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GAS CHROMATOGRAPHY – MASS SPECTROMETRY (GC- MS) ANALYSIS OF ANTIMICROBIAL COMPOUNDS IN HENNA (*Lawsonia inermis* L.) AND BITTER MELON (*Mormodica charantia* L.) LEAVES EXTRACTS

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ABSTRACT

Phytochemical analysis using GC-MS is a novel approach in analysis of bioactive compounds in plant materials. The study was carried out with the objective of investigating the phytochemical constituents of leaves extracts of Henna and Bitter melon using GC-MS analysis to identified the bioactive compounds there in. The plant materials were obtained in Maiduguri Metropolitan Council (MMC) in March 2024, air dried in shade for two days and pulverized into fine powder, 100g of each of the plants powder was dissolved in methanol to obtain the extract. An aliquot of the extracts of Henna and Bitter melon were then divided into two part. One part was used for phytochemical analysis while the second part use in the GC-MS analysis. Results for the phytochemical analysis indicate the presence of eight phytochemical compounds in Henna, they includes the following; Flavonoid, Tannins, Saponin, Alkaloids, Glycosides, Anthraquinones, Phenols and Steroids while Bitter melon had six phytochemical compounds; Flavonoid, Tannins, Saponin, Alkaloids, Anthraquinones and Phenols. The GC-MS analysis indicate the presence of 14 bioactive compounds in each of the leaves samples of Henna and Bitter melon identified to have antimicrobial activity. It can be concluded that both Henna and Bitter melon could be used effectively in the integrated management of plant diseases in the study area as both plants are readily available, safe, cheap and easy to obtained.

Keywords: Bioactive compounds, GC-MS, *Lawsonia inermis*, *Mormodica charantia*, Phytochemicals

INTRODUCTION

Plant diseases are better controlled using synthetic chemicals, because of their quick action and effectiveness they are used against destructive crop pest and diseases (Bobate, *et al*., 2023). Despite their importance continuous use of these chemicals have been proven to cause problems in the environment (Giannousi *et al*., 2013), development of resistance by organisms, contamination of food chain, effect on non-target organisms (Islam *et al*., 2017).

Botanical pesticides have been used since time immemorial, their use gradually declined when the synthetic pesticide emerged as alternative and a quick remedy for the control of plant diseases. In recent times, the use of botanical pesticides in the developed world is slowly increasing again becoming more popular in organic farming (Misra, *et al.,* 2019). Unlike in the developing countries where the use of botanical pesticide has not been properly adopted by farmers due to improper dissemination of information about its importance as do the conventional synthetic pesticide.

Botanical pesticides components are naturally occurring chemical derivatives of plants that function as deterrents, attractants, anti-feedants and growth inhibitors (Hikal, *et al*., 2017). In the control of plant pathogens, plant extract from different plants have been shown to contain very useful bioactive compounds (Sani and Gwa, 2018). Plant extracts contains phytochemical compounds such as alkaloids, flavonoids, tannins, glycosides, anthraquinones, and other related compounds (Adeyemi, 2010; Etaware, 2019) and a complex mixtures of monoterpenes and phenols (Farone *et al*., 2015). Majority of botanical pesticides are used in the management of insect pests, nematodes, fungi, bacteria, and virus diseases (Idris, *et al*., 2024). Studies have shown that several plant extracts have inhibitory effects on plant pathogenic organisms (Lum and Takor, 2021; Ndifon and Lum, 2023). They also possess pharmaceutical and therapeutic potentials in the treatment and prevention of infectious diseases in clinical medicine (Mancini *et al*., 2014; Thomsen *et al*. 2013), they are known to conserved biodiversity (Ogendo *et al*., 2008) in sustainability and resilience in farming systems while promoting a balanced relationship between humans and their environment, as they have been shown to have minimal toxicity to mammals and low danger of building resistance in target pests. (Liu *et al*., 2017). They break down easily in the environment and are rapidly metabolized by animals (Lengai, 2020; Khan *et al*. 2014; Khaliq *et al*. 2014).

