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Evaluation of Elemental and Volatile Compounds of Three Selected Plants of Genus *Hibiscus*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study aims to evaluate the minerals, bioactive compounds of 3 selected *Hibiscus* Genus *i,e. Hibiscus sabdariffa* L., *Hibiscus cannabinus* L., and *Hibiscus acetosella* Welw.
Place and Duration of Study: The selected plants were collected during May to October 2018 from Imphal (24°37'N and 93°39'E) Manipur North Eastern State of India, which lies 2590 feet above sea level, and study were carried out in Genetics Laboratory, Department of Life Sciences, Manipur University.
Methodology: The minerals composition and bioactive compounds were evaluated by using Graphite Flame Atomic Absorption Spectrometer (GF-AAS) method and Gas Chromatography-Mass Spectrometry (GCMS), respectively.
Results: The elemental analysis shows the presence of Calcium, Magnesium, Iron, Zinc, Copper, Sodium, Potassium, Selenium, Chromium, Cobalt. By using the GC-MS method, the compounds are identified with Retention time (RT) and area percentage. The two compounds are identified for methanol extract and four compounds for chloroform extract of *Hibiscus sabdariffa* L. For *Hibiscus cannabinus* L., three compounds are identified for methanol extract and four compounds for chloroform extract and for *Hibiscus acetosella* Welw. eleven compounds for methanol extract and three compounds for chloroform extract.
Conclusion: The selected plants are good source of Sodium, Potassium, Selenium, Chromium, Cobalt, Calcium, Magnesium, Iron, Zinc, Copper and bioactive compounds which had antibacterial, anticancer, antioxidant properties and renal related disorders protection effects. However, it is needed to study the pharmacological activity for further evaluation.

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Keywords: GF-AAS; Hibiscus; GCMS; minerals.

1. INTRODUCTION

Medicinal plants have been used for treating various diseases and other beneficial purposes from ancient times. Natural products play an important role in treating various diseases by acting as a source of the drug discovery process. Investigation of elements and bioactive compounds composition of every medicinal plant is very much needed as its deficiency or excess may affect human health. People believe that natural medicines are much safer than synthetic drugs, have led to exceptional growth in the usage of plants and plant products as traditional or folk medicine in primary health care.

Hibiscus cannabinus L., *Hibiscus acetosella* Welw. and *Hibiscus sabdariffa* L. plants belong to Malvaceae. In the Malvaceae family, the *Hibiscus* genus is the largest consisting of 300 species approximately [1]. In the North-Eastern Region of India, mainly Manipur above plants used in making soup in summer and consumed as medicinal plants for urolithiasis problems. The three selected plants are serve as source of medicine [2] and apart from medicinal value *H. sabdariffa* L. flower is traded and used for tea and beverages [3]. Aqueous extract of *H. sabdariffa* L. using calyces' part effectively prevented the development of urolithiasis in male albino rats and has antimicrobial and antioxidant, anti-urolithiasis activity [4] anticancer [5]. *H. cannabinus* L. has free radical scavenging activity and antibacterial [6,7] and haematinic properties [8] and inhibits smooth muscle cell migration and calcification in rabbits' blood vessels, inhibiting the development of atherosclerosis [9]. The edible oil of *H. cannabinus* L. seeds extracts has high antioxidant activity. It contains alpha-linolenic acid, as essential omega-3-fatty acid, which has anti-inflammatory and anti-thrombotic properties [10].

H. acetosella Welw. used to treat pile patients [11] and have potential acts as an antigenotoxicity and antimutagenicity in mice induced by alkylating agents [1] and also antioxidant and anti-inflammatory activity [12], anti-anaemia, antipyretic properties [13]. Selected plants are traditionally used for anti-urolithiasis problems in the region above, mainly *H sabdariffa* L. Therefore, the present study aims to evaluate and analyse the elemental composition of minerals and volatile bioactive compounds of *Hibiscus sabdariffa* L., *Hibiscus cannabinus* L. and *Hibiscus acetosella* Welw.

