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GC-MS analysis of phytochemical compounds in the ethanolic extract of flower of three species of Ipomoea

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Abstract
The aim of this study was to phytochemically screen the plant metabolites present in the plant material(flower) in ethanolic extract of three species of Ipomoea namely *Ipomoea bereviensis*, *Ipomoea cairica* and *Ipomoea hederacea*, qualitatively using gas chromatography-mass spectroscopy (GC-MS). The shade dried flowers were extracted with ethanol. The three samples were filtered and derivatised using MoX reagent and BSTFA: TMCS. The analysis showed peaks with low and high molecular weight revealing the presence of 28 compounds in *I. bereviensis*, 36 compounds in *I. cairica* and 29 compounds in *I. hederacea* which were identified in comparison to the fragmentation patterns in the resulting mass spectra with those published in literature and using the National Institute of Standards and Technology (NIST), Mass Spectral Database of the gas chromatograph–mass spectrometer. Phytochemical screening affirmed the presence of mostly sugars, sugar alcohols, ethers and lipids. These results will consequently be discussed in the light of their putative biological or therapeutic relevance and if pursued further can lead to drug development. The outcome of this preliminary screening, which is a valuable step in the detection of bioactives will also help in discovering the bioactive profile of these particular species.

Keywords: phytochemical screening, bioactives, GC-MS, TIC, ethanolic extract, Rf

Introduction

In recent decades a significant development has been the huge influx of pharmaceutical products [3]. It is reported that these drugs carry with them many dangers especially when used as self-medication. These include severe side effects, drug allergies, self-poisoning and accidental overdoses. To stop this growing dependence on these synthetic drugs which have such serious implications, researchers have turned their focus on plant based medication.

The practice of using plants as therapeutic agents has long been in practice and the developing countries still rely on and depend heavily on plant sources for alleviating diseases. Though traditional forms of plant based medication are based entirely on beliefs, practices and skills which have been handed down from older generations, the modern, revolutionized seeks to use this knowledge but by trying to maximize the benefits.

Therefore screening of active components from plants has direct relation to the development of new medicinal drugs which have efficient protection and treatment role against various diseases [13].

Gas Chromatography- Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [15].

The genus *Ipomoea* includes approximately 500-600 species, comprising the largest number of species in the Convolvulaceae family [4]. Commonly, called the 'morning glories' these twining climbers are both much sought after primarily for their ornamental value and are also studied and researched for their diverse uses. Several New World species of *Ipomoea* have successfully adapted to tropical and warm temperate regions in Asia, Australia, and Europe, as well as North and South America, due to high phenotypic plasticity and genetic adaptability [2].

Various species of *Ipomoea* are also used extensively as food, medicines or in religious ritual. These species are used in different parts of the world for the treatment of several diseases, such as, diabetes, hypertension, dysentery, constipation, fatigue, arthritis, rheumatism, hydrocephaly, meningitis, kidney ailments and inflammations. Some of these species have showed antimicrobial, analgesic, spasmolitic, spasmogenic, hypoglycemic, hypotensive, anticoagulant, anti-inflammatory, psychotomimetic and anticancer activities [11]. Further investigation using more modern techniques for separation, isolation and identification of the bioactives present revealed that many other species e.g., *I. involucrata* and *I. triloba* are also excellent and nutrition filled sources of food. The appreciable amounts of protein, carbohydrates, crude fibre, lipid and calorific values along with low toxicant levels have been found [5].

Alkaloids, phenolic compounds and glycolipids are the most common biologically active constituents from Ipomoea plant extracts [11].

Additional studies have shown the presence of saponins, tannins, flavanoids, cardiac glycosides and phlobatannins which serve as useful therapeutic agents [5]. This particular genus is synonymous with secondary metabolite production. Alkaloids and phenolic compounds are present in large quantities. Studies have shown the importance of these constituents form a medical point of view. Apart from this many workers in the past have proved that these secondary metabolites are excellent taxonomic markers to detect the minor variations among the taxa [6, 7, 8, 9]. Extracts of *Ipomoea hederacea* which is commonly called 'ivy-leaf'

morning glory showed a high antimicrobial effect against a host of bacterial species which include *B. subtilis, P. multocida, S. aureus, E. coli, A. niger, A. flavus, A. alternate* and *R. solani.* The higher total phenolic content present in the stem showed antioxidant properties as well, thus claiming its position for usage in a range of phytochemical preparations owing to its high antimicrobial and antioxidant potential [18].

