

LEAF ESSENTIAL OIL OF *SENNA ALATA* LINN FROM SOUTH EAST NIGERIA AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

The chemical constituents of the essential oil (extracted with isopropanol) of the leaves of *Senna alata* Linn were characterized using Gas Chromatography-Mass Spectrometry (GC/MS) technique and seven compounds were identified which include (6Z)-7,11-dimethyl-3-methylidenedodeca-1,6,10-triene (2.42%), 4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene (3.80%), 4,4,7a-trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one (2.91 %), 3,7-dimethylocta-1,6-diene (3.94%), hexadecanoic acid methyl ester (8.59%), hexadecanoic acid (3.31%) and octadecanoic acid methyl ester (75.03%). The extract exhibited marked antimicrobial activity against *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* and *Proteus mirabilis*. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique. The presence of these compounds in the leaves of *S. alata* might be responsible for its antimicrobial activity as well as its use in the treatment of dermal diseases and other infections in herbal medicine in Nigeria.

Keywords: *Senna alata* Linn, Chemical constituents, GC/MS analysis, Essential oil.

INTRODUCTION

The dependency of man on plant resources for the treatment and management of health problems has inestimably gained recognition. The use of plant extracts in form of concoction, decoction and infusion to treat a variety of diseases and infections has received tremendous appreciation and has been locally testified to be efficacious against certain traditional health challenges that have shown resistance to orthodox medicine. Demands of traditional herbal medicines are increasing by the day by both the developing and the developed countries of the world¹. The demand is due to the increased acceptance of traditional herbal medicines as a result of their absence of side effects. Studies on the ethno-medicinal uses of our wild medicinal plants and investigations regarding enhanced productivity of medicinal plants is one of the frontier areas of modern research¹.

S. alata is a shrub that belongs to the family *Fabaceae*. It is widely distributed in the tropical countries and is native to South America, but has been planted widely for medicinal and ornamental purposes and is now pan-tropical.

In many countries, including most countries of tropical Africa, it has become naturalized and is often considered a weed². The shrub stands 3–4 m tall, with leaves 50–80 cm long. The inflorescence looks like a yellow candle. The fruit, shaped like a straight pod is up to 25 cm long. Its seeds are distributed by water or animals³. The seed pods are nearly straight, dark brown or nearly black, about 15 cm long and 15 mm wide. On both sides of the pods there is a wing that runs the length of the pod. Pods contain 50 to 60 flattened, triangular seeds³. *S. alata* is often called the Ringworm Bush because of its very effective fungicidal properties, for treating ringworm and other fungal infections of the skin. The leaves are ground in a mortar to obtain a kind of "green cotton wool". This is mixed with the same amount of vegetable oil and then rubbed on the affected area 2-3 times a day³.

S. alata has been reported to have very high medicinal values like –antimicrobial property particularly against fungal dermatophytes and traditionally being used in the treatment of skin infections in man^{1,4,5}. Leaf extract is also credited for the treatment of constipation,

inguinal hernia, intestinal parasitosis, syphilis and diabetes^{1,6,7,8}. Leaf extract is a good antioxidant¹. The juice of the fresh leaf of *S. alata* is universally recognised as a remedy for parasitic skin diseases, and is used in many eruptive and pustular skin infections by simply rubbing the crushed leaves alone or mixed with lime juice or oil⁹. In Sierra Leone, the leaves in form of an infusion are used as a purgative, and a strong decoction is sometimes given as an abortifacient or during labour to hasten delivery. The juice expressed from the fresh leaves is taken along with lime juice for worms⁹. The bark has been recommended as a tanning material. The juice of the root is rubbed into cuts for tattooing or tribal markings. The plant is highly decorative and of unusual and interesting appearance⁹. The leaves of *S. alata* have been reported to be useful in treating convulsion, gonorrhoea, heart failure, abdominal pains, oedema and it's also used as a purgative^{10,11}. It has been observed that antimicrobial activity of the plants is associated with the presence of some chemical components such as phenols, tannins, saponins, alkaloids, steroids, flavonoids and carbohydrates¹¹. Skin problems treated with *S. alata* include ringworm, favus and other mycoses, impetigo, syphilis sores, psoriasis, herpes, chronic lichen planus, scabies, shingles, eczema, rash and itching. In veterinary medicine too, a range of skin problems in livestock is treated with leaf decoctions. Such decoctions are also used against external parasites such as mites and ticks². Other ailments treated in tropical Africa with *S. alata* include stomach pain during pregnancy, dysentery, haemorrhoids, blood in the urine (schistosomiasis, gonorrhoea), jaundice, headache, hernia, one-sided weakness or paralysis². In this study, the leaf essential oil of *S. alata* from South East Nigeria is probed for its chemical constituents and antimicrobial activity with a view to substantiate claims of its efficacy as an antimicrobial agent.

