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EVALUATION OF BIOACTIVE COMPOUNDS IN THE EDIBLE *HIBISCUS ROSA-SINENSIS* L FLOWER PETALS BY GC-MS ANALYSIS

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ABSTRACT

Nature is always a golden sign to show the prominent phenomena of coexistence. Natural products from edible flowers are the basis for treating human diseases. Medicinal flowers are presently in demand and their acceptance is increasing progressively. The purpose of the current study is to monitor the bioactive compounds in the ethanolic extract of *Hibiscus rosa-sinensis* L flower petals by GC-MS analysis. Totally 15 compounds were identified and the chromatograph showed peaks with individual compounds. The major constituents identified in ethanol extract of petals were Morpholine, 4,4'- (phenylmethylene) bis- Morpholine (22.39%), Decane, 2,3,5,8-tetramethyl (19.90%), Octadecane, 6-methyl- (9.88%), Undecanoic acid, ethyl ester (8.17%), 7,9-Di-tert-butyl-1- oxaspiro(4,5)deca-6,9- diene-2,8-dione (8.13%), 1-Iodo-2-methylundecane (6.61%), Sulfurous acid, pentyl tetradecyl ester (4.00%), d-Mannose (3.08%), Z-(13,14- Epoxy)tetradec-11-en-1-ol acetate (2.89%), 9,12-Octadecadienoyl chloride, (Z,Z) (1.81%) and various other compounds were identified as low level. Among the 15 compounds, Morpholine was found to be the major bioactive compound in *Hibiscus rosa–sinensis* L flower petals which shows (22.39%) peak area with retention time (23.03). The result of this study offer a platform of using *Hibiscus rosa-sinensis* L flower petals as herbal alternative for various diseases and it can be used as functional and pharmaceutical food.

KEYWORDS: *Hibiscus rosa-sinensis* L flower petals, GC-MS, Bioactive compounds, Morpholine.

INTRODUCTION

Human societies have been in close contact with their environments since the beginning of their formation and used the ingredients of the environment to obtain food and medicine. Awareness and application of plants to prepare food and medicine have been realized through trial and error, and gradually human became able to meet his needs from his surroundings. Information about medicinal plants has long been transmitted gradually and from generation to generation, a human knowledge has gradually become complete with the formation of civilizations and the provision of more facilities. Medicinal plants are used as a medical resource in almost all cultures. Ensuring the safety, quality and effectiveness of medicinal plants and herbal drugs very recently became a key issue in industrialized and developing countries. By standardizing and evaluating the health of active plant-derived compounds, herbal drugs can help the emergence of a new era of the healthcare system to treat human diseases in the future. Awareness of traditional knowledge and medicinal plants can play a key role in the exploitation and discovery of natural plant resources (Fatemeh Jamshidi-Kia *et al*., 2018).

Phyto-constituents are the complex mixture of component so they are able to become more effective than chemically synthesized pure compounds. The phyto-constituent present in the plant has physical or chemical effect in nature which has the ability to minimize the human illness (Deepak Bhattarai and Meena Rajbhandari, 2016).

Hibiscus rosa-sinensis L is an evergreen woody, glabrous, showy shrub, leaves bright green, ovate, entire below, coarsely toothed above; flowers solitary, axillary, bell shaped, with pistil and stamens projecting from the centre; capsules roundish, many seeded. (Nadkarni, 1998).

Flower powder shows spheroidal, pentoporate, pore-circular pollen grains ; stellate trichomes single, elongated, conical or twisted and convoluted; glandular trichomes uni or bi- seriate, multicellular- cylindrical and bi-or multiseriate, multicellular-globose or clubshaped; ranunculaceous type of stomata; sphaeraphide calcium oxalate crystals. In Ayurvedic medicine an infusion of petal is widely used as a demulcent refrigerant drink in fever. The flowers have also been found to be effective in the treatment of arterial hypertension and to have significant antifertility effect (Pekamwar *et al*., 2013).

To identify the bioactive constituents such as long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino, nitro compounds etc., GC-MS is an effective tool. Hence, it is

associated with particular detection techniques which have become a sophisticated means for analysis of various compounds (Emasushan *et al*., 2018). The main aim of the present study is focused on the GC-MS Analysis of ethanolic extract of *Hibiscus rosa-sinensis* L flower petals belongs to family malvaceae having a tremendous therapeutic potential which is not fully utilized.

MATERIALS AND METHODS

Collection and identification of flower material

Hibiscus rosa-sinensis L flowers were freshly collected from in and around Tiruvarur, Tamil Nadu and India. The plant was identified with the help of Flora of Presidency of Madras and authenticated with the Voucher specimen deposited at Rapinat Herbarium, Department of Botany, St.Joseph's College, Trichy.