Since *Ipomoea* has long been discussed as a store house for bio constituents this study has also sought to find out quantitative and qualitative details in these particular species.

Gas Chromatography Mass Spectrometry (GC-MS) is a technique for the analysis and quantitation of organic volatile and semi-volatile compounds. Gas chromatography (GC) is used to separate mixtures into individual components using a temperature-controlled capillary column.

Described as one with a high efficiency rate and versatility, this analytical technique has numerous applications in the field of science and technology namely quality control, impurity profiling, analytical research and maintenance for human welfare.

This analytical technique combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify various substances in a test sample. GC is used primarily to separate the volatile and thermally stable substitutes while an enhanced GC-MS fragments the analyte to be identified on the basis of its mass.

Material and methods

The three species of Ipomoea used in this study are Ipomoea bereviensis Vatke, Ipomoea cairica (L.) Sweet and Ipomoea hederacea Jacq. These have been labeled as I-1, I-2 and I-3 respectively.

Plant material

The selected three species of *Ipomoea, I. bereviensis, I. cairica* and *I. hederacea* were collected from different parts of Bangalore, capital city of Karnataka.

I. bereviensis was collected from the St. Joseph's College campus, Bangalore. It was planted as an ornamental to adorn the railing.

I. hederacea was collected from private gardens in and around Langford town, Bangalore which is interestingly called the "Morning glory" town by the locals owing to the large number of blooms of a variety of Ipomoea species.

I. cairica was collected from local gardens in St. Thomas Town, Bangalore a relatively greener belt in the city.

Sample preparation

Since literature showed that the extraction is usually carried out by using polar solvents, the use of, ethanol was used.

The flower samples were air or shade dried for 4-5 days. This was then powdered and macerated in 100% Ethyl alcohol and left to stand for 48 hours after which it was dried to evaporation. The residue left behind was collected and taken for GC-MS.

The three samples were filtered and derivatised using MoX reagent and BSTFA: TMCS.

These derivatised samples were diluted 1:10 dilution with ethanol.

For sample 1, 0.2ul was injected without dilution.

Gas chromatographic analysis was performed on a Thermo Scientific trace GC ultra-instrument equipped with a DB-5 MS with dimensions 30m×0.25mm×0.25µm film thickness. The GC settings are given in Table 6

The samples (1µL) were injected with a split ratio of 1:10. The carrier gas was Helium as a flow rate of 1.0 ml/min.

Spectra were scanned from 30m/z to 600m/z.

Most constituents were identified by comparison of their retention indices with those found in literature. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 2011.

The instruments used

GC: Thermo Scientific, Trace GC ultra
MS: DSQII
Ionization for MS: Electron Impact Ionization
Mass Analyzer: Quadrupole
Software: X Calibur
Library: NIST 2011
Experimental conditions:
Column: DB 5ms with integrated guard column

Dimensions: 30mL×0.25mm ID×0.25µm film thickness

Table 1: Temperature Ramp

	Rate (ºC/min)	Temperature(ºC)	Hold time(min)
Initial		70	2
Ramp	5	150	0
	3	250	2
	1	350	3

The program details are given below:
Carrier gas: Helium
Flow (ml/min): 1.0
Mode: Split ratio 1: 10
Injection volume: 1µl
Scan Mass range: 30m/z – 600m/z
Polarity: +ve

Identification of Compounds**:** Interpretation of mass spectrum of GC-MS was conducted using the mass spectral database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name and molecular weight of the components was ascertained from available data.

Results and Discussion

The GC-MS chromatogram of ethanolic samples of Ipomoea revealed the presence of 28 compounds in I-1 (*I. bereviensis*), 36 compounds in I-2 (*I.cairica*) and 29 compounds in I-3 (*I.hederacea)* which were identified in comparison to the fragmentation patterns in the resulting mass spectra with those published in literature and using the National Institute of Standards and Technology (NIST), Mass Spectral Database of the gas chromatograph–mass spectrometer.

The RT for three species was *I.hederacea* the RT was between 5.65 - 63.37, I-2 – *I.cairica* the RT was 7.45-58.68 and I-3 – *I.bereviensis* the RT was 7.45-58.6.