2. MATERIALS AND METHODS

2.1 Sample Collection

The leaves of the selected plants were collected during May to October from Imphal (24°37'N and 93°39'E) Manipur North Eastern State of India which lies 2590 feet above sea level. The leaves were washed thoroughly with tap water and rinsed with distilled water and kept for 72 hrs for shade dried. Identification of *H. cannabinus* L., and *H. sabdariffa* L. was done at Botanical Survey of India, Shillong, Meghalaya *H. acetosella* Welw. was identify at Department of Life Sciences (Botany), Manipur University, Canchipur, Manipur, India.

2.2 Elemental Analysis

The dried leaves were ground into fine powdered by using mortar and pestle. The powdered sample(0.5gm) was digested in Teflon digestion vessel using HNO_3 and volume was made up to 50ml with double distilled water and analysed by using GF-AAS.

2.3 GC-MS Analysis

The dried leaves (150 g) were extracted by using Soxhlet extractor with methanol and chloroform. The extract was vacuum dried by using rotary vacuum evaporator. Analysis was performed by using GC-MS Perkin Elmer (USA) in Guwahati Biotech Park inside IIT Guwahati campus and the GC-MS model were Clarus 680GC and Clarus 600MS. The capillary column(60.0m×250µm) was used and initial temperature was maintained at 70ºC for 3min, ramp 6ºC/min to 200ºC and hold 3min, ramp 6ºC/min to 300ºC hold 10min. The injection temperature was maintained at 280ºC. Helium was used as carrier Gas and ratio of 10:1 was used as split injection and solvent delay was 9 min. The transfer and source temperature were maintained at 200ºC and 180ºC respectively. The mass spectral scan range was at the rate of 40 to 600Da. The compounds were matched with the compounds listed in National institute of Standards and Technology (NIST) library.

3. RESULTS AND DISCUSSION

3.1 Elemental Analysis

Iron, Calcium, Magnesium, Zinc, Copper, Sodium, Potassium, Selenium, Chromium, Cobalt were revealed in *H. cannabinus L*, *H. acetosella* Welw. and *H. sabdariffa* L. Iron (1.23±0.27ppm), Potassium (1.83±0.013ppm), Magnesium (0.04±0.009ppm), Selenium (0.35±0.002ppm), Chromium (0.87±0.050ppm) were found highest concentration in *H. sabdariffa* L. Calcium (0.92±0.011ppm) , Zinc (0.12±0.002ppm), Sodium (0.65±0.082ppm), Cobalt (0.06±0.001ppm) were found highest concentration in *H. acetosella* Welw and Copper (0.34±0.001ppm) is the only element that is found highest in *H. cannabinus* L. Graphical representation analysis of the elemental composition of *H cannabinus* L., *H acetosella* Welw and *H sabdariffa* L. are below in [Fig. 1].

As reported, Iron, Calcium, Magnesium, Zinc, Copper, Sodium, Potassium, Phosphorous were found in *H. sabdariffa* L [14,15]. In previous study, it has been reported that Calcium, Potassium, Iron, Zinc, Phosphorous were found in *H. cannabinus* L [15]. Calcium is an essential component for bone, deficiency increase with at-risk populations [15]. Increased potassium intake is associated with a lower incidence of urolithiasis, and Magnesium is an inhibitor of calcium oxalate and calcium phosphate [16]. It has been reported that Magnesium and Calcium combination supplement avoids the rise of kidney stone formation [17]. Zinc plays a role in cell proliferation, differentiation, and metabolism [18] and Zinc deficiency or low intake may increase the risk of chronic kidney disease [19]. Prolonged copper deficiency during active growth stages leads to anaemia, growth retardation, defective keratinization and pigmentation of hair, hypothermia, and mental retardation changes in the skeletal system [18]. Sodium maintains normal cellular homeostasis and regulates fluid and electrolyte balance and blood pressure [20]. Selenium plays a vital role as an antioxidant in human health and protects the thyroid from oxidative damage [21]. Chromium helps in the biosynthesis of glucose tolerance factors, and Cobalt deficiency produces cardiomyopathy, congestive cardiac failure, pericardial effusion, polycythemia and thyroid enlargement. The deficiency of iron causes anaemia [22].