MATERIALS AND METHODS

Plant Materials

S. alata leaves were collected from the compound of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, where it was planted as ornamental plant. Identification and authentication were done at the Taxonomy Section of Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, where a herbarium specimen is on file with voucher number FHI 137631,14701. The leaves were then dried under a shade and

thereafter milled into a uniform and fine powder by a mechanically driven attrition mill.

Extraction of Plant Materials

The powdered leaves (500 g) were successfully extracted with 2 L of ethanol (8h /3 times/30°C). The extract was concentrated under reduced pressure and the supernatant extract was decanted (9.48g) after complete removal of the solvent. The extract was centrifuged at 10,000 rpm for 20 min and the clear supernatant extract was subjected to systematic GC/MS analysis¹².

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

GC analysis was carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm x 50 m) and the conditions were as follows: temperature programming from 60-280°C held at 60°C for 1 min, and at 160°C for 2 min (rate 10°C/min), at 220°C for 2 min (rate 10°C/min) and finally at 280°C for another 2 min (rate 10°C/min). The injection temperature was 220°C. GC/MS analysis was conducted using GCMS-QP 2010 Plus Shimadzu Japan with column oven temperature of 60°C. The carrier gas was Helium with a pressure of 100.2 Kpa and linear velocity of 46.3 cm/s. Total flow was 20.7 mL/min, column flow was 1.61 mL/min, injection mode was split, flow control mode was linear velocity, purge flow was 3.0 mL/min and split ratio was 10.0. Also, ion source temperature was 200°C, interface temperature was 250°C, solvent cut time was 2.5 min., detector gain was 0.00 KV, detector gain mode was relative and the threshold was 1000. For the mass spec., start time was 3.0 min., end time was 27.0 min, event time was 0.5 s, scan speed was 1250, and start m/z was 50 while end m/z was 600. The mass spectrum was also equipped with a computer fed mass spectra data bank. Hermle Z 233 M-Z centrifuge, Germany, was used. All solvents used were of analytical grade and were procured from Merck, Germany.

Components Identification

The components of the extract were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature^{13,14}.

Antimicrobial Activity

The *in vitro* antimicrobial activity of the leaf essential oil of *S. alata* was carried out for 24 h

culture of four selected microorganisms. The organisms used were *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli* and *Proteus mirabilis*. All the test organisms were clinical isolates of human pathogens obtained from stock cultures at the Federal Medical Centre, Umuahia, Abia State, Nigeria. With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminium foil and sterilized in an autoclave at 121°C for 15 min. They were however, used within 48 h of production. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique^{15,16}. A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the inoculum was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the extract bearing paper discs was carefully placed on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 h in an incubator

at 37°C. They were examined daily for growth and for the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimetre rule. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of the extract having different zones and selecting the lowest concentration. Gentamycin was used as a standard antimicrobial agent.

RESULTS AND DISCUSSION

The isopropanolic extract of *S. alata* leaves showed seven peaks from the chromatogram of the extract (Fig. 1). These peaks indicated the presence of seven compounds in the extract (1-7, in Fig. 2). The nomenclature, molecular formula, molecular weight, retention time and percentage peak area of these compounds are shown in Table 1. The chemical constituents comprise sesquiterpene (6.22 %), monoterpene ketone (2