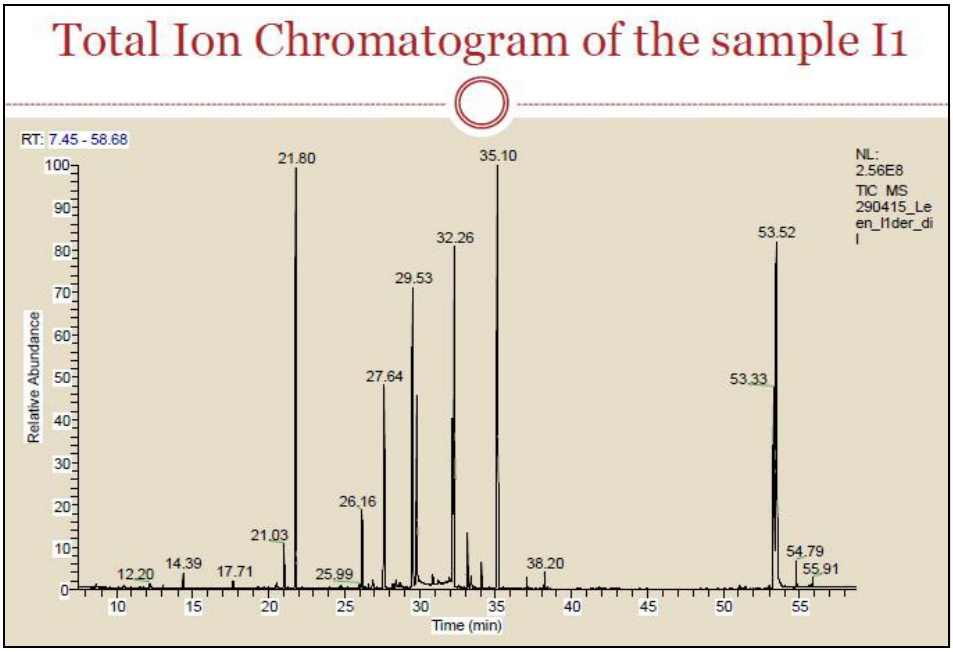

Fig 1: Total Ion Chromatogram of I-1 *(I. bereviensis*)

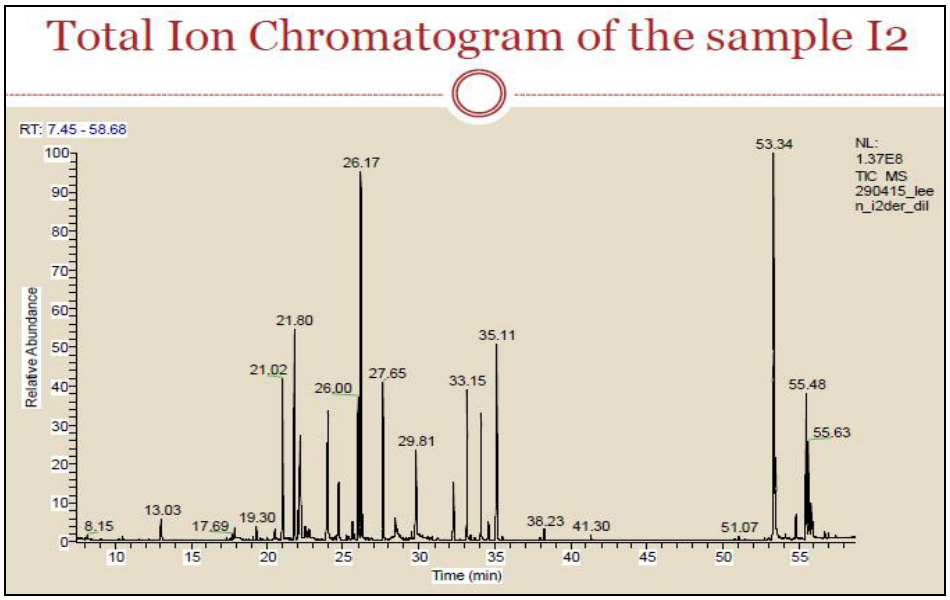

Fig 2: Tic of I2 (*I. bereviensis*)

