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Phytochemical Evaluation and GC-MS Analysis of Leaf Extract of *Manihot Esculenta* Crantz

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ABSTRACT

Plants contain a variety of bioactive compounds which makes them an important foundation of pharmaceutical research. *Manihot esculenta* Crantz is a perennial shrub with medicinal prospects. The current research was conducted to qualitatively estimate the phytochemicals and Gas Chromatography-Mass Spectroscopy analysis of its methanol extract to identify the compounds present. The qualitative phytochemical estimation was done using standard tests. The GC-MS study of the methanolic extract was done following standard protocol. The phytochemical analysis detected alkaloids, flavonoids, saponins, phenols, steroids, tannins, carbohydrates, glycosides, triterpenoids, ketones, and proteins. A total of 24 bioactive compounds were revealed through GC-MS analysis, including hentriacontane, tetrapentacontane, 1,54-dibromo-, dotriacontyl isobutyl ether, 3-methyl-2-(2 oxopropyl) furan, 4-dodecene-6,8,10-triyn-3-one, (e)-, z,z-6,27-hexatriactontadien-2-one, cholest-5-en-3-ol (3.beta.)-, and propanoate. The findings of this study support its traditional use in management of diabetes. Nevertheless, more research is needed to determine the specific mechanism of action and to characterise the active chemicals found in the extract.

Keywords: GC-MS; *Manihot esculenta*; Medicinal use; Methanol; Phytochemicals

NOMENCLATURE

GC-MS : Gas chromatography-Mass spectroscopy
m/z : Mass range
MW : Molecular weight
NIST : National institute standard and technology
RT : Retention time

1. INTRODUCTION

Plants are packed with antioxidants, which contains flavonoids and phenolic compounds. The World Health Organisation states that the traditional medicine system will remain a crucial part of healthcare, because of the fact that 80 % of the population relies on the use of herbal medicine to treat several ailments. The testing of plant extracts is indispensable for pharmaceutical research as they are a major source of drugs at present[1]. The proper identification, characterisation as well as and understanding of mechanisms involved in the active molecules of plant species are an exclusive part of therapeutics development[2-3].

Manihot esculenta Crantz (Euphorbiaceae) is a dicotyledonous plant suitable for consumption, commonly found in South America[4]. Cassava leaves are used as a native edible vegetable and are abundant in carbohydrates, proteins, vitamins, and minerals[5-7]. *M. esculenta* is used indigenously in Nigeria and its parts are traditionally employed for curing fever, headache, rheumatism, hemorrhoids, ringworms, tumors, conjunctivitis, sores, and lesions[8]. In addition, its leaves have been therapeutically used in, aches, cancerous affections, dysentery, hypertension, irritable bowel syndrome, and outgrowth of the eye and tumor. They are also used to treat prostatitis, spasms, flu, snake bites, and marasmus and is effective as diuretic, skin disinfectant, demulcent, and cyanogenic substance[9-11]. Gas Chromatography-Mass Spectroscopy (GC-MS) is an investigative method for the identification of chemical components present in a plant extract[12]. Hence, this analysis was carried out to detect the phytoconstituents present in *M. esculenta* and a GC-MS study of its methanolic extract was performed to identify the compounds present in it.

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2. MATERIALS AND METHODS

2.1 Collection of Leaves

Leaves of *Manihot esculenta* were gathered from local areas in and around Guwahati, Assam. The plant specimen was identified and validated in the Herbarium deposited at Botanical Survey of India (Voucher Specimen No.: BSI/ERC/Tech/2023-24/1191).

2.2 Preparation of Plant Material

The fresh leaves of *Manihot esculenta* were kept for air dry at room temperature and powdered. The powder was mixed with methanol at a ratio of 1:10 for maceration and permitted to stand for 2 days under normal conditions. The prepared mixture is then filtered and the filtrate is concentrated to obtain a dark green extract[13].

2.3 Phytochemical Screening

The methanolic extract of *Manihot esculenta* was exposed to a thorough screening for detecting the presence of various phytochemicals, including phenols, flavonoids, alkaloids, steroids, tannins, glycosides, carbohydrates, reducing sugars, saponins, and ketones[14-18].

