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Preliminary phytochemical screening and GC-MS analysis of *Anogeissus leiocarpus* stem bark extract

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Abstract
The aim of the study was to determine the phytochemicals presence in the plant extract of *Anogeissus leiocarpus* and analyze the extract using GC-MS analysis. The stem bark of *Anogeissus leiocarpus* was collected, dried and grounded. Extraction was conducted using successive extraction technique with three different solvents that include n-hexane, methanol, and distilled water. The extract was then screened for the presence of phytochemicals that include saponins flavonoids phytosterols and tannins phenols. GC-MS analysis of the extract was also conducted in order to validate the qualitative phytochemical test. The result of the phytochemical screening revealed the presence of saponins, phytosterols, and tannins phenols, whereas the GC-MS analysis of the extract showed the presence of 29 chemical compound that include Ethanimidic acid, ethyl ester, Chloroformate, 1, 2-Propanediol diformate, (R)-(-)-2-Amino-1-propano, 1H-Imidazole-4-ethanamine, 2-methy, Furan, 2, 5-dimethyl, Thiirane, 2, 3-dimethyl-, trans, R(-)-1-Cyano-2-methylpyrrolidine, 2,4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one, Pentanoic acid, 4-methy, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl, 4-Piperidinemethanamine, 5-Hydroxymethylfurfural, 1-Allyl-cyclohexane-1, 2-diol, 1, 2, 3-Benzenetriol Sucrose, Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl. The findings of this study suggest the use of stem bark extract of *Anogeissus leiocarpus* for treatments of various ailments, since it contain various compounds which have various medicinal properties.

Keywords: Preliminary phytochemical, GC-MS analysis, *Anogeissus leiocarpus* stem

Introduction
Anogeissus leiocarpus is a deciduous tree belonging to the family combretaceae which are commonly found in tropical and subtropical regions across the globe. It is also distributed throughout central and west African regions where it is used as medicinal herb. The plant has been characterized with greyish and scaly bark, branches which are often dropping and slender, leaves alternate, Ovate-lancelet in shape, 2 – 8cm long and 1.3 – 5cm across (Ahmad, 2014) [2]. In Nigeria the plant (*Anogeissus leiocarpus*) has been reported to be used in the treatments of various diseases such as ulcers, body pain, asthma, blood clots, coughing, tuberculosis, diabetics, pile and jaundice (Ayeni and Nuhu 2018; Victor, 2013; Abubakar *et al.*, 2017) [6, 1, 31]. However, several studies have reported that the therapeutic importance of the plants have been linked to it biologically active secondary metabolites such as flavonoids, alkaloids, tannins, polyphenols, saponins presence in the plant (Mann *et al.*, 2014) [16]. Despite that several studies have been conducted on the phytochemicals screening of *Anogeissus leiocarpus* plant (Ezeonu and Ejikeme, 2016; Ibrahim *et al*., 1997; Burkill 1985) [11, 12, 13], still the plant is yet to be fully explored for its phytochemical constituents. Therefore, the aim of the present study was to determine the phytochemicals compounds present in the plant extract of *Anogeissus leiocarpus* and analyze the extract using GC-MS analysis.

Materials and Methods
Plant materials collection and preparation
The *Anogeissus leiocarpus* stem bark was obtained from local timber market in Damaturu Metropolis, Yobe State, Nigeria. The stem bark collected were cleaned up with tap water and followed by distilled water to remove the dust particles and were shade dried at room temperature for 48 hours. The stem bark was then grinded into fine powder using mortar and pestle and stored at room temperature for further analysis.

Plant extraction
The extraction was conducted using successive extraction technique previously described by (Roopalatha and Vijay, 2013) [26] with slight modification.

Five hundred gram (500 g) of the plant extract powder was weighed into 1000 ml of n-hexane and shake continuously for 24hours on a laboratory universal shaker. It was then suspended in 250 ml of methanol and then kept for another 24 hours, followed by filtration. The filtrate was further re-suspended in 250 ml of distilled water and kept for 24hours. These was then filtered and freeze dried, kept in hot air oven to remove all solvent, the extract was filtered and freeze dried to complete dryness.

Phytochemical screening

The extracts of *Anogeissus leiocarpus* stem bark were tested for the presence of saponins, flavonoids, phytosterols, tannins, and phenols. The results of the analysis are expressed as (+) positive for the presence and (-) negative for the absence of phytochemicals. The qualitative phytochemical analysis was carried out by using standard procedures as described by Ezeonu and Ejikeme, (2016) [11] and Daskum *et al.*, (2019) [10].

Test for Saponins

Three milliliters (3 mL) of *Anogeissus leiocarpus* extract was added into 10 mL of distilled water in test a tube. The solution was then shaken vigorously for about 5 minutes and allowed to stand for 30 min. The sample was then observed for honeycomb froth, which indicate the presence of saponins.