3.2 GC-MS Analysis

In GC-MS analysis two solvent are used viz chloroform and methanol for extraction and identified high peaks by NIST library search. *H. cannabinus* L chloroform fraction extract revealed the presence of Carbazic acid, 3-(1-propylbutylidene)-, ethyl ester (5.622%), Hexadecanoic acid, ethyl ester (2.342%), (E)-9-octadecenoic acid ethyl ester (2.924%), Heptacosane (2.807%), and methanol fraction of *H. cannabinus* L revealed the presence of Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15, 15, -hexadecamethyl (0.761%), Heptasiloxane (0.423%), Hexasiloxane (0.460%).

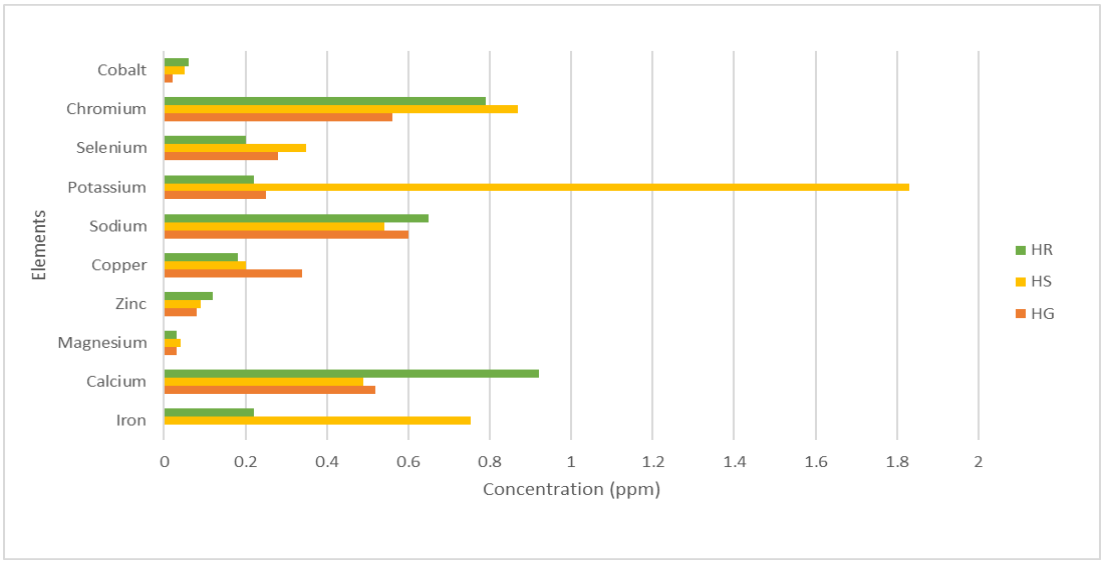

Fig. 1. Analysis of elemental composition of HR, HG, HS. HR- *Hibiscus acetosolla* Welw, HG- *Hibiscus cannabinus* L, HS- *Hibiscus sabdariffa* L

Chloroform fraction of *H. sabdariffa* L. revealed the presence of Phytol (2.222%), (+)-. Alpha. -Tocopherol Acetate (3.173%) and methanol fraction of *H. sabdariffa* L revealed the presence of Phenol, 3,5-Bis(1,1-Dimethylethyl) (2.556%), Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl (2.033%), Octasiloxane,1,1,3,3,5,5,7, 7,9, 9,11, 11,13 ,13,15, 15-Hexadecamethyl (3.347%), (+)-Alpha-Tocopherol acetate (7.952%).