2.4 Percentage Yield

The percentage of the extract was determined as the percentage of the weight of the extract to the original weight of the dried sample used following the formula below:

$$\text{Percentage yield} = \frac{\text{Weight of dry extract}}{\text{Weight of dry plant material}} \times 100$$

2.5 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS study was performed using Clarus 680 GC and AMP, Clarus 600C MS. Helium (99.99 %) was used as the carrier gas at 1 ml/min. A 1 µl injection was made in splitless mode, with the injector at 280 ºC and the ion source at 180 ºC. The oven temperature ramped from 60 ºC to 200 ºC at 7 ºC/min, and afterward, it was again increased to 10 ºC/min (held for 5 min). The run time was 39 min, with a 7 min solvent delay. Mass spectra were recorded in Electron Impact Positive (EI+) mode at 70 eV, with an 8 min solvent delay and a mass range (m/z) of 50-600 m/z.

2.6 Identification of Compounds

The peaks observed in the Chromatogram were interpreted through a library search of their mass spectra using the database software from the National Institute of Standards and Technology (NIST). The mass spectra of the unknown components were compared with those of known compounds in the NIST library to identify the compounds.

3. RESULTS

3.1 Phytochemical Screening and Yield

Qualitative analysis of the methanolic extract of *M. esculenta* leaves showed the presence of alkaloid, flavonoid, phenol, saponin, steroid, tannin, carbohydrate, glycosides, cardiac glycoside, triterpenoid, ketone, and protein (Table 1). The percentage yield was determined as 14.7 % (w/w).

3.2 GC-MS Profiling of Methanolic Extract of *Manihot esculenta* Crantz

The GS-MS analysis of *Manihot esculenta* Crantz leaves revealed the presence of 24 compounds (Fig. 1), along with few mass spectra (Fig. 2) . The identified phytoconstituents are presented in Table 2. The phytocompounds found in the GC-MS analysis of the methanolic extract of *M. esculenta* leaves include hentriacontane, tetrapentacontane, 1,54-dibromo-, dotriacontyl isobutyl ether, 3-methyl-2-(2 oxopropyl)furan, 4-dodecene-6,8,10-triyn-3-one,

Table 1. Qualitative biochemical determination of methanolic extract of *M. esculenta* leaves

Secondary metabolite	Test conducted	Presence/absence
Alkaloid	a. Mayer's test	+
	b.Dragendorff's test	+
	c. Wagner's test	+
Flavonoid	a. Alkaline reagent test	+
	b. Shinoda test	+
Phenol	Ferric chloride test	+
Saponin	a. Froth test	+
Steroid	a.Liebermann-Burchard test	+
	b. Salkowski's test	+
Tannin	Ferric chloride test	+
Carbohydrate	a. Molisch's test	+
	b. Fehling's test	+
Glycosides	Borntager's test (quinones & anthraquinone)	+
Cardiac Glycosides	Keller Killani test	+
Triterpenoids	Salkowski's test	+
Ketone		+
Protein	Ninhydrin test	+