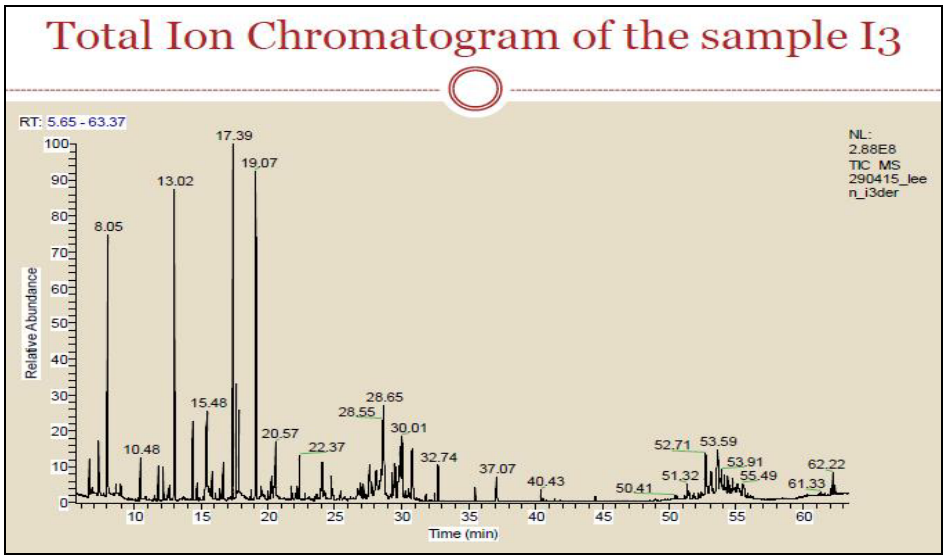

Figure 3: Tic of I3 (*I. bereviensis*)

Relative percentage amounts of separated compounds were calculated automatically from peak areas of the total ion chromatograms (TIC). As shown in Figures 1, 2 and 3 This is done in to determine whether these plant species contain any individual compound or group of compounds. It also helps to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their putative biological or therapeutic relevance.

The major compounds identified and present were mostly sugars, sugar alcohols, ethers and lipids. A few compounds were found commonly present in all or at least two of the species. These include n-Hexadecanoic acid, Xylitol, 1.2.3.4.5-pentakis –O-TMS, Propanoic acid, D-Psicofuranose pentakis (TMS) ether.

The following tables display the various compounds found and identified in the three different species.

Table 2: List of a few of the 28 metabolites in *Ipomoea bereviensis*

Sl. no.	RT	Name of the compound	Molecular formula	Mol.wtg/mol	Purity (%)
1	8.64	Propanoicacid,2-[(trimethylsilyl)]-oxy, Trimethyl ester	$C_9H_{22}O_3Si_2$	234	56
2	14.3	Glycerol, tris(trimethylsilyl) ether	$C_{12}H_{32}O_3S_3$	308.64	84
3	17.7	Ethyl 2,3,4,6-tetrakis-o-(Trimethyl)-D-glycopyranoside	$C_{29}H_{60}O_7Si_5$	661.217	76
4	21.8	Isophthalic acid,2-bromo-4-fluorophenyl pentyl ester	$C_{26}H_{32}BrFO_4$	507.43	28
5	24.8	Arabinopyranose, tetrakis-O-(trimethylsilyl)-	$C_{17}H_{42}O_5Si_4$	438.85	63
6	26.1	Xylitol,1,2,3,4,5,-pentakis-o-TMS	$C_{20}H_{52}O_5Si_5$	513.0	94
7	26.6	Mannose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, D-	$C_{21}H_{52}O_6Si_5$	541.06	56
8	28.2	Arabinose, 2,3,4,5-tetrakis-O-TMS	$C_{17}H_{42}O_5Si_4$	438	70
9	29.5	D-(-)-Fructofuranose, pentakis (trimethylsilyl) ether (isomer 1)	$C_{21}H_{52}O_6Si_5$	541.0	100
10	29.8	D-fructose, Pentakis-TMS Ether	$C_{21}H_{52}O_6Si_5$	541.06	95
11	32.1	D-psicose, pentakis (TMS) ether	$C_{22}H_{55}NO_6Si_5$	570.1	84
12	32.2	a-D-(+)Mannopyranose, pentakis TMS ether	$C_6H_{12}O_6$	180.1	91
13	33.1	Mannose, 6-deoxy-2,3,4,5-tetrakis-O-(trimethylsilyl)-, L-	$C_{18}H_{44}O_5Si_4$	452.8	71
14	33.3	D-Mannitol,1,2,3,4,5,6-hexakis-o-(TMS)	$C_{24}H_{62}O_6Si_6$	615.2	81
15	33.3	Trimethylsilyl ether of glucitol	$C_{24}H_{62}O_6Si_6$	615.2	50
16	34.0	d-xylose tetrakis(trimethylsilyl)-	$C_{18}H_{45}NO_5Si_4$	467.8	89
17	35.0	a-D-Allopyranose, pentakis (TMS) ether	$C_{21}H_{52}O_6Si_5$	541.06	98
18	35.0	*D-Glucose,2,3,4,5,6-pentakis-o-TMS	$C_{22}H_{55}NO_6Si_5$	570.1	91
19	35.1	Talose,2,3,4,5,6-pentakis-o-(TMS)	$C_{22}H_{55}NO_6Si_5$	570.1	85
20	37.0	Hexadecanoic acid trimethylsilyl ester	$C_{19}H_{40}O_2Si$	328.6	83
21	38.1	Myo-inositol,1,2,3,4,5,6 hexakis-o-(TMS)	$C_{24}H_{60}O_6Si_6$	613.2	91
22	53.4	*Sucrose, octakis (trimethylsilyl)teeth	$C_{35}H_{84}O_{11}Si_8$	905.7	38
23	53.4	Piscofuranose, pentakis (trimethylsilyl)ether	$C_{21}H_{52}O_6Si_5$	541	71
24	53.4	d-(+) turanose, octakis (TMS)ether	$C_{37}H_{89}NO_{11}Si_8$	948.7	82

