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(RESEARCH ARTICLE)

Phytochemical studies of *Cissus populnea* in Benue State, Nigeria

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Abstract

Phytochemical studies of *Cissus populnea* in Benue State Nigeria was carried out to determine the presence of different chemicals in the stem, based on locality. The state was stratified into three zones using the already existing political grouping. From each of the zones samples of plants were collected with a minimum of 100 m apart. This is to avoid collection from the same siblings. The collected samples were air dried and pounded to paste. The obtained paste was soaked in hexane (150 ml), ethyl acetate (150 ml) and methanol (150 ml) for 48 h each. The mixtures were agitated every 4 h and then filtered in glass jars (1 L) representing the locations for each zone. The filtrates were left open to evaporate to dryness. After that, extracts were transferred into vials. The ethyl acetate extracts were coded for identification and sent for GC-MS analysis. The results obtained confirmed the presence of different chemicals in the stem of the plants and there was variation in the type of chemical obtained based on location.

Keywords: Phytochemical; Ethyl acetate; GC-MS; *Cissus populnea*

1. Introduction

Plants serve as medicine for our bodies and materials for drug manufacturing [1]. Apart from essential nutrients, there are non-essential nutrients or chemical compounds that occur naturally in plants. They contribute to the changes in colour, flavour, and smell in plants. In addition, they provide protection and prevention against diseases affecting the human system.

Plant products that are derived from plant parts such as bark, leaves, fruits, seeds and even roots, have been part of phytomedicine. This suggest that almost all parts of plants contains important chemicals. Many phytochemicals have been found, the most important of these chemical compounds of plant are glycosides, carotenoids, steroids, tannins, flavonoids and tepenoides [1]. Some antimicrobial and antibiotic substances like glycosides, flavonoids and saponinis are found in many plants, however, these compounds are not well established due to poor knowledge and practical techniques in extracting them.

So many indigenous medicinal plants are used as spices and food plants [2]. The knowledge of photochemistry is very important in knowing the composition of the chemical constituents present in plants organs because such information will be of value in the synthesizing of complex chemical substances [3-5].The screening of this phytochemicals also helps in the reasons why some plants could be edible and others poisonous, others good for pregnant mothers and others not. As good as photochemistry is to our world today, there are techniques which detect the most inert and volatile compounds in a plant extract, hence gas chromatography mass spectrometry is very important technique of analyzing chemical compounds. The objective of the study is to determine the different kinds of chemicals present in *Cissus populnea*.

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Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical method that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within an extract sample. It is a dual detection technique of analyzing extracts which identifies and quantifies accurately. GC is used to separate the volatile and thermally stable substitutes in a sample while MS fragments the extracts to be identified on the basis of its mass. Its combination coined the name GC-MS. A further addition of mass spectrometer in it leads to GC-MS/MS. Better result is obtained when single and triple quadruple modes are used [6-8].

Cissus populnea Guill and Perr is one of the several climbing tropical shrubs that is believed to promote fertility in both male and female, even though the mechanisms are not clear. Extracts from the stem of *Cissus populnea* is used to improve fertility in men with low sperm count [9].Thickening agents from plants like *Cissus polpulnea,* Ogbono, Melon and Okro are used as soup thickener in different parts of Nigeria. It has been reported that *C. populnea* can survive in all seasons. When leaves are squeezed, it becomes slimy and gelatinous. These thickening agents contain nutrients beneficial to health. Also, soups are very important as they make our meals more balanced in nutrients. Soup as a liquid food serves as "appetizer" as they are taken few minutes before the main meal [7].

2. Material and methods

2.1. Study area and sample collection

The stem of *Cissus populnea* Guills and Perr plant was collected from three local governments (Ukum, Makurdi and Oju Local Government Areas), one local government per senatorial zone in Benue State, Nigeria, in November 2016. From each zone, three samples of *Cissus populnea* were collected. A space of at least 100 meters apart was ensured to avoid collecting the same siblings.

