves and flowers from petroleum ether extract of *Catharanthus roseus* with GC-MS analysis.

EXPERIMENTAL

Materials and methods

Collection of plant material

Fresh leaves and flowers of *C. roseus* were collected from the gardens of Chemistry Department of Dhaka University, Bangladesh in June, 2013 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. ≠ 39512) has been deposited.

Solvents

Petroleum ether (b.p. 40-60^{o}C, Merck, Germany) of AR grade, under normal atmospheric pressure was employed for extraction of plant material. Solvent from extract were recovered under distillation and the dried extracts were preserved in a refrigerator.

Extraction of fatty acids and preparation of methyl ester (FAMEs)

The fresh plant material of leaves and flowers of *Catharanthus roseus* was collected and washed individually from running tap water to remove soil particles and other dust. Then, they were dried at room temperature and powdered by Fritsch mortar grinder, Germany. The natural fatty acids were extracted separately from the powder (100 g) of both leaves and flowers of the plant with petroleum ether (b.p. 40^{o}C-60^{o}C) in a Soxhlet apparatus for 72 hr. The extracts were concentrated under reduced pressure in a rotary evaporator. The extracts were filtered using Whatman No. 1 filter paper and then vacuum distilled to remove solvent completely. The extracts from the leaves of *Catharanthus roseus* was 7.90 g (7.90% w/w) and for the extract from the flowers of *Catharanthus roseus* was 5.53 g (5.30% w/w). Petroleum ether extracts for both the leaves and flowers of *Catharanthus roseus* were kept in a nitrogen atmosphere in a refrigerator. The fatty acids present in the extracts were converted to fatty acid methyl esters (FAMEs) first and analyzed according to the method reported by Griffin[15] for GC-MS analysis.

The fatty acid composition was determined by analysis of their methyl esters. The fatty acid methyl esters (FAMEs) were prepared by esterification reaction by using BF_3-MeOH complex according to AOAC method[16]. 10 mg of extract of leaves/flowers was taken in a screw capped glass tube. 1 mL of BF_3-MeOH complex were added and then heated at 100^{o}C for 1 hr in a water bath. After that it was cooled at room temperature and 1 mL of deionized water & 2 mL of hexane were added. The glass tube was vortexed and

centrifuged at low RPM for two minutes. The upper layer was collected by means of syringe and kept in closely tight glass vial in refrigerator. Then the prepared FAMEs were ready to analyze.

Gas chromatograph-Mass spectrum analysis

GC-MS analysis of the fatty acids of leaves and flowers of *Catharanthus roseus* from petroleum ether extracts were carried out on a Agilent 7890. A system equipped with Mass Spectrophotometer detector and split less injection system. The GC was fitted with a HP-5MS capillary column (30 m × 0.25 mm: film thickness: 0.25 μm). The temperature program was as follows: injector temperature 260°C, initial oven temperature at 70°C, then increased at 10°C/min to 150°C for 5 min., then 12°C/min to 200°C for 15 min. and then 12°C/min to 220°C for 15 min. Helium was used as the carrier gas at 17.69 psi pressure with flow 0.6 mL/min. Samples were dissolved in methanol and 1 μL aliquot was injected automatically. MS was set in scan mode. The ionization was electron ionization. The mass range was set in the range of 50-550 m/z. MS spectra of separated components were identified on NIST libraries for fatty acid compositions.

RESULTS AND DISCUSSION

GC-MS analysis of fatty acids of leaves and flowers of *Catharanthus roseus* from petroleum ether extract showed the presence of 5 compounds for the case of leaves and 5 compounds in the case of flowers. GC analyzed results which include the active principles with their retention time, molecular formula, molecular weight and composition of the fatty acids of leaves and flowers of *Catharanthus roseus* from petroleum ether extract are presented in Table 1 and 2.

Table 1: GC-MS analysis of fatty acids from petroleum ether extract of leaves of *C. roseus*

S. No.	Retention time (min)	Name of the compound	Molecular weight	Molecular formula	Conc. (%)
1	17.62	Methyl myristate	228.37	$C_{15}H_{30}O_2$	0.74
2	21.38	Methyl palmitate	256.42	$C_{17}H_{34}O_2$	9.78
3	26.50	Methyl Linoleate	294.47	$C_{19}H_{34}O_2$	2.56
4	26.75	Methyl elaidate	296.47	$C_{19}H_{36}O_2$	8.57
5	27.79	Methyl stearate	312.53	$C_{19}H_{38}O_2$	2.53

Table 2: GC-MS analysis of fatty acids from petroleum ether extract of flowers of *C. roseus*

S. No.	Retention time (min)	Name of the compound	Molecular weight	Molecular formula	Conc. (%)
1.	21.39	Methyl Palmitate	270.45	$C_{17}H_{34}O_2$	13.13
2.	24.45	Butyl methyl phthalate	290.85	$C_{17}H_{34}O_4$	5.79
3.	26.55	Methyl Linoleate	294.47	$C_{19}H_{34}O_2$	12.13
4.	26.79	Methyl Elaidate	296.48	$C_{19}H_{36}O_2$	12.92
5.	27.80	Methyl Stearate	298.50	$C_{19}H_{38}O_2$	11.56

Total 5 fatty acids were identified as their methyl esters in the case of leaves of *Catharanthus roseus*. The major constituents was Methyl Elaidate (8.57%) with retention time 26.75 and in the case of flowers 5 fatty acid compositions were identified. The major constituent was Methyl Palmitate (13.13%) with retention time 21.39, respectively.