Henna (*Lawsonia inermis* L.) synonyms Alba in Arabic. In Nigeria, the Hausas from the North called it "Lalle". It belongs to the family Lythraceae. It is a tall shrub of between 2.6m to 5.8m high with multi branched spine tipped branchlets, fruits are small brownish capsule containing 32-49 seeds per fruits. The leaves are the most important part of the plant containing a chemical compound called lawsone, it is use for dying cloth and for beautification of the skin, use as antimicrobial (Borade *et al*., 2011; Rahmatullah *et al*., 2009).

Bitter melon (*Momordica charantia* L.) belongs to the family cucurbitaceae. It is a creeping plant known as balsam pear, bitter melon or bitter apple. The hausas in Northern Nigeria called it "Garahunu" while the Kanuris from Borno called it "Daddau". It is widely used as a medicine due to its several ethnopharmacological use for the treatment of diabetic (Rahmatullah, *et* al., 2012), immunomodulatory (Deng *et al.*, 2014), as antidengue (Tang *et al*., 2012) and as an antioxidant, (Aljohi *et al*., 2016) it also promote allelopathic activity (Singh, 2014).

Botanical pesticides varies in their chemical composition depending on the geographical location, botanical origin, genetics and extraction techniques (Elshafie, 2015). Many

plants contains various volatile chemicals of high value that needs special methods of extraction to avoid loss during the course of high hydro-distillation. Therefore the need for Gas-Chromatography Mass Spectroscopy was conceived in this study. The aim of this study was therefore, to find out the phytochemical constituents in leaves extracts of Henna and Bitter melon and to analysed the leaves extracts using GC-MS analysis so as to identified the bioactive compounds present in the Henna and Bitter melon leaves.

MATERIALS AND METHODS

Location, Period of Study, Collection and Preparation of Plant Material

The experiment was conducted at the Department of Microbiology, Yobe State University, Damaturu, from January to March 2024. Fresh matured leaves of Henna (*Lawsonia inermis* L.) and Bitter melon (*Momordica charantia* L.) were harvested from Henna tree Henna and Bitter melon plants in Maiduguri Metropolis. The specimen (leave samples) were authenticated at the herbarium laboratory, Department of Botany, University of Maiduguri. The samples were air dried at ambient temperature and then grinded into powder using a blender. 100g of the dried leaf samples were extracted in a 100ml of methanol. The concentrated extracts were divided into two and the first part used for phytochemical analysis while the second part used for GC-MS analysis.

Phytochemical analysis

The qualitative and quantitative phytochemical analysis of Henna and Bitter melon leaves were carried out for the determination of phytochemical compounds using standard procedure as described by Sofowora and Trease (1993), Onwuka (2018), Banu and Catherine (2015)

GC – MS Plant extract profile.

The GC – MS profile of the plant extracts were carried out where a standared stock solution were dissolved in berberine hydrochloride in methanol. Each solution was filtered through a 0.45 micrometer nylon membrane filter before injection into the Chromatographic column. High Performance Liquid Chromatography (HPLC) analysis was performed on a Dionex Ultimate 3000 HPLC system that comprised a quarternary pump, an autosampler, a column thermostat, temperature controlled sample trays, an online degasser and a UV detector. The analytical column was a waters symmetry C18 (4.6 x 250 mm, 5micrometer). A full wave scanning of the plant samples and a 265 nm absorbing wavelength of the standard solution was chosen as the detection wavelength. A series of solutions were analyse with different ratios of acetonitrile – 0.02% potassium dihydrogen phosphate solution to choose the mobile phase. Finally, the ratio of 26:74 (v/v) was selected for the mobile phase with a flow rate of 1.0ml/min. The temperature of the column was maintained at 30^{o}C and the injection column was 10microlitre. The chromatographic conditions were optimized to separate the primary marker peaks of each sample with a resolution of (R > 1.5) and theoretical plate numbers. The plant sample peaks were identified by comparing their retention times of the peaks with that of the standard. The concentration of the plant samples were calculated according to the equation of the calibration curves. Three parallel operations were performed for each sample.

Identification of compounds

The bioactive compounds obtained from the GC-MS analysis were identified based on their retention indices and interpretation of mass spectrum using the database of National Institute of Standared and Technology (NIST). The spectra of the unknown compounds of Henna and Bitter melon fractions were compared with the standared mass spectra of the known compounds stored in NIST Library.