*= compounds that have eluted more than once with different RT and base peaks.

Table 3: List of a few of the 36 metabolites found in *Ipomoea cairica*

Sl. No	RT	Name of the compound	Molecular formula	Mol.wt g/mol	Purity (%)
1	17.6	Ethyl 2,3,4,6-tetrakis-o-(Trimethyl)-D-glycopyranoside	$C_{29}H_{60}O_7Si_5$	661.217	54
3	19.3	1,2-butandiol,bis(TMS)	$C_{10}H_{26}O_2Si_2$	234.4	*75*
4	21	Propanetriol, 2-methyl-, tris-O-(trimethylsilyl)-	$C_{13}H_{34}O_3Si_3$		*94*
5	21.8	Propanoic acid, 2,3-bis [(trimethylsilyl) oxy]-, trimethylsilyl ester	$C_{12}H_{30}O_4Si_3$	234	96
6	24.6	Xylitol,1,2,3,4,5,-pentakis-o-TMS	$C_{20}H_{52}O_5Si_5$	513.0	90
7	25.9	D-(+)Xylose, tetrakis(TMS)ether, trimethyloxime	$C_{18}H_{45}NO_5Si_4$	467.8	98
8	26.1	Adonitol, pentakis(TMS)ether	$C_{20}H_{52}O_5Si_5$	513.0	71
9	26.6	Mannose, 6-deoxy-2,3,4,5-tetrakis-O-(trimethylsilyl)-, L-	$C_{18}H_{44}O_5Si_4$	452.8	38
10	28.4	Arabinofuranose,1,2,3,4,5-tetrakis-o-(TMS)	$C_{17}H_{42}O_5Si_4$	438.8	59
	29.8	D-fructose, Pentakis-TMS Ether	$C_{21}H_{52}O_6Si_5$	541.06	88
11	33.1	Talose,2,3,4,5,6-pentakis-o-(TMS)	$C_{22}H_{55}NO_6Si_5$	570.1	98
12	34	d-Xylose tetrakis(trimethylsilyl)-	$C_{18}H_{45}NO_5Si_4$	467.8	94
13	35.1	D-Glucose,2,3,4,5,6-pentakis-o-TMS	$C_{22}H_{55}NO_6Si_5$	570.1	98
14	53.3	d-(+) turanose, octakis (TMS)ether	$C_{37}H_{89}NO_{11}Si_8$	948.7	87
15	35.4	Hexadeconic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4	63
16	41.2	Octadecane,1-isocyanato	$C_{19}H_{37}NO$	295.5	69