Test for Phytosterols

A small quantity of *Anogeissus leiocarpus* extract was added into a beaker containing 5mL of chloroform. The mixture as then filtered using filter paper. Few drops of concentrated sulphuric acid was added to the filterate. The mixture was then shaken and allowed to stand for few minutes. It was then observed for appearance of golden yellow color which indicates the presence of triterpenes

Test for Phenols

Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Tannins

Two milliliters (2 mL) of *Anogeissus leiocarpus* extract was poured into a test tube and few drops of ferric chloride (10%) solution were added into the test tube. The mixture was then observed for the formation of white precipitate which indicates the presence of tannins.

Test for Flavonoids

Five milliliters (5 mL) of sodium hydroxide (20%) were added into 5 ml of *Anogeissus leiocarpus* extract in a test tube. The mixture was then observed for color change. Formation of yellow color indicates the presence of flavonoids.

Gas Chromatography-Mass spectrometry (GC-MS)

The GC-MS analysis of the sample was conducted using GC-MS (QP 2010 series, Shimadzu, Tokyo, Japan). The inert gas helium (99.999%) was used as carrier gas with the flow rate of 1 ml/min. HP5 column with specification length 30 mm, internal diameter 0.32 mm, film of 0.25 mm and temperature limit -60 °C to 325 °C (350 °C) was used. The total run time of GC was 35 minutes. The oven temperature raised from 70 °C up to 280 °C with the rate of 8 °C per min rise in temperature. The sample size of 4 µl was injected through the injector. The MS was taken at 70eV. The identification of compounds was done by comparing the spectrum of unknown compounds with NIST mass spectrum library. The name, molecular weight and structure were also determined (Omoregie, 2016) [21].

Results and Discussion

The phytochemical screening of *Anogeissus leiocarpus* stem bark extract showed the presence of four different secondary metabolites that include saponins, phenols, tannins, phytosterols, while flavonoids was found to be absent (Table 1).

Table 1: Phytochemical screening of *Anogeissus leiocarpus* stem bark extract.

Phytochemicals	Result
Saponins	+
Flavonoids	-
Phytosterols	+
Tannins	+
Phenols	+

In a similar study conducted by Aliyu and Sani (2011) [4], also reported presence of saponins, phenols, tannins, and phytosterols. Contrary to our finding flavonoids were found to be present in aqueous and ethanol stem bark extracts of *Anogeissus leiocarpus.* All the chemical components found in the present study have been reported to possess therapeutic values (Tyler *et al*., 1988, Robinson 1967) [29, 25]. For example, tannins can induce an antidiarrheal effect and this substance may precipitate on the enterocytes reducing intestinal secretion and peristaltic movement (Yu *et al*., 2000) [33]. Saponins have also been reported to have anti-diabetic and hypoglycemic effects which are very useful in the management of diabetes mellitus (Anila *et al.,* 2000, Sui *et al.*, 1994) [5, 27].

In this study, the result of GC-MS analysis identified different compounds and their molecular formula from the stem bark extract of *Anogeissus leiocarpus.* The results in table 2 show all the compounds identified and their peaks with the retention time. The compound (R)-(-)-2-Amino-1-propanol found to have the highest peak value with retention time of 1.886 which make it the most active ingredient or compounds of the stem bark extract of *Anogeissus Leiocarpus* respectively. Furthermore, the finding of the present study revealed the presence of ethanimidic acid, ethyl ester with molecular formula C_4H_9NO. 1, 2-propanediol diformate with molecular formula $C_5H_8O_4$. (R)-(-)-2-Amino-1-propanol with molecular formula C_3H_9NO. 1H-imidazol-4-ethanamine, 2-methyl with molecular formula $C_6H_{11}N_3$. Furan, 2, 5-dimithyl with molecular formula C_6H_8O. Thiirane 2, 3-dimithyl-trans with molecular formula C_4H_8S. Sucrose with molecular formula $C_{12}H_{22}O_{11}$. Pentanamide, N-(aminocarbonyl) with molecular formula $C_6H_{12}N_2O_2$. Therefore, GC-MS analysis technique identified compound and their derivative have very rich pharmacological activities ethanimidic acid, ethyl ester shown to have antioxidant, cancer preventive, anti-microbial, analgesic (Chidozie and Adoga, 2020) [7]. Thiirane, 2, 3-dimithyl-, trans, thiirane derivatives possess anti-microbial and cytotoxic activities, thiirane is also used in synthesis of various intermediate compounds for industrial purposes. These compounds have different functionalities in their