RESULTS AND DISCUSSION

The result in Table 1. Show the Phytochemical analysis for Henna (*L. inermis*) which revealed the presence of eight compounds; Flavonoids, Tannins, Saponins, Alkaloids, Glycosides, Anthraquinones, Phenols and Steroids while six compounds were present in Bitter melon (*M. charantia*) they includes; Flavonoids, Tannins, Saponins, Alkaloids, Anthraquinones and Phenols. The Flavonoids, Tannins and Phenols contents were higher in Henna than in the Bitter melon. The quantitative analysis further shows that the phytochemicals are present in this order for Henna; Flavonoid 3.08%, Tannins 5.40%, Phenols 4.49% while all the other phytochemicals in the Henna were less than 1%. Similarly, for Bitter melon; Flavonoid was 1.03%, Tannins 2.33%, Alkaloids, 2.10% while phenols 1.50% the rest of the other phytochemicals were less than 1%.

Table 1: Qualitative and Quantitative phytochemical analysis of Henna and Bittermelon leaves extracts

S/No.	Phytochemicals	Henna g/100g	Bittermelon g/100g
1	Flavonoids	3.08	1.03
2	Tannins	5.40	2.33
3	Saponins	0.11	0.04
4	Alkaloids	0.60	2.10
5	Glycosides	0.28	-
6	Anthraquinones	0.01	0.72
7	Phenols	4.49	1.50
8	Steroids	0.16	-

Source: field study (2024)

The chemical constituents, retention time, molecular formula and the molecular weight and concentration of each of the bioactive compounds in Henna and Bitter melon is presented in Tables below; The results in Table 2 indicate the presence of fourteen bioactive compounds present in Henna (*Lawsonia innermis L.*). The chemical components with their retention time and amount of compounds (%).The result indicate that α – Aminoxypropionic acid (30.5%) and squalene (22%) were the most concentrated bioactive compounds in Henna with their respective retention time (RT) of 2 minutes respectively. The bioactive compounds present in Bitter melon is presented in Table 3. Results showed that Bitter melon has about 14 bioactive compounds with N,N-Dimethyl-10-undecen-1-amine (34.4%) as the dominant compounds followed by tetradecanoic acid (11.2%) with their retention time (RT) in column of 2 and 12 minutes, respectively.

Table 2: Bioactive Compounds of Henna

S/No.	RT* (mins)	Component	Formula	Area	Area (%)
1	1.39	Carbonic acid, 2-chloroethyl 2-methoxyethyl ester	$C_6H_{11}ClO_4$	414973	4.0
2	2.03	α-Aminoxypropionic acid	$C_3H_7NO_3$	3186590	30.5
3	3.23	Pyrimidine, 5-methyl-	$C_5H_6N_2$	495537	4.7
4	5.42	2,3,7-Triazaindolizine	$C_5H_4N_4$	413047	3.9
5	6.11	4-Acetoxy-3-methoxystyrene	$C_{11}H_{12}O_3$	55712	0.5
6	7.20	N-[2-(4-Methylphenylthio)ethyl]propionamide	$C_{12}H_{17}NOS$	53873	0.5
7	7.75	Octanal, (2,4-dinitrophenyl)hydrazone	$C_{14}H_{20}N_4O_4$	176489	1.7
8	7.81	2-Formyl-9-[β-d-ribofuranosyl]hypoxanthine	$C_{11}H_{12}N_4O_6$	50603	0.5
9	8.25	Levoglucosan	$C_6H_{10}O_5$	64672	0.6
10	8.91	Retinal	$C_{20}H_{28}O$	101437	1.0
11	9.18	5-Methyl-3-phenyl-1-adamantanecarboxylic acid	$C_{18}H_{22}O_2$	211940	2.0
12	9.96	2,4-Dimethyl-6-(2-furyl)pyridine	$C_{11}H_{11}NO$	104879	1.0
13	10.30	1,3-Diphenylbuta-1,2-diene	$C_{16}H_{14}$	175071	1.7
14	26.88	Squalene	$C_{30}H_{50}$	2302990	22.0

Source: field study, (2023)
*RT=Retention time in column

The bioactive compounds present in Bitter melon is presented in Table 3. Results showed that Bitter melon has about 14 bioactive compounds with N,N-Dimethyl-10-undecen-1-amine (34.4%) as the dominant compounds followed by tetradecanoic acid (11.2%) with their retention time (RT) in column of 2 and 12 minutes, respectively.