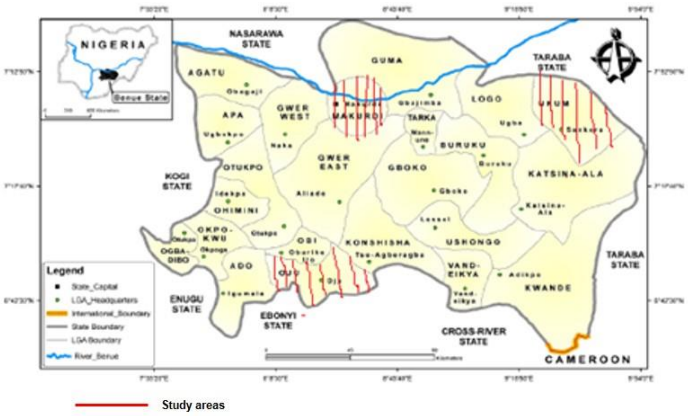

Figure 1 Map of Benue State, Nigeria (ministry of lands and survey, Makurdi) (Source: [10])

2.2. Preparation of samples

The stem of the *C. populnea* plant was sliced into strands of smaller size and air dried. The dried stem strands were pounded and ground into powder using a local mortar and pestle, after which they were sieved and stored in an air tight container prior to analysis, following the method of [6].

2.3. Procedure for n-hexane, ethyl acetate and methanol extracts

The powdered plants (10 g) from each of the three locations were macerated in hexane (150 ml), ethyl acetate (150 ml) and methanol (150 ml) for 48 h each. The mixtures were agitated every 4 hours and then filtered in glass jars (1L) representing the locations for each zone. The filtrates were left open to evaporate to dryness. After that, the extracts were transferred into vials. The ethyl acetate extracts were coded for identification and sent for GC-MS analysis [11].

2.4. Gas chromatography mass spectrometry analysis

The GC-system model 7890B and a Mass spectrometric detector model 5977A were used for the analysis. It was set at a temperature of 60 ˚C – 325 ˚C: 30 m × 250 µm × 0.25 µm with an initial oven temperature of 60 ˚C and a holding time of 3 min. For every ramp rate it was 5˚C/min holding time of 0 min and from 60 ˚C to 300 ˚C and the total run time was 51 min. The injector temperature was set at 250 ˚C (mass analyzer), having a mode split ratio of 20:1, split flow of 14

ml/min, pressure 4.4867psi and a total of 17.7ml/min. The different parameters involved in the operation of Mass spectra were taken at a scan start interval of 50.00; end mass 550.00 and a threshold of 150[12].

2.5. Identification of chemical compounds

The mass spectrum GC-MS was conducted using the database of National Institute of Standards and Technology, which has more than 62,000 patterns. The spectra of the known components stored in the NIST14 Library include the name, area, percentage quality and structures of the chemical compounds of the extracts, which were ascertained by the mass hunter software [13].

3. Results

Tables 1, 2 and 3 show the principal chemical compounds present per zone figure 2, 3 and 4 show charts of compounds identified from the GC-MS analysis of Ethyl acetate stem extracts of *Cissus populnea* from the three senatorial zones of Benue State. Table 4 compares result obtained for all three zones considered for the study. The GC-MS results obtained indicated the peaks, retention time, area, molecular formula and chemical compound quality match of the data base library of National Institute of Standards and Technology.

Table 1 Compounds identified ethyl acetate stem extracts of *Cissus populnea* collected from Zakibiam, Ukum using GC-MS analysis

Sr. No	Compound name	Peak	Retention Time (m)	Area (%)	Molecular Formula	Quality (%)
1	Hexadecanoic acid methyl ester	1	33.681	20.91	$C_{17}H_{34}O_2$	98
2	cis-Octadecanoic acid, methyl ester	2	37.205	24.05	$C_{19}H_{36}O_2$	99
3	Heptadecanoic acid, 16-methyl, methyl ester	3	37.679	11.74	$C_{19}H_{38}O_2$	97
4	1,8 Naphthyridin-2-amine,5,7-dimethyl	5	46.648	2.51	$C_{10}H_{11}$	25
5	6-Octadecanoic acid, (Z)-		46.795	5.43	$C_{18}H_{34}O_2$	44
6	Nonacos-1-ene	7	50.276	2.12	$C_{29}H_{58}$	53

Note: Principal chemical compounds deduced from GC-MS.

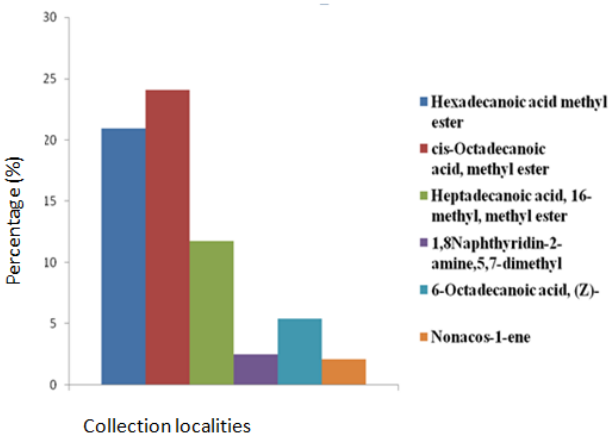

Figure 2 Compounds identified in ethyl acetate stem extracts of *Cissus populnea* collected from Zakibiam, Ukum