(+) = presence; (-) = absence

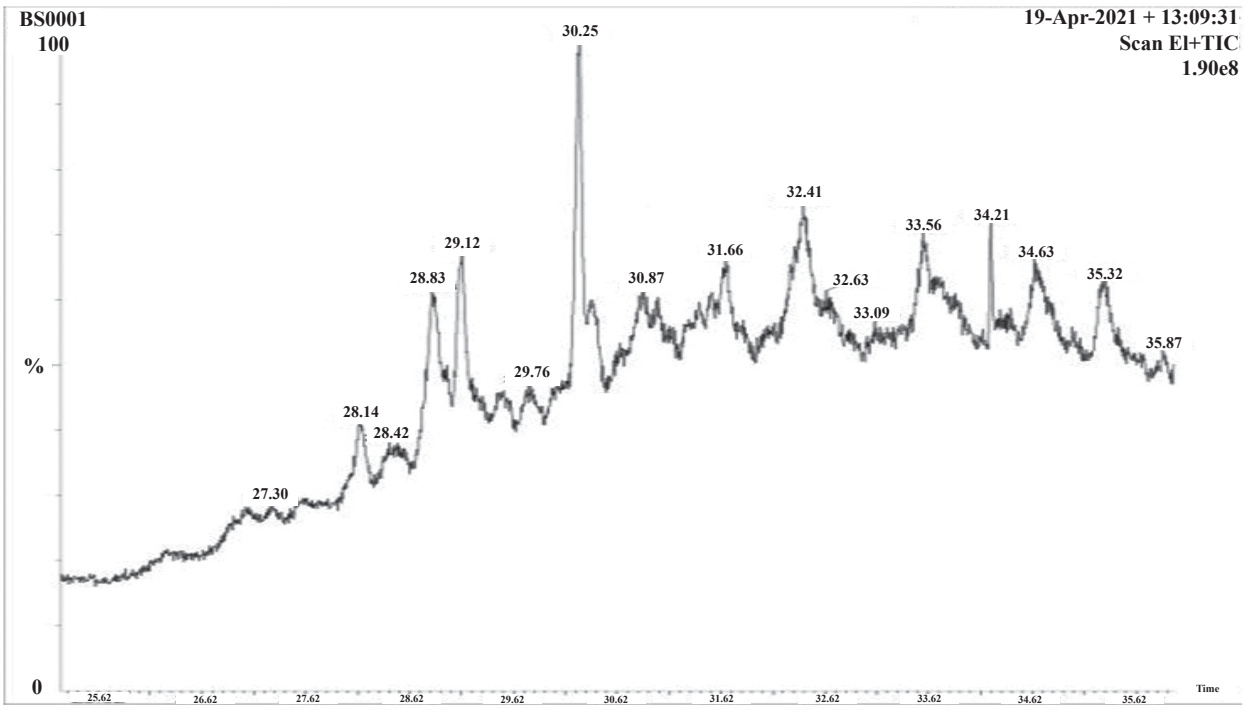

Figure 1. GC-MS chromatogram of methanolic extract of *Manihot esculenta* crantz.