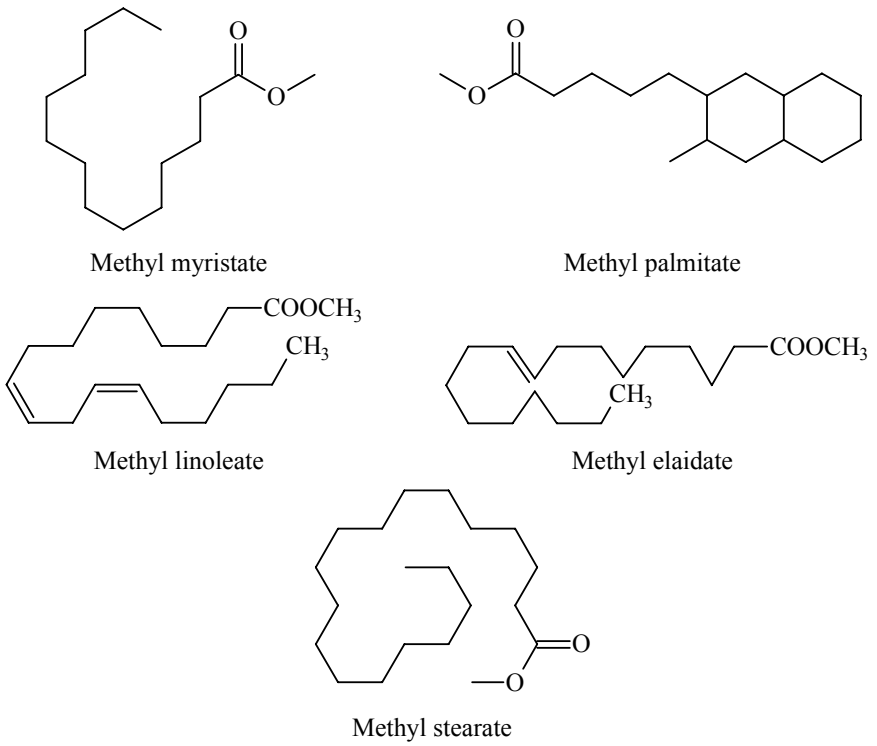

Fig. 1: Structure of the identified fatty acid esters from GC-MS analysis of petroleum ether extract of leaves of *C. roseus*

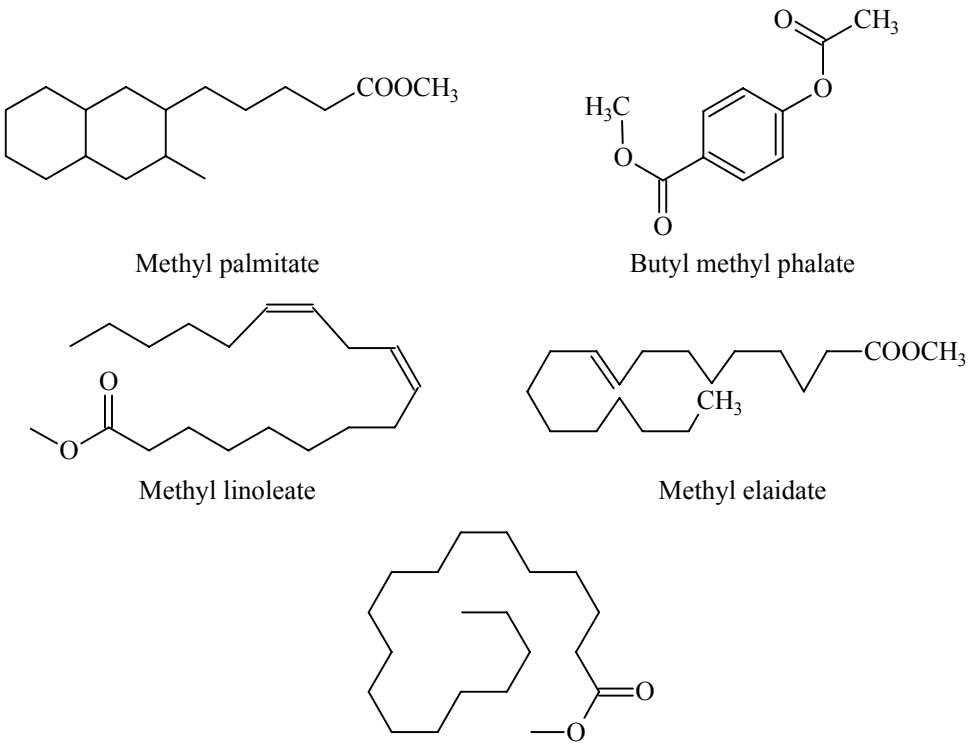

Fig. 2: Structure of the identified fatty acid esters from GC-MS analysis of petroleum ether extract of flowers of *C. roseus*

CONCLUSION

The present study found 5 constituents from leaves and 5 constituents from flowers of petroleum ether extract of *Catharanthus roseus* by gas chromatography-mass spectroscopy (GC-MS) analysis. The presence of these chemical compounds justified the extensive uses of leaves and flowers of the plant by traditional practitioner to treat various ailments. It could be concluded that *Catharanthus roseus* contains various chemical constituents that can be bioactive compounds of medical importance. However, further studies are needed to evaluate its bioactivity and toxicity profile.

ACKNOWLEDGEMENT

We are grateful to DRICM, BCSIR for giving us the opportunity to do analysis of the petroleum ether extract of plant materials. We are also thankful to the Division-in-Charge,

Chemical Research Division, BCSIR Laboratories, Dr. Ismet Ara Jahan, Dhaka, for providing necessary facilities to carry out this research work.

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Accepted : 18.04.2015