Table 2 Compounds identified in ethyl acetate stem extracts of *Cissus populnea* collected from Makurdi (EB1) using GC-MS analysis

Sr. No	Compound name	Peak	Retention Time (m)	Area (%)	Molecular Formula	Quality (%)
1	Dodecanoic acid, methyl ester	1	24.414	13.94	$C_{13}H_{26}O_2$	98
2	Methyl tetradecanoate	2	29.284	6.39	$C_{15}H_{30}O_2$	98
3	Undecanoyl chloride	3	32.940	5.44	$C_{11}H_{21}ClO$	43
4	Hexadeconoic acid, methyl ester	4	33.670	7.83	$C_{17}H_{34}O_2$	99
5	9,12-Octadecanoic acid, methyl ester	5	37.082	2.07	$C_{19}H_{34}O_2$	97
6	9 -Octadecanoic acid (z)- methyl ester	6	37.199	7.60	$C_{19}H_{36}O_2$	99
7	Methyl stearate	7	37.670	3.26	$C_{19}H_{38}O_2$	98
8	2-Methyl –z,z-3,13-Octadecadienol	8	43.449	12.51	$C_{19}H_{36}O$	98
9	Octadec-9-enoic acid	10	43.706	3.52	$C_{18}H_{34}O_2$	53
10	7- Pentadecyne	11	43.902	8.70	$C_{15}H_{28}$	96
11	i-propyl 9-Octadecenoate	13	46.251	6.30	$C_{21}H_{40}O_2$	22
12	Carbonic acid, But-3-en-1-yl, pentadecyl ester	14	46.647	2.70	$C_{10}H_{18}O_3$	35
13	6- Octadecenoic acid, (z)-	15	48.184	2.18	$C_{18}H_{32}O_2$	40
14	i-Propyl 9-tetradecenoate	16	48.578	1.39	$C_{17}H_{34}O_2$	44
15	Benzo(h) quinoline, 2,4-dimethyl-	7	50.663	1.23	$C_{15}H_{13}N$	50

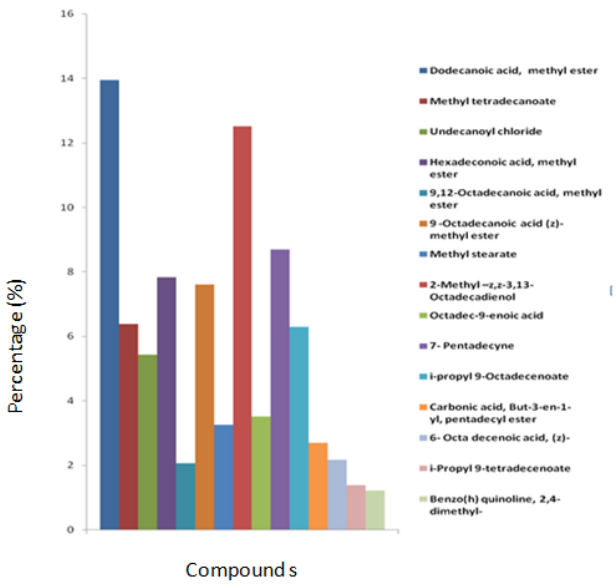

Figure 3 Compounds identified in ethyl acetate stem extracts of *Cissus populnea* collected from Makurdi

Table 3 Compounds identified in ethyl acetate stem extracts of *Cissus populnea* collected from Oju (EC1) using GC-MS analysis

Sr. No	Compound name	Peak	Retention Time (m)	Area (%)	Molecular Formula	Quality (%)
1	Hexadecanoic acid methyl ester	1	33.680	18.09	$C_{17}H_{34}O_2$	97
2	9-Octadecanoic acid (Z)-, methyl ester	2	37.208	21.30	$C_{19}H_{36}O_2$	99
3	Methyl stearate	3	37.674	9.45	$C_{19}H_{38}O_2$	97
4	n- Hexadecanoic acid	4	47.686	6.55	$C_{16}H_{32}O_2$	60

