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Research Article

Phytochemical constituents of ethanolic extracts of fruits of *Acacaia nilotica* and flowers of *Calotropis procera*

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ABSTRACT

The fruits of *Acacia nilotica* and flowers of *Calotropis procera* are widely used in the treatment of various health conditions in traditional medicine. This study was carried out to investigate the phytochemical constituents of ethanolic extracts of fruits of *Acacia nilotica* and flowers of *Calotropis procera*. Fruits of *Acacia nilotica* was purchased from old market in Wukari, Nigeria, while the flowers of *Calotropis procera* was harvested from road side in Wukari, Nigeria. The plant parts were air-dried and ground into powder. The phytochemical analysis was carried out using GC-MS. The result of the GC-MS analysis showed constituents in both plant parts that have nutritional function and possess other activities such as antimicrobial and anticancer. Some of these phytoconstituents include: 9-octadecanoic acid, (Z)2,3-hydroxypropyl ester, Hexadecanoic acid, methyl ester, Methyl tetradecanoate, 9-Octadecenoic acid (Z)-, methyl ester and Methyl 11-oxo-9-undecenoate. The phytochemical result of the ethanolic extracts of both fruits of *Acacia nilotica* and flowers of *Calotropis procera* showed that both plant parts possess medicinal and nutritional properties.

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Introduction

Plants have been shown to have a lot of potential in the treatment and management of certain diseases. Many plants have been employed by tribal and folklore healers in various nations to treat a variety of ailments. Plants' therapeutic value is derived from bioactive phytochemical components (Imo and Zaaku, 2019). For decades, natural products, particularly those of plant origin, have been utilised successfully to treat a variety of ailments. Medicinal plants produce a diverse spectrum of compounds with medicinal properties, making them promising candidates for the development of new antibacterial and antioxidant medications (Sibanda and Okoh, 2007; Yigezu, 2014).

Acacia nilotica, known as Egyptian mimosa, was once a member of the Mimosaceae family. According to the revised Leguminosae subfamily classification based on a taxonomically thorough phylogeny that includes the taxa of the mimosoid clade in Caesalpinioideae, *Acacia nilotica* is now known as *Vachellia nilotica* (Amadou *et al.*, 2020). *A. nilotica* is a leguminous tree that can grow up to 20 metres tall, with a trunk diameter of 60 cm and a dense canopy. It is a complicated species with nine subspecies, six of which are found in Africa's tropics. Subsp. *nilotica*, subsp. *tomentosa*, and subsp. *adstringens* are three subspecies of *A. nilotica* that are native to the Sahel region of West Africa (Raj and Chandrawanshi, 2015). *Acacia nilotica* is a worldwide multipurpose leguminosae plant. *A. nilotica*, for example, is used in traditional pharmacopoeia for animal and human, agricultural, pastoral, industrial, and food in the West African Drylands. It was also mentioned that *A. nilotica* bark is one of the main sources of natural aphrodisiacs (Abdalla *et al.*, 2020). *A. nilotica* is capable of phytomediation of cadmium from

metal contaminated saline and nonsaline soils, in addition to its medical properties (Issoufou *et al.,* 2020). *Acacia nilotica* has therapeutic properties in all of its parts (Rather *et al.,* 2015). Furthermore, every portion of the plant has therapeutic properties and is used to cure a variety of ailments (Abdalla *et al.,* 2020). Mahesh and Satish (2008) reported the plant part to have antibacterial and antifungal activities, Singh *et al.* (2009) reported its antioxidant activity, while its anti-inflammatory activity was reported by Abdalla *et al.* (2020).