The GC-MS analysis of the leaf extracts of Henna and Bitter melon resulted in the identification and quantification of 14 compounds exhibiting various phytochemical activities. The chromatogram of both Henna and Bitter melon leaves each with identified compounds and their peak representing a compound is shown in figures 1 and 2 respectively.

Table 3: Bioactive Compounds of Bitter melon

S/No.	RT* (mins)	Component	Formula	Area	Area (%)
1	1.36	Topotecan	$C_{23}H_{23}N_3O_5$	841684	6.5
2	2.04	N,N-Dimethyl-10-undecen-1-amine	$C_{13}H_{27}N$	4486474	34.4
3	3.06	4-Vinyl-imidazole	$C_5H_6N_2$	438125	3.4
4	6.11	4-Acetoxy-3-methoxystyrene	$C_{11}H_{12}O_3$	80798	0.6
5	6.69	Phenol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	153072	1.2
6	7.90	Retinal	$C_{20}H_{28}O$	112340	0.9
7	10.31	E,Z-2,15-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	49839	0.4
8	10.91	Methyl ursolate	$C_{31}H_{50}O_3$	797459	6.1
9	11.62	Palmitic acid	$C_{16}H_{32}O_2$	296410	2.3
10	12.41	Tetradecanoic acid	$C_{14}H_{28}O_2$	1457819	11.2
11	12.52	Phytol	$C_{20}H_{40}O$	878666	6.7
12	12.61	Octadecanoic acid	$C_{18}H_{36}O_2$	287147	2.2
13	14.79	Methyl 3β-hydroxyolean-18-en-28-oate	$C_{31}H_{50}O_3$	7408	0.1
14	15.77	Phthalic acid	$C_{24}H_{38}O_4$	149361	1.1