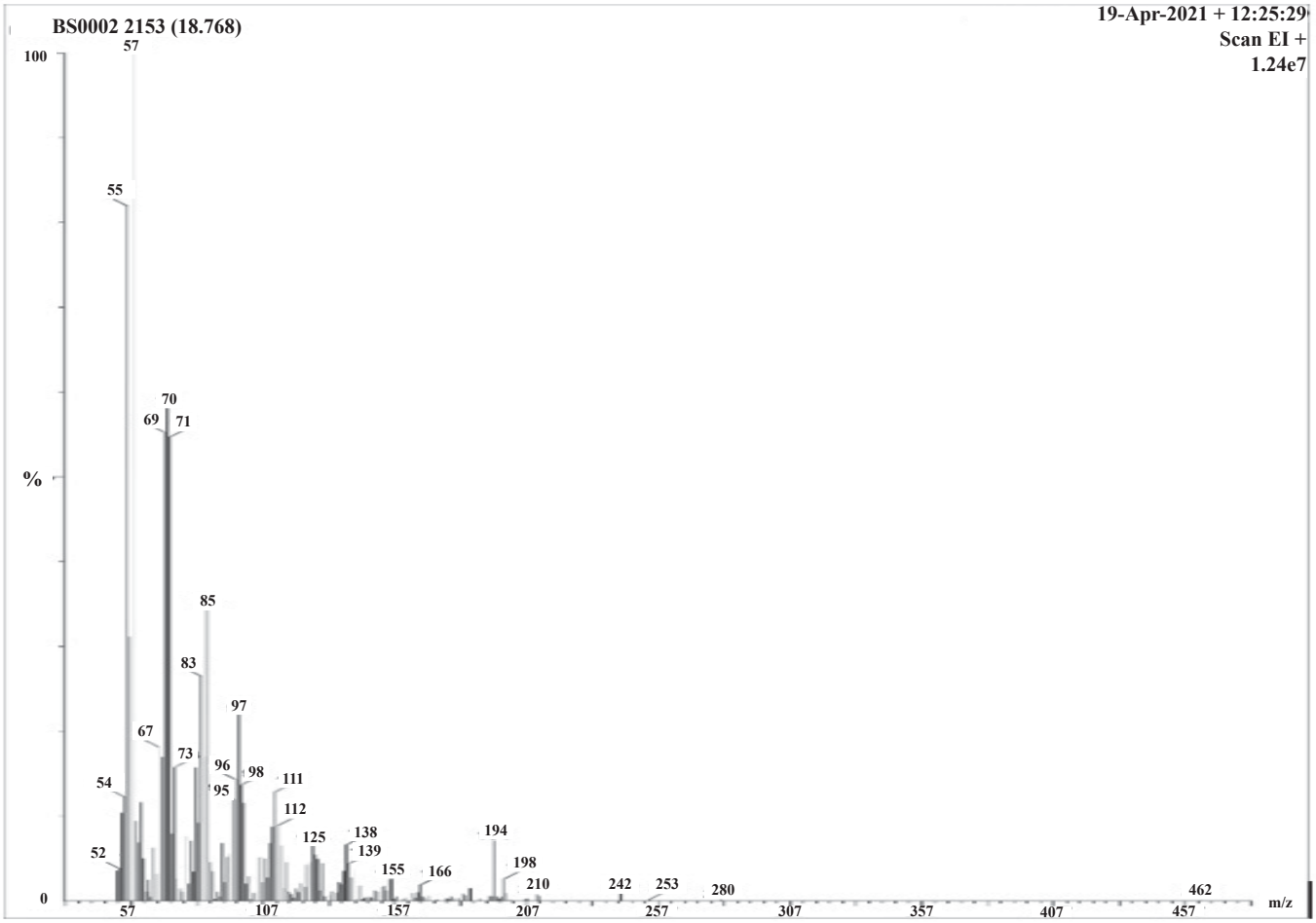

Figure 2. Mass spectras of methanol extract of *Manihot esculenta* crantz leaves.

Table 2. Phytocompounds found in *Manihot esculenta* crantz leaves

S. No.	RT (min)	Name of the compound	Molecular formula	MW(g/mol)	Peak area (%)
1	28.832	HENTRIACONTANE	$C_{31}H_{64}$	436	3.345
2	28.832	TETRAPENTACONTANE,1,54DIBROMO-	$C_{54}H_{108}Br_2$	914	3.345
3	28.832	DOTRIACONTYL ISOBUTYL ETHER	$C_{36}H_{74}O$	522	3.345
4	29.117	3-METHYL-2-(2-OXOPROPYL) FURAN	$C_8H_{10}O_2$	138	3.163
5	29.117	4-DODECENE-6,8,10-TRIYN-3-ONE, (E)-	$C_{12}H_{10}O$	170	3.163
6	29.117	Z,Z-6,27-HEXATRIACTONTADIEN-2-ONE	$C_{36}H_{68}O$	516	3.163
7	30.252	BETULIN	$C_{30}H_{50}O_2$	442	4.187
8	30.252	3.BETA.-HYDROXY-5-CHOLEN-24-OIC ACID	$C_{24}H_{38}O_3$	374	4.187
9	30.252	RHODOPIN	$C_{40}H_{58}O$	554	4.187
10	31.658	CIS-1-CHLORO-9-OCTADECENE	$C_{18}H_{35}Cl$	286	3.495
11	31.658	1-HEPTACOSANOL	$C_{27}H_{56}O$	396	3.495
12	31.658	HEXATRIACONTYL PENTAFLUOROPROPIONATE	$C_{39}H_{73}F_5O_2$	668	3.495
13	32.408	1-DECANOL, 2-HEXYL-	$C_{16}H_{34}O$	242	7.302
14	32.408	TRIACONTYL TRIFLUOROACETATE	$C_{32}H_{61}F_3O_2$	534	7.302
15	32.408	1-HENTETRACONTANOL	$C_{31}H_{64}O$	592	7.302
16	33.559	CARBONIC ACID, OCTADECYL VINYL ESTER	$C_{21}H_{40}O_3$	340	5.295
17	33.559	HEXACOSYL ACETATE	$C_{28}H_{56}O_2$	424	5.295
18	33.559	HEPTYL TRIACONTYL ETHER	$C_{37}H_{76}O$	536	5.295
19	34.214	OCTYL TETRADECYL ETHER	$C_{22}H_{46}O$	326	0.592
20	34.214	OCTACOSANOL	$C_{28}H_{58}O$	536	0.592
21	34.214	DOCOSYL HEPTAFLUOROBUTYRATE	$C_{26}H_{45}F_7O$	522	0.592
22	35.314	TETRATRIACONTYL HEPTAFLUOROBUTYRATE	$C_{38}H_{69}F_7O_2$	690	1.630
23	35.314	5-METHYL-Z-5-DOCOSENE	$C_{23}H_{46}$	322	1.630
24	35.314	DODECANE, 1-FLUORO-	$C_{12}H_{25}F$	188	1.630