Figure 1: *Acacia nilotica* fruit

Calotropis procera is a perennial shrub with soft wood that is evergreen. It has one or two stems, a few branches, and a small number of leaves, all of which are concentrated towards the developing point. The bark is light grey, corky, and furrowed. When the stems or leaves are severed, a large amount of white sap oozes (Amit *et al.,* 2013). *C. procera* prefers open, sparsely populated areas. This species' plant grows in dry habitats with annual precipitation ranging from 150 to 1000 mm, as well as in areas with excessively drained soil with up to 2000 mm of yearly precipitation. It can also be found in common habitats such as roadside and seaside dunes, as well as being commonly disturbed in urban areas. *C. procera* can also be found at elevations of up to 1,000 metres. The plant is sometimes grown as an attractive plant in dry or coastal settings since it is easy to propagate and care and may grow in a xerophytic environment (Ahmed *et al.,* 2005). *Calotropis procera*'s therapeutic usefulness has been documented in previous pharmacopoeias. Leprosy, hepatic and splenic enlargements, dropsy, and worms are among conditions where it is highly suggested. Fresh leaves are also used for the same purpose as latex when it comes to sore joints and swelling (Amit *et al.,* 2013). The antioxidant activity of *C. procera* dried latex (DL) and its antidiabetic effect in alloxan-induced diabetes rats were reported by Tsala *et al.* (2015) and Sayed *et al.* (2016). The plant part also have been reported by Meena *et al.* (2010) to have antimalarial activity.

Figure 2: *Calotropis procera*

Materials and methods

Plant material

The dried fruits of *Acacia nilotica* was purchased from old market in Wukari, Nigeria. It was ground into powder. The fresh flowers of *Calotropis procera* was harvested at Aguwan Roger Road, Wukari, Nigeria. It was air-dried and then ground into powder. The chemical constituents of the ground samples were extracted using 70% ethanol.

Extract preparation

Seventy percent (70%) ethanol was prepared and used to soak the two ground samples (fruits of *Acacia nilotica* and flowers of *Calotropis procera*) separately. The mixtures were filtered after 48 hours. The filtrates were concentrated using a water bath set at 68°C in order to eliminate the ethanol. The crude plant parts extracts were used for phytochemical analysis.

Quantitative determination of some phytochemical constituents of ethanolic extracts of fruits of *Acacia nilotica* and flowers of *Calotropis procera*

The ethanolic extracts of fruits of *Acacia nilotica* and flowers of *Calotropis procera* were quantitatively analyzed for phytoconstituents using Gas Chromatography-mass Spectrometry (GC-MS).

Results

Table 1: GC-MS Phytochemical Profile of Ethanolic Extract of Fruit of *Acacica nilotica*

S/N	Compound name	Retention time (min)	Area %
1	4-Hydroxy-2-methylacetophenone	6.35	0.25
2	Nonanoic acid, methyl ester	6.71	0.14
3	Cyclopentaneundecanoic acid	7.41	0.11
4	Octadecanoic acid	8.24	0.78
5	n-Decanoic acid	8.37	0.07
6	cis-1,4-Cyclohexanediamine, N,N'-diacetyl	8.46	0.17
7	Pentadecanoic acid	8.49	0.10
8	Tetradecanoic acid	8.65	0.37
9	Undecanoic acid	8.72	0.62
10	Octanoic acid, methyl ester	10.77	0.08
11	Dodecanoic acid, methyl ester	11.22	1.26
12	Methyl 11-oxo-9-undecenoate	11.48	0.04
13	13-Docosenoic acid, methyl ester	11.52	0.04
14	Dodecanoic acid	12.40	0.54
15	2- Chloropropionic acid, octadecyl ester	12.50	0.29
16	5-Thio-D-glucose	12.87	0.69
17	Dodecanoic acid, 1-methylethyl ester	13.18	0.02
18	Methyl tetradecanoate	15.77	0.29
19	2-Heptadecenal	17.03	0.15
20	Decanoic acid, ethyl ester	17.25	0.12
21	Hex-5-enoic acid, ethyl ester	18.55	1.21
22	trans-4-Aminocyclohexanol, N,O-diacetyl	18.72	0.71
23	Oxacyclopentadecan-2-one	18.84	0.12
24	Heptadecanolide	19.08	1.37
25	Undecylenic acid	19.18	0.27
26	Heptanoic acid, ethyl ester	19.31	0.86
27	9-Eicosenoic acid, (Z)-	19.37	0.98
28	Hexadecanoic acid, methyl ester	19.55	1.88
29	Pentadecanoic acid, 14-methyl-, methyl ester	19.93	12.97
30	Tridecanoic acid	20.57	0.09
31	n-Hexadecanoic acid	21.09	1.92
32	tetradecanoic acid, ethyl ester	21.28	1.25
33	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	22.96	0.59
34	9-Octadecenoic acid, methyl ester	23.17	8.19
35	Phytol	23.50	1.78
36	Methyl stearate	23.75	8.60
37	Linoelaidic acid	24.05	4.35
38	9,17-Octadecadienal, (Z)-	24.20	5.72
39	Oleic Acid	25.87	0.05
40	9-Methyl-Z-10-tetradecen-1-ol acetate	26.59	2.58
41	cis-Vaccenic acid	26.69	1.06
42	cis-10-Nonadecenoic acid	26.84	0.20
43	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	27.26	0.87
44	15-Hydroxypentadecanoic acid	29.96	0.22
45	9-Octadecenal, (Z)-	32.65	1.56
46	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	33.12	0.95
47	Cycloeicosane	33.30	0.17
48	9-Octadecanoic acid (Z)-, 2,3-dihydroxypropyl ester	35.13	17.04
49	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	35.34	8.96