Table 4: List of a few of the 29metabolites found in *Ipomoea hederacea*

Sl.no.	RT	Name of the compound	Mol. Formula	Mol. Wt.	Purity
1	62.2	a-Sitosterol (TMS)ether	$C_{32}H_{58}OSi$	486.9	52
2	35.4	Hexadeconic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4	89
3	11.7	Erythrofuranose, tris(TMS)ether	$C_{13}H_{32}O_4Si_3$	336.6	90
4	14.7	Acetoacetic acid, bis(TMS)ether	$C_{10}H_{22}O_3Si_2$	246.4	81
5	17.38	3-methyl-1,3-bis (TMS)butane	$C_{11}H_{28}O_2Si_2$	248.5	96
6	24.7	Xylitol,1,2,3,4,5,-pentakis-o-TMS	$C_{20}H_{52}O_5Si_5$	513.0	81

7	54.1	Piscofuranose, pentakis (trimethylsilyl) ether	$C_{21}H_{52}O_6Si_5$	541	46
8	10.4	* Glycerol, tris(trimethylsilyl) ether	$C_{12}H_{32}O_3S_3$	308.6	87
9	37.0	Hexadecanoic acid trimethylsilyl ester	$C_{19}H_{40}O_2Si$	328.6	87
10	40.4	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.4	84
11	19.0	Bis (TMS)(2R,3R)2-hydroxy3-(TMS) oxybutanedioate	$C_{16}H_{38}O_6Si_4$	438.8	53
12	20.2	2-ketohexanoic acid, (TMS)ester	$C_{16}H_{25}NO_3Si$	307.4	78
13	12.5	Phloroglucinol, (TMS)ether	$C_{15}H_{30}O_3Si_3$	342.6	75
14	13.0	3,7-dioxa-2,8disilanon-5-one 1,2,2,8,8(TMS)	$C_9H24O2Si2$	220.4	99

The tables 2, 3 and 4, display the allocation of metabolites. The GC-MS profiling revealed commonalities across the three species. I-2 and I-3 showed several common compounds among each other while I-3 showed a few unique compounds. Of a total of 28 compounds in I-1, only 9 were unique to the species. 4 were common with I-3 and 11 common with I-2. In I-2, 12 were unique and 4 common with I-3.In I-3, of the 29 listed, 20 were found exclusively in this species with 4 each common to I-1 and I-2. Figure 17
There were only 2 compounds common to all the three species. They were D-Psicofuranose, pentakis (TMS) ether, with a CAS no-EPA-380127, and Xylitol, 1, 2, 3, 4, 5-pentakis-O-(TMS) bearing the no.14199725.
This data is represented in the Venn diagram below.

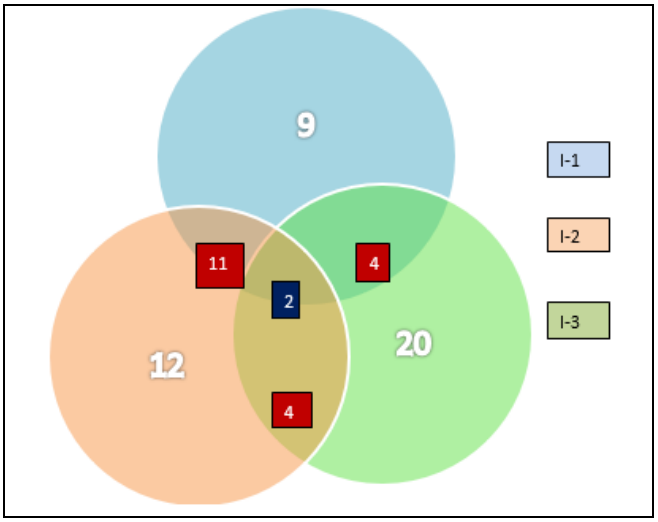

Fig 4: Allocation of metabolites in three species of Ipomoea

Common and exclusive metabolites across I-1, I-2 and I-3 are shown in Venn diagram. The numbers indicate the differential display of a few metabolites among the three species.