Extraction

The petals of *Hibiscus rosa-sinensis* L flowers were used for this study. Petals powder of *Hibiscus rosa-sinensis* L flowers were shade dried. 20 g of the powdered petals were soaked in 95% ethanol for 12 h. The extract was then filtered through Whatmann filter paper No.41 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the flower material used (Muthukumaran Pakkirisamy *et al*., 2017).

GC-MS Analysis

The sample was investigated through Gas Chromatography Mass Spec- trometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS/MS is a Scion 436- GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/95% Dimethyl polysiloxane) and Length: 30m; Internal diameter: 0.25 mm; Thickness: 0.25 µm. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The 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, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C and total GC running time was 41 min.9 This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode

with ionization energy of 70eV. The solvent delay was 0-3.0 min. A scan interval of 0.5 seconds and fragments from m/z 50 to 500 Da was programmed. The inlet temperature was set at 280°C, source temperature 250°C. 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 MS Work station 8. The NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components. The GC-MS/MS was performed by Food Safety and Quality Testing Laboratory, Indian Institute of food processing technology, Thanjavur.

RESULTS AND DISCUSSION

GC-MS analysis is the one of important step towards understanding the nature of active principles in edible medicinal flowers. The compounds present in the ethanolic extract of *Hibiscus rosa–sinensis* L flower petals, were identified by GC-MS analysis (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 1. Fifteen compounds were identified in ethanolic extract by GC-MS. The major components present in the *Hibiscus rosa–sinensis* L flower petals were Morpholine, 4,4'- (phenylmethylene) bis- Morpholine (22.39%), Decane, 2,3,5,8-tetramethyl (19.90%), Octadecane, 6-methyl- (9.88%), Undecanoic acid, ethyl ester (8.17%), 7,9-Di-tert-butyl-1- oxaspiro(4,5)deca-6,9- diene-2,8-dione (8.13%), 1-Iodo-2-methylundecane (6.61%), Sulfurous acid, pentyl tetradecyl ester (4.00%), d-Mannose (3.08%), Z-(13,14- Epoxy)tetradec-11-en-1-ol acetate (2.89%), 9,12-Octadecadienoyl chloride, (Z,Z) (1.81%) and various other compounds were identified as low level . These phytochemicals are responsible for various pharmacological actions like antimicrobial, anti-oxidant, antifertility, antimutagenic, anti-inflammation, Anticancer, Hepato protective, Diuretic, Antiasthma activities etc. *Hibiscus rosa–sinensis* L flower petals has medicinal value due to the presence of these major constituents (Sonjit *et al*., 2013).

Among the 15 compounds identified, Morpholine was found to be the major bioactive compound *Hibiscus rosa–sinensis* L flower petals which show 22.39% peak area with retention time 23.03. Morpholine is an organic chemical compound (O(CH2CH2)2NH) containing nitrogen and oxygen heterocyclic six membered ring and is considered an important building blocks in the field of medicinal chemistry field (Zhou *et al*., 2014).

Morpholine derivatives are very essential in the drug discovery process and stimulate research in broad spectrum of biological activity study (Achari et al., 2004). This class of heterocyclic compounds having characteristic functional groups of amine and ether and great industrial importance. Chemical manipulations on morpholine based molecules through structure - activity relationship strategy could help developing many interesting candidates of therapeutic significance to tackle broad range of medical ailments(Kumar rupak *et al*., 2016) It have found great significance in modern years due to their variety of pharmacological activities including analgesic, anti-inflammatory, anticancer, antidepressant, HIV-protease inhibitors, appetite suppressant, local anaesthetic, antiplatelet, selective inhibitor of protein kinase C, antitumor, neuroprotective, antifungal, anti-tuberculosis, anti- parasitic, anti-malarial, hypolipidemic and hypocholesterolemic activities (Duhalde *et al*., 2007).

Due to presence of such large number of useful metabolites in these *Hibiscus rosa – sinensis* L flower petals, this plant are recommended as phytopharmaceutically important. Also, this type of GC-MS analysis is the first step towards understanding the nature of active principles. In *in-vitro* and *in-vivo* parts of these petals would be helpful for large scale isolation of such pharmaceutically important compounds from *Hibiscus rosa–sinensis* L flower petals.

Table 1: Phytocomponents Identification in Ethanolic Extract of *Hibiscus Rosa-Sinensis* L Flower Petals by GC-MS.