(e)-, z,z-6,27-hexatriactontadien-2-one, cholest-5-en-3-ol (3.beta.)-, propanoate, 3. beta.-hydroxy-5-cholen-24-oic acid, rhodopin, Cis-1-chloro-9-octadecene, 1-heptacosanol, hexatriacontyl pentafluoropropionate, 1-decanol, 2-hexyl-, triacontyl trifluoroacetate, 1-hentetracontanol, carbonic acid, octadecyl vinyl ester, hexacosyl acetate, heptyl triacontyl ether, ctyl tetradecyl ether, octacosanol, docosyl heptafluorobutyrate, tetratriacontyl heptafluorobutyrate, 5-methyl-z-5-docosene, and dodecane, 1-fluoro-.

4. DISCUSSION

Phytochemical investigation of the methanolic extract of *M. esculenta* disclosed the existence of alkaloid, flavonoid, phenol, saponin, steroid, tannin, carbohydrate, glycosides, cardiac glycoside, and triterpenoid, which are known to possess several therapeutic properties[19]. It has been demonstrated that flavonoids are extremely potent scavengers of a majority of oxidizing molecules, including singlet oxygen and other free radicals linked to several disorders[20]. Antioxidant and mucosa protective properties of flavonoids are well reported by various studies[21,22].

Vegetables high in flavonoid content are common nutraceutical foods because they are effective in treating heart problems[23]. Due to their high bioavailability, flavonoids have been shown to produce pharmacologically significant plasma concentrations in humans when consumed consistently through diet[24]. Additionally, multiple studies have revealed that flavonoids may protect the heart against ischemia and reperfusion[25, 26]. Tannins help to lower the permeability of the mucosa to chemical irritants and saponins may activate protective mechanisms for the mucosa by acting as an astringent and are known to lower acidity. Moreover, it has been found that terpenoids and alkaloid substances have strong anti-ulcer properties[27,28].

Among the bioactive components identified, hentriacontane is known to possess anti-inflammatory, antitumor, antimicrobial, antitubercular, anticancer, antioxidants, and antidiabetic activities. Likewise, tetrapentacontane, 1,54-dibromo- are known to possess antimicrobial, anticancer, antioxidant, antidiabetic, and anti-inflammatory properties[29]. It is also established beta-hydroxy-5-cholen-24-oic acid and tetratriacontyl heptafluorobutyrate possesses strong antimicrobial activity[30]. Also, rhodopin identified in the extract is a major compound of phototrophic bacteria[31]. 1-heptacosanol has been established to show antimicrobial and antioxidant activity[32]. Similarly, dodecane, 1-fluoro- possesses anti-inflammatory, antioxidant, antiseptic, antifungal, analgesic, antibacterial, antipyretic properties and octacosanol is known to possess antinociceptive and anti-inflammatory activity[33].

5. CONCLUSION

In the current investigation, it was noticed that leaves of *M. esculenta* contain a variety of secondary metabolites, which are known to possess diverse pharmacological activities. GC-MS analysis identified 24 phytoconstituents, and they contribute to its multiple activities, some of which are antibacterial, antioxidant, anticancer, anti-inflammatory, antidiabetic, analgesic, and antipyretic properties. Further research is necessary to determine if some of these compounds could be used to create innovative medications.

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