Chloroform fraction of *H. acetosella* Welw revealed the presence of Alpha-Amyrin (2.430%), Lupan-3-Ol (1.539%), octadecane,9-ethyl-9-heptyl (1.861%) and methanol fraction of *H. acetosella* Welw revealed the presence of Pentadecanoic acid, 14-methyl, methyl ester (2.960%), 9,12,15-octadecatrienoic acid, methyl ester (z,z,z) (2.557%), Docosanoic acid (3.271%), 3-beta-myristoylolean-12-en-16.beta-ol (2.852%), 1-naphthalenepropanol, alpha-ethyldecahydro-5-(Hydroxymethyl) (2.396%), Lupeol (3.647%), Squalene (5.167%), Isoledene (6.444%), Tau-cadinol (8.632%), Beta-Guaiene (2.310%), 9-Octadecenoic acid(Z)-,9-octadecenyl ester,Z (2.245%). Compounds with area %, molecular formula, molecular weight and retention time are given below in Table 1.

Table 1. Compounds Identified Of HG-C, HG-M, HS-C, HS-M, HR-C, HR-M with retention time (RT), area percentage, molecular formula and molecular weight

	AREA	Compounds	RT	Molecular formula	Molecular weight
HG-CHL	5.622	Carbazic acid, 3-(1-propylbutylidene)-, ethyl ester	9.018	$C_{10}H_{20}N_2O_2$	200.28 g/mol
	2.924	(E)-9-octadecenoic acid ethyl ester	37.039	$C_{20}H_{38}O_2$	310.5145
	2.807	Heptacosane	46.708	$C_{27}H_{56}$	380.7 g/mol
	2.342	Hexadecanoic acid, ethyl ester	33.543	$C_{18}H_{36}O_2$	284.5 g/mol
HG-MET	0.761	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15, -hexadecamethyl	43.377	$C_{16}H_{48}O_7Si_8$	577.2 g/mol
	0.460	Hexasiloxane	51.240	$C_{12}H_{38}O_5Si_6$	430.94 g/mol
	0.423	Heptasiloxane	51.125	$C_{16}H_{48}O_6Si_7$	533.1472
HS-CHL	3.173	(+)-. Alpha. -Tocopherol Acetate	51.106	$C_{31}H_{52}O_3$	472.7 g/mol
	2.222	Phytol	35.644	$C_{20}H_{40}O$	296.5 g/mol
	1.561	Dl-Alpha. -Tocopherol	50.190	$C_{29}H_{50}O_2$	430.7 g/mol
HS-MET	7.952	(+)-Alpha-Tocopherol acetate	50.995	$C_{31}H_{52}O_3$	472.7 g/mol
	3.347	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl	49.644	$C_{16}H_{48}O_7Si_8$	577.2 g/mol
	2.556	Phenol,3,5-Bis(1,1-Dimethylethyl)	42.167	$C_{14}H_{22}O$	206.32 g/mol
	2.033	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl	49.259	$C_{12}H_{36}O_5Si_6$	428.92 g/mol
HR-CHL	2.430	Alpha-Amyrin	43.397	$C_{30}H_{50}O$	426.7 g/mol
	1.861	Octadecane,9-ethyl-9-heptyl	50.140	$C_{27}H_{56}$	380.7 g/mol
	1.539	Lupan-3-Ol	45.713	$C_{30}H_{52}O$	428.7 g/mol
HR-MET	8.632	Tau-cadinol	47.123	$C_{15}H_{26}O$	22.37 g/mol
	6.444	Isoledene	46.853	$C_{15}H_{24}$	222.37 g/mol
	5.167	Squalene	45.707	$C_{30}H_{50}$	410.7 g/mol
	3.647	Lupeol	45.447	$C_{30}H_{50}O$	426.7 g/mol
	3.271	Docosanoic acid	41.991	$C_{22}H_{44}O_2$	340.6 g/mol
	2.960	Pentadecanoic acid, 14-methyl, methyl ester	31.982	$C_{17}H_{34}O_2$	270.4507
	2.852	3-beta-myristoylolean-12-en-16. beta-ol	44.042	$C_{44}H_{76}O_3$	653.1 g/mol
	2.557	9,12,15-octadecatrienoic acid, methyl ester (z,z,z)	35.423	$C_{19}H_{32}O_2$	292.5 g/mol
	2.396	1-naphthalenepropanol, alpha-ethyldecahydro-5-(Hydroxymethyl)	44.967	$C_{20}H_{36}O_2$	308.5 g/mol
	2.310	Beta-Guaiene	47.888	$C_{15}H_{24}$	204.35 g/mol
	2.245	9-Octadecenoic acid(Z)-,9-octadecenyl ester,Z	50.524	$C_{38}H_{72}O_3$	577 g/mol