Source: field study, (2023)
*RT=Retention time in column

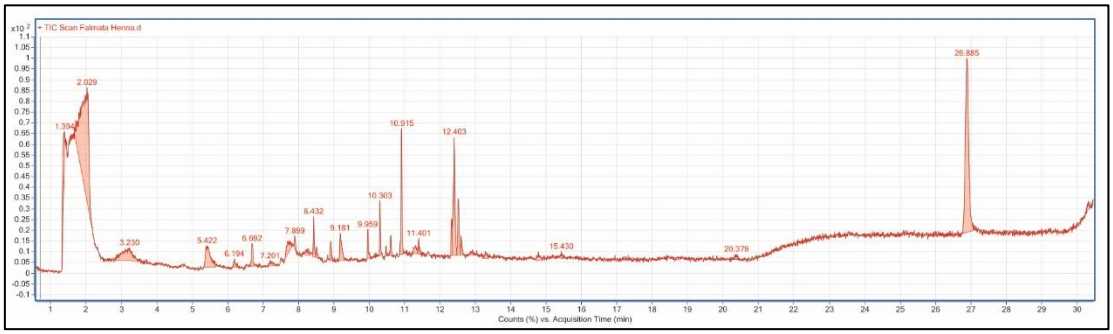

Figure 1: GC-MS chromatogram of Henna leaves

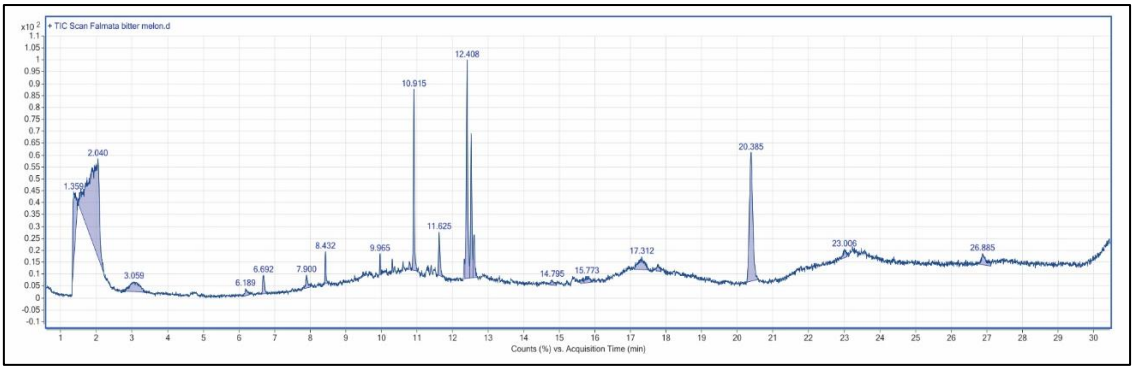

Figure 2: GC-MS chromatogram of bitter melon leaves

Discussion

The modern system of complete plant disease control against pathogenic microorganisms is the use of synthetic chemicals (Avasthi *et al*., 2010). This research found a wide variety of phytochemical substances in Henna and Bitter melon they includes; flavonoids, saponins, tannins, alkaloids, and many more. Thus, these findings corroborate those of Ahmed *et al.,* (2017), who demonstrated that phytochemicals found in plants had antioxidant, antifungal, and antimicrobial properties. Fini *et al.* (2011) have shown that flavonoids are essential for the colouration of flowers and other plant components and thereby protecting them from infections. Flavonoids play a crucial role in scavenging and reducing damage to the chloroplast membrane's outer envelope (Agati *et al.,* 2012). Flavonoids rich vegetables are widely used as functional foods for treatment of cardiovascular disease (Stoclet and Schain, 2011), increases plasma concentration in humans (Cao, et al., 2010). Alkaloids, saponins, tannins, glycosides, and flavonoids have been found in henna as reported in other studies (Chukwu *et al.,* 2011; Wangini *et al.,* 2014). This study also shows that henna leaf extracts can kill fungi. There are a plethora of beneficial chemicals found in bitter melon as well. In this study, the phytochemical tests showed that Bitter melon contain alkaloids, tannins, saponins, and flavonoids too, studies by Mada *et al.,* (2013) and the one by Oragwa *et al.,* (2013) found comparable results. Saponins and Tanins containing compounds are implicated in the treatment of mucous membrane and prevention of permeability of mucosa to chemical irritation thereby reducing excess acidity in stomach (Li *et al*., 2014).They can also disrupt fungi and bacterial cell by membranes leading to leakage of cellular components or they bind to proteins on microbial cell leading to denaturation and inhibiting enzyme activity (Alina *et al.,* 2023) Alkaloid too are used in human medicine for the treatment of ulcers. They are known to disrupt bacterial cell and interfering with DNA function and inhibiting protein synthesis in many bacteria species (Garba and Oeniyi, 2012). Among the bioactive compounds found in Henna and α-aminoxypropionic acid and and Squalene were the most abundant bioactive compounds analysed, they were the ones with the highest peak respectively. The amino acids are responsible for numerous biological functions in living organisms used in the preparation of homones, enzymes, and as source of fragrance in food industries (Azad, 2018). Squalene on the other hand is known to be responsible for biosynthesis of cholesterol in humans and as well in the treatment of cancer, enhances immune response to various associated antigens in humans (Reddy and Patrick, 2009). Similarly, Fang, *et al.* reported that squalene have been proven to inhibit and kill microbial organisms by direct induction of cells membrane leading to rupture and consequent leakage of cytoplasmic contents. N,N-Dimethyl-10-undecen-1-amine is also one of the compound obtained from analysis bioactive compounds in Bitter melon, it is generally used in the synthesis of various compounds such as surfactants and polymers as a catalysts (Li *et al*., 2014), in the preparation of AST-type zeolite having an antioxidant and antibacterial properties (Starlin, *et al*., 2019). Bitter melon also has Tetradecanoic acid in abundance in the leaves extracts studied. Tetradecanoic acid also refer to as myristic acid has a mechanism of action by integrating it into the bacterial cell membrane causing disruption and cell death more often in gram positive bacteria. It also has anti imflamatory,anti-arthritic, antieczema, insecticidal and nematicidal properties(Mary and Giri 2018).

CONCLUSION

It can be concluded that, in the present study the leaves of Henna and Bitter melon have show various types of phytochemicals having many antimicrobial and pharmacological properties. The GC – MS analysis show the presence of fourteen (14) bioactive compounds in both Henna and Bitter melon which has antimicrobial, anticancer, anti-inflammatory, antioxidant activities. Therefore, the type of phytochemical in these plants could be said to be responsible for their potent effect. Investigation in the flowers, stem/bark, and roots is recommended. Furthermore, the same plants from different ecology is advocated for using other extraction solvent is recommended for further study too.

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