A total of 49 compounds were found from the GC-MS analysis of ethanolic extracts of fruits of *Acacia nilotica* showing various phytochemical activities. The phytochemical constituents with their retention time (RT) and area (%) are presented in table 1 while the chromatogram is presented in figure 3. The following are some of the phytoconstituents present in the GC-MS analysis of fruit of *Acacia nilotica*; 4-Hydroxy-2-methylacetophenone, Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate, 9-Octadecenoic acid, methyl ester, Dodecanoic acid, 1-methylethyl ester, Octadecanoic acid, cis-1,4-Cyclohexanediamine, N,N'-diacetyl, Dodecanoic acid, methyl ester, 13-Docosenoic acid, methyl ester, Methyl tetradecanoate, Pentadecanoic acid, 14-methyl-, methyl ester, Methyl stearate, 9-Eicosenoic acid, (Z)-, Linoelaidic acid.

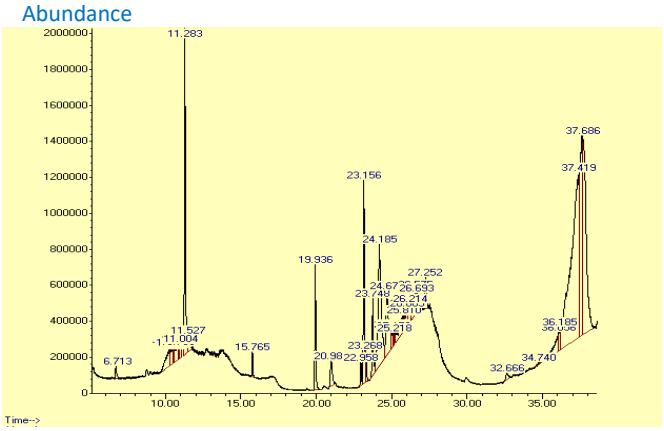

Figure 3: GC-MS chromatogram of ethanolic extract of fruit of *Acacia nilotica*

Table 2: GC-MS Phytochemical Profile of Ethanolic Extract of Flower of *Calotropis procera*

S/N	Compound name	Retention time (min)	Area %
1	Pentanoic acid, 4-methyl-, methyl ester	6.71	0.30
2	1,2,3-Benzenetriol	10.26	1.30
3	2-Pyridinemethanamine	10.86	0.17