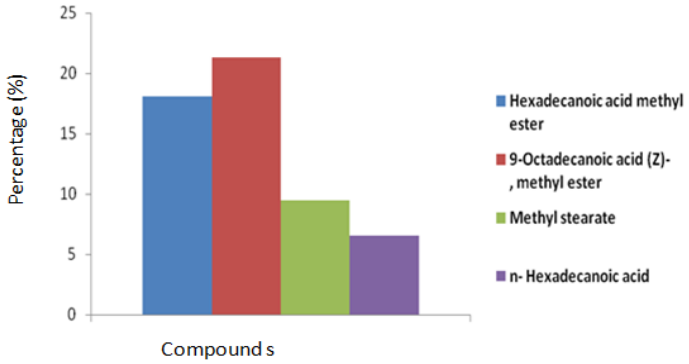

Figure 4 Compounds identified in ethyl acetate stem extracts of *Cissus populnea* collected from Oju

Table 4 Comparison of compounds identified in ethyl acetate stem extracts of *Cissus populnea* in region of Benue State using GC-MS analysis

Sr. No.	Compound name	Peak area (%)		
		Zakibiam	Makurdi	Oju
1	Hexadecanoic acid., methyl ester	20.19	7.83	18.09
2	Methyl stearate	11.74	3.26	9.45
3	6-Octadecanoic acid, (Z)-	5.43	2.18	1.42
4	Nonacos-1-ene	15.42	-	5.35
5	Octadec-9-enoic acid	5.43	3.52	-
6	cis-Octadecanoic acid, methyl ester	24.05	-	-
7	Dodecanoic acid methyl ester	-	13.94	-
8	methyl tetradecanoate	-	6.39	-
9	Undecanoyl chloride	-	5.44	-
10	9,12-Octadecanoic acid, methyl ester	-	2.07	-
11	9 -Octadecanoic acid (z)- methyl ester	-	7.60	-
12	2-Methyl –z,z-3,13-Octadecadienol	-	12.51	-
13	Heptadecanoic acid, 16-methyl, methyl ester	11.74	-	-
14	7- pentadecyne	-	8.70	-
15	i-propyl 9-Octadecenoate	-	6.30	-
16	Carbonic acid, But-3-en-1-yl, pentadecyl ester	-	2.70	-
17	Benzo(h) quinoline, 2,4-dimethyl-		1.23	-

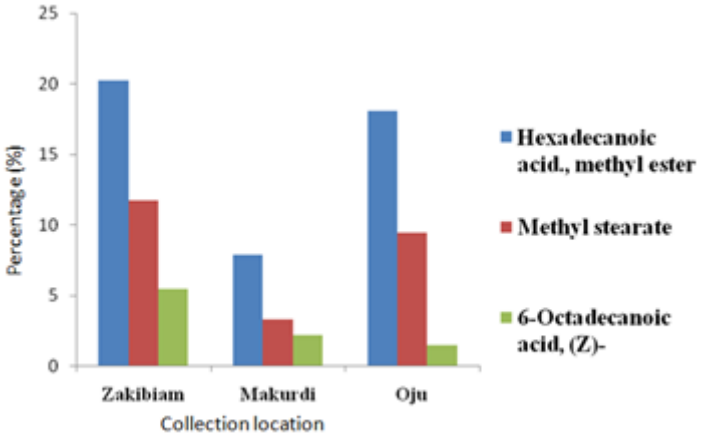

Figure 5 Three abundant chemical compounds present in the ethyl acetate stem extracts of *Cissus populnea* in Benue State

4. Discussion

A total of 17 chemical compounds were identified from the stem of *C. populnea* collected from the different localities in Benue State. This shows how abundant the chemical compounds are, in the stem of the plant. This finding agrees with the results of Onojah *et al* (14) on the abundance of chemical contents in the fresh stem exudates and the dried pulp of the root of *C. populnea*. Since there is no marked geographical difference in the zones it could be inferred that the variation in the chemical present could be due to soil type. Some of the chemicals found in the three zones, hexadecanoic acid., methyl ester, methyl stearate, 6-Octadecanoic acid, (Z)-, nonacos-1-ene, octadec-9-enoic acid, can serve as a product in perfumes, and cosmetics in industries, medicine for pregnant mothers and infertility problems, spices and protein for proper growth and body maintenance. Some of the chemicals obtained agree with the findings of [15]. In their studies Methyl stearate was observed as a major component occurring in *Cissus populnea*.

5. Conclusion

The result obtained from this study revealed that *Cissus populnea* is edible as the compounds found in them are not known to be harmful to man. In all the zones, haxdecanoic acid, methyl stearate and octandecanoic acid were found to be common and present in all the samples collected for the zones. However, hexandecanoic acid is abundant in Zakibiam, Ukum and Oju but very scanty in Makurdi. This calls for chemotaxonomic studies of the plants to confirm their taxonomic status as there could be subspecies or varieties among them.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

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