structure. It is widely used as anti-cancer (Mohammed, 2014) [18]. Literature surveys proved 1H-imidazole-ethanamine and its derivatives have various pharmacological activities like anti-bacterial and anti-fungal (Ajani *et al.*, 2021) [3]. Chloroformates has been applied in analytical chemistry as a derivatization reagent for reactions with amines and alcohols to form carbamates. They are also useful in analysis of drinking/ wastewaters and for pharmaceutical and environmental areas (Parkinson, 2012) [24]. They also serve as synthetic intermediates for use in perfumes, drugs, pesticides, polymers, dyes and fuel additives (Kreutzberger, 2001) [14]. 1, 2-propanediol diformate with molecular formula $C_5H_8O_4$ is widely used as lubricants and solvents in food; as a hygroscopic agent, an antifreeze; and also in pharmaceutical and cosmetic fields (Tao *et al.*, 2021) [28]. 2,4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one was reported as the main flavourant principle in pineapple (Van-Cott, 1954) [30] This compound is used in pharmaceutical industry, it also has a gustatory activity and thus can be used in food industry as flavourant (Chukwu, 2017) [8]. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) confirmed the use of Pentanoic acid, 4-methyl as a flavoring agent (National Center for Biotechnology Information 2022) [19]. 3, 5-dihydroxy-6-methyl-2, 3-dihydro-4*H*-pyran-4-one is of great benefit to the food industries owing to their satisfying sensory qualities. (Odutayo, 2020) [20]. It is also present in cakes, toasted oak wood, tartary buckwheat tea, brown sugar, garlic oil, cocoa and cocoa containing products (Papageorgiou, 2020) [23]. 1, 2, 3-Benzenetriol has been reported to have anticancer properties (Yang *et al.*, 2009) [32]. 1-Allyl-cyclohexane-1,-diol numerous studies have been conducted to demonstrate wide range of biological activities of cyclohexane derivatives. This includes anti-cancer, cytotoxic, antioxidant, analgesic and antimicrobial activities (Mamza *et al.*, 2022) [17]. (R)-(-)-2-Amino-1-Propanol the biological result clearly indicated the importance of aminol propanol groups to have anti-malarial activity and cytotoxic activity (Tao *et al.*, 2021) [28]. This compound is also used as a chiral intermediate for the synthesis of agrochemicals and pharmaceutical products. It acts as additives in healthcare products and animal feed (Contini *et al.*, 2013) [9]. 5-hydroxymethylfurfural is a member of the class of furans that is furan which is substituted at positions 2 and 5 by formyl and hydroxymethyl substituents, respectively. It is used to make aminoalcohols, glycols, dialdehydes, ethers and acetals. It is also a flavoring agent that naturally occurs in some foods and alcoholic beverages (O'Neil *et al.*, 2013) [22]. It has also been shown to have antioxidant properties as well as anticancer activities (Zhao *et al.*, 2013). Sucrose is a sweetener found in a variety of foods and soft drinks, used to manufacture syrups and jams, source of invert sugar, pharmaceutical products, chemical intermediate for detergents and emulsifying agents among others (Lewis, 1997) [15].

Table 2: The GC-MS analysis below showed the components of *Anageissus leiocarpus* stem extract.

Peak	RT	Components	Hight	Width	FWHM
1	1.199	Ethanimidic acid, ethyl ester	504998	0.105	0.056
2	1.296	Chloroformate	203537.68	0.126	0.082
3	1.405		65900.47	0.2	0.069
4	1.777	1, 2-Propanediol diformate	279049.11	0.148	0.025
5	1.886	(R)-(-)-2-Amino-1-propano	263037.87	0.052	0.026
6	1.937		320830.89	0.073	0.035
7	2.092		171240.54	0.071	0.023
8	2.223	1H-Imidazole-4-ethanamine, 2-methy	126276.48	0.186	0.052
9	2.458	Furan, 2, 5-dimethyl	192071.52	0.199	0.06
10	2.63	Thiirane, 2, 3-dimethyl-, trans	127488.69	0.189	0.03
11	2.841		241013.55	0.12	0.067
12	2.939	R(-)-1-Cyano-2-methylpyrrolidine	154359.46	0.171	0.1
13	3.082	2,4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one	469325.25	0.093	0.019
14	3.265	Pentanoic acid, 4-methy	39152.74	0.238	0.175
15	3.631		105683.73	0.143	0.121
16	3.82		239565.73	0.24	0.128
17	4.226	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl	877428.63	0.24	0.067
18	4.615	4-Piperidinemethanamine	71894.84	0.13	0.078
19	4.798	5-Hydroxymethylfurfural	3574957.49	0.269	0.074
20	5.29	1-Allyl-cyclohexane-1, 2-diol	60764.94	0.175	0.086
21	5.903	1, 2, 3-Benzenetriol	758973.54	0.419	0.129
22	6.372		944283.45	0.372	0.189
23	7.264	Sucrose	227674.74	0.598	0.278
24	8.054		164098.79	0.062	0.26
25	8.317		104478.72	0.148	0.037
26	9.279	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)	93399.13	0.152	0.035
27	9.668		68599.31	0.182	0.074
28	9.874		58980.61	0.191	0.07
29	19.864		13062.3	1.31	1.17

Conclusion

Based on the findings of the present study, it is concluded that *Anogeissus leiocarpus* stem bark extract contains several bioactive compounds that may be possibly used for treatments of various ailments. It is therefore recommended that further study should be conducted to determine the efficacy of the plant extract against different bacterial strains.

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