S.No.	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
1.	5.04	d-Mannose	C H O 6 12 6	180	3.08
2.	8.20	Decane, 2,3,5,8-tetramethyl-	C H 14 30	198	19.90
3.	10.88	Octadecane, 6-methyl-	C H 19 40	268	9.88
4.	13.22	1-Iodo-2-methylundecane	C H I 12 25	296	6.61
5.	13.99	1,2-Benzenedicarboxylic acid, butyl octyl Ester	C H O 20 30 4	334	1.17
6.	14.45	Albuterol	H NO C 13 21 3	239	6.51
7.	14.58	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9- diene-2,8-dione	C H O 17 24 3	276	8.13
8.	15.63	Undecanoic acid, ethyl ester	C 13 26 2 H O	214	8.17
9.	17.93	9,12-Octadecadienoyl chloride, (Z,Z)-	C18 H31 C10	298	1.81
10.	18.03	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	H O C 16 28 3	268	2.89
11.	18.40	Hexadecanoic acid, ethyl ester	H O C 18 36 2	284	3.02
12.	23.03	Morpholine, 4,4'-(phenylmethylene) bis-	H N O C 15 22 2 2	262	22.39
13.	27.22	β-D-Mannofuranoside, farnesyl-	H O C 21 36 6	384	1.04
14.	28.40	Sulfurous acid, pentyl tetradecyl ester	H O S C 19 40 3	348	4.00
15.	35.98	Ethyl 6,9,12,15-octadecatetraenoate	H O C 20 32 2	304	1.41

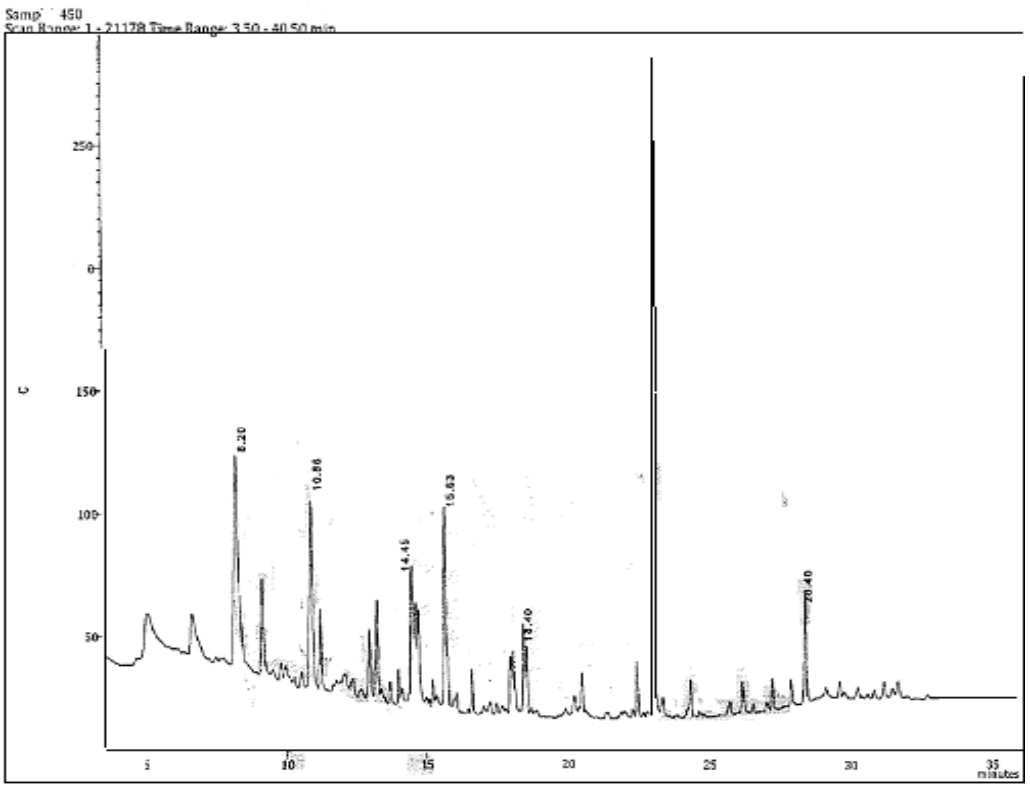

Figure 1: GC-MS Chromatogram of Ethanolic Extract of *Hibiscus Rosa-Sinensis* L Flower Petals.

CONCLUSION

The present work has been performed to establish the various bioactive compounds which could serve as important and has commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the innovative drugs. In this study, 15 bioactive compounds from the ethanol extract of *Hibiscus rosa–sinensis* L flower petals were identified by Gas-chromatography with Mass spectrometry (GC-MS) analysis. These findings have provided scientific basis to the ethno medical usage of the plant. This primary information will facilitate in conducting further studies on discovery of bioactive constituents, resolve of their efficacy by *in vivo* studies and demonstration of their safety and efficacy in clinical trials.

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