HR- Hibiscus acetosolla Welw, HG- Hibiscus cannabinus L, HS- Hibiscus sabdariffa L, M-methanol, C-chloroform

As reported in previous study, β-sitosteryl-β-D-galactoside, hibiscitrin, sabdaritrin, gossypitrin, gossytrin and other gossypetin glucosides, quertin and luteolin from the *H. sabdariffa* L. leaves [3] and Cyclohexane carboxylic acid ethyl ester, Cyclopropane carboxylic acid methyl ester, Hexanoic acid-4-octyl ester, Hexadeca-2,11-dienoic acid, Oleic acid, Octadecanoic acid, E-13 Docosenoic acid, E-11-Hexadecanal, n-Hexadecanpoc acid were reported as chemical composition of *H. sabdariffa* L. oil extract by GCMS analysis [23].

It has been reported that Lupeol has protection effect in the injury of renal associated with hypercholesterolemia and minimizes the formation of kidneystones in the urolithiatic animals [24,25] and there is no toxicity in rats and induces immunity and protects against visceral leishmaniasis [26,27]. *H. acetosella* Welw content of polyphenols, coumarins and flavonoids [27] and *H. sabdariffa* L. calyces' content the flavonoids, gossypetine, hibiscetine and sabdaretine, alpha-tocopherol as it rich in anthocyanins and protocatechuic acid as reported [28]. As reported, Hexadecanoic acid has anti-inflammatory and Lupan-3-ol has anti-microbial, anti-inflammatory and antitumor bioactivities [29].

Phytol belongs to diterpene has diuretic properties and possess antimicrobial and antioxidant [30,31]. Alpha-tocopherol acetate is fat soluble compound and has significant reduction of hydrophobicity of E. coli and antioxidant properties also. Alpha amyrin acts as a growth inhibitor of Straptococcus in oral cavity [32]. Squalene has inhibitory effect on carcinogenesis in animal models [33] and anticancer, antioxidant, detoxifier activities have been reported [34].

4. CONCLUSION

From this study, a good source of Iron, Calcium, Magnesium, Zinc, Copper, Sodium, Potassium, Selenium, Chromium, Cobalt are revealed in three selected Genus Hibiscus plants. Among these three plants, *i.e., H. acetosolla* Welw*., H. cannabinus* L*., H. sabdariffa* L*., H. sabdariffa* L. found the highest concentration(ppm) in five elements out of ten elements. Micro or macronutrients play an essential role as people consume diet or medicine to live healthily. Mineral deficiency causes diseases and disorders in humankind.

In GCMS analysis, *H. acetosolla* Welw report highest bioactive compounds as compared to other two plants. In this study, the three selected plants have shown to have various bioactive compounds which posses antioxidant, anticancer, antibacterial, anti-inflammatory, antitumor, detoxifier activities and protective effect in the injury of renal-related disorders.

Thus, this study clearly shows the presence of some useful minerals and bioactive compounds which has a potential for treating the diseases and deficiency. So, it is needed to study on the pharmacological activity for further evaluation.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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