4	Pyrazine, (methylthio)-	11.00	0.52
5	Dodecanoic acid, methyl ester	11.28	7.95
6	Pyrazole-5-carboxylic acid, 3-methyl-	11.52	0.53
7	Methyl tetradecanoate	15.76	0.49
8	Pentadecanoic acid, 14-methyl-, methyl ester	19.93	2.66
9	n-Hexadecanoic acid	20.98	1.08
10	Methyl 10-trans,12-cis-octadecadienoate	22.95	0.47
11	9-Octadecenoic acid (Z)-, methyl ester	23.15	4.56
12	Methyl stearate	23.74	1.75
13	Cis-13-Octadecenoic acid	24.18	7.85
14	octadecanoic acid	24.67	3.73
15	cis-Vaccenic acid	24.95	0.65
16	2-Methyl-Z,Z-3,13-octadecadienol	25.15	0.34
17	Cyclopentadecanone, 2-hydroxy-	25.73	1.55
18	Palmitoleic acid	26.06	0.78
19	E-11-Hexadecenal	26.21	0.74
20	9,12-Octadecadienoyl chloride, (Z, Z) -	26.57	1.75
21	Tridecanoic acid, methyl ester	27.25	0.89
22	Oleic Acid	36.05	1.68
23	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	36.18	0.79
24	9-Octadecanoic acid (Z)-, 2,3-dihydroxypropyl ester	37.41	27.72
25	9-Methyl-Z-10-tetradecen-1-ol acetate	37.68	14.01

The GC-MS phytochemical analysis of ethanolic extracts of flowers of *Calotropis procera* revealed a total of 25 compounds were found showing various phytochemical activities. The phytochemical constituents with their retention time (RT) and area (%) are presented in table 2 while the chromatogram is presented in figure 4. The following are some of the phytoconstituents present in the GC-MS analysis of flowers of *Calotropis procera*; Cyclopentadecanone, 2-hydroxy-, 9-Methyl-Z-10-tetradecen-1-ol acetate, 9-Octadecanoic acid (Z)-, 2,3-dihydroxypropyl ester, 9,12-Octadecadienoyl chloride, (Z, Z) -, Oleic Acid, Tridecanoic acid, methyl ester, Palmitoleic acid, Methyl stearate, 9-Octadecenoic acid (Z)-, methyl ester, Pentanoic acid, 4-methyl-, methyl ester, Dodecanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-, methyl ester, n-Hexadecanoic acid.

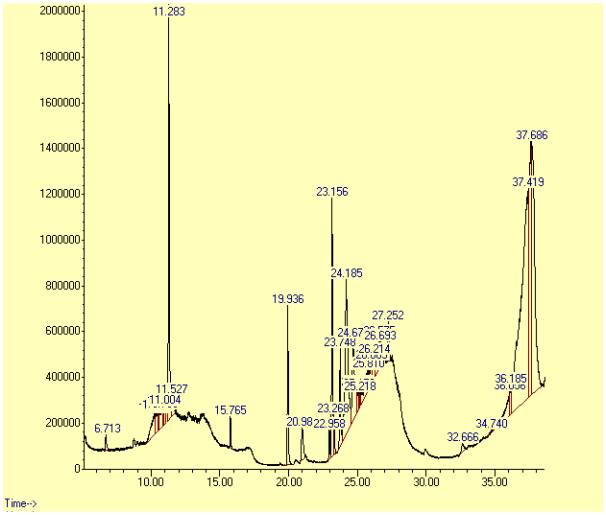

Figure 4: GC-MS chromatogram of ethanolic extract of flower of *Calotropis procera*

Discussion

The phytochemical constituents of both plant parts (fruits of *Acacia nilotica* and flowers of *Calotropis procera*) were analysed using a GC-MS. The phytochemical analysis of the ethanolic extracts of both plant parts showed the presence of various compounds in which most of them have been reported for various properties and functions.

In fruits extract of *Acacia nilotica*, the most abundant compound, 9-octadecanoic acid, (Z)2,3-hydroxypropyl ester (17.04%) belongs to the family of mono acylglycerols and is used as a food additive and also possess antimicrobial and anticancer activities (Sunita and Ganesh, 2017). Dodecanoic acid is a food additive (flavouring agents). Tetradecanoic acid, ethyl ester is used as flavouring agent and has been reported to possess hypercholesterolemic activity by increasing LDL-cholesterol (Imo *et al*., 2018). Linoelaidic acid is a geometric isomer of linoleic acid and it is useful in oil paints, it is also used in beauty products industry because of its benefits on the skin and its consumption is associated with lowering cardiovascular disease. Hexadecanoic acid, methyl ester has wide applications in food, syrups and perfume industries (Ezekwe *et al*., 2021). Tridecanoic acid is important in the food industry as a flavouring agent or adjuvant. Methyl stearate is used as a food additive and in the production of cosmetics and soaps. Methyl tetradecanoate is a food additive permitted for direct addition to food for human consumption as synthetic flavouring substances (Ezekwe *et al*., 2021). The presence of these phytochemicals in the ethanolic extract of fruits of *Acacia nilotica* is an indication that the fruit may be useful in the food industry.

n-hexadecanoic acid has been reported to have anti-inflammatory, antioxidant, hypocholesterolemic and antibacterial activities (Mustapha and Runner, 2016) and is also used in personal care and cosmetics (Kavitha, 2021). Tetradecanoic acid has surfactant properties and is also used in beauty and cosmetic industries (Ezekwe *et al*., 2021). 9,12-Octadecadienoic acid (Z, Z)-, methyl ester was reported to have anti-inflammatory, anti-arthritic, antiacne, anti-histaminic, anti-eczemic, anti-androgenic, anti-coronary, anti-cancer, antihypercholesterolemic and 5-Alpha reductase inhibitor activities (Kavitha, 2021). Oleic acid is a fatty acid that occurs naturally in animals and plants and it is used as a component of normal human diet and a major component of soap as an emulsifying agent. 9-Eicosenoic acid is involved in the process of lipid metabolism and plays a role in energy storage and as a membrane stabilizer. Phytol was reported to have antioxidant, neuroprotective, antimicrobial, anticancer, anti-inflammatory, and anti-diuretic activities (Njoku *et al*., 2021). Nonanoic acid, methyl ester is used in agricultural veterinary products and also used in therapeutic goods and fragrances.

Octadecanoic acid (also known as stearic acid) was reported to have antimicrobial, anti-inflammatory, hepatoprotective, hypercholesterolemic and anticancer activity (Sirigiri and Kandru, 2017). Pentadecanoic acid is an essential fatty acid that have clinical benefits in stem chronic cardiometabolic, liver and inflammatory diseases (Doman *et al*., 2021). Srivastva *et al*. (2021) reported that the fruits of *Acacia nilotica* exhibits antibacterial activity and inhibits the growth of some β-lactamase producing bacteria. The presence of these phytochemicals in the extract used in this present study suggests that the constituents of ethanolic extract of fruit of *Acacia nilotica* could exhibit some medicinal properties.

The phytoconstituents found in the ethanolic extract of flower of *Calotropis procera* includes Methyl tetradecanoate reported to have wide usage in the food industry as a flavouring agent and methyl stearate which is an antifoaming agent and fermentation ingredient. Octadecanoic acid earlier discussed is also found in ethanolic extract of flower of *Calotropis procera*. In clinical studies, it has been reported to be associated with lowered LDL cholesterol (Imo *et al*., 2018). Oleic acid has been reported to have anti-inflammatory, anti-oxidant and antimicrobial activities (Imo *et al*., 2018). 9-Octadecenoic acid (Z)-, methyl ester has been shown to have anti-carcinogenic activity (Raginee *et al*., 2013).

The result of the GC-MS analysis showed that the ethanolic extract of both plant parts may be utilized in clinical studies for their antimicrobial, anticancer, anti-inflammatory and antioxidant activities. They may also be useful as raw materials in food industries.

Conclusion

The phytochemical results of both ethanolic extracts of fruits of *Acacia nilotica* and flowers of *Calotropis procera* showed that the plant parts possess a lot of phytoconstituents that can be useful in medicinal, pharmaceutical and food industries.

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