Table 5: Biological activities of phytochemical compounds identified in Ethanol extract of flowers in the three species of Ipomoea

Sl. no.	Name of the compound	I-1	I-2	I-3	Biological activity
1	9,12-Octadecadienoic acid, ethyl ester	_	_	+	Nematicide, hepatoprotective, antihistaminic, anticoronary
2	Hexadecanoic acid, ethyl ester	_	+	+	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor
3	D-Allopyranose pentakis (TMS) ether	+	_	_	Anti-oxidative activity
4	n- Hexadecanoic acid				Anti-oxidant, Hypocholesterolemic, Nematicide, Anti-androgenic, Hemolytic, Pesticide, Lubricant, 5-Alpha reductase inhibitor, antipsychotic.
5	á-Sitosterol	_	_	+	Antimicrobial Anticancer Anti-inflammatory, Antiasthma, Diuretic, Antidiabetic, anti diarrhoal, antiviral.

Activity source: Dr. Duke's Phytochemical and Ethno botanical Databases

Table 6: Displaying the different categories of metabolomes

Class	Name of the compound	*I.bereviensis*		*I.cairica*		*I.hederacea*	
		Amt (%)	Purity (%)	Amt (%)	Purity	Amt (%)	Purity
Lipids	Propanoic acid, 2,3-bis(trimethylsilyl)oxy]-, trimethylsilyl ester	0.05	56	2.45	96	_	_
	Hexadecanoic acid, trimethylsilyl ester	0.17	83	_	_	0.25	87
	9,12-Octadecadienoic acid, ethyl ester	-	-	-	-	0.12	84
	Hexadecanoic acid, ethyl ester	-	-	0.05	63%	0.15	89
Sugars	D-Fructose, 6-O-[2,3,4,6-tetrakis-O-(trimethylsilyl)-.alpha.-D-glucopyranosyl]- 1,4,5,6-tetrakis-O-(trimethylsilyl)-	-	-	0.36	74	-	-
	a.-D-Glycopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-alpha-D-	-	-	0.89 0.29	72 F80	-	-

	fructofuranosy l 2,3,4,6-tetrakis-O-(trimethylsilyl)-						
Sugar alcohol	Xylitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)	1.15 0.11	94 76	5.07	98	0.20	81
	Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	0.25	91	_	_	_	_
Others	a,-Sitosterol trimethylsilyl ether	-	-	-	-	0.09	52

Discussion

The presence and subsequent identification of metabolites in this study could be compared to a similar investigation carried out by J. Senthil in the plant of their choice-*Ipomoea sepiaria* Koenig Ex. Roxb [16]. While most of the compounds identified here, in all three species of Ipomoea, were the same as those found in sepiaria, a parallel could be drawn about their adaptation, habitat and a few phenotypic characters. A few compounds found in common were Octadecene and 1-Hexadecene which are olefins or alkenes and have antimicrobial properties. They also find their use in the production of detergents and biodegradable surfactants respectively [10]. (Kurt Koss wig 2005).

Conclusion

Among the different classes of compounds identified in the three species chosen, fatty acids were present in large amounts. The diverse and potential biological activities of fatty acids possess the ability to kill or inhibit bacterial growth. The properties which make them desirable antibacterial agents are their non-specific mode of action and a broad spectrum. These biological capabilities could find its use in agriculture, food preservation and in medicine where the use of antibiotics is overused. (Olena Konovalova *et al*., 2013) [14].

Among the other compounds were phytochemicals such as Hexadecanoic acid, Sitosterol and 9, 12-octadecadienoic acid which show antioxidant, antibacterial and anti-inflammatory properties. (Sudha *et al*., 2013) [17].

Hexadecanoic acid,(TMS) which was seen all three species of the study is also called Palmitic acid and is found to be an intermediate in the biosynthesis of sexual pheromones of some insects. It is used in the preparation of the ingredients of some drugs to decrease the hydrophobicity of Virginia mycin, a drug used against Mycobacterium avium and it is well known as insecticide and anti-microbial agents. (Reported by Mohamed Zaky Zayed *et al*., 2014) [12].

GC-MS analysis showed the presence of many phytochemical constituents, but a detailed investigation should be carried out determine the mechanism and pathway they follow to corroborate with the claims of folklore (Mustapha N. Abubakar and Runner R. T. Majinda**,** 2016) [1].

With the identification of bioactive compounds present in the species of Ipomoea used in this study, one could further study the isolation and purification of compounds for other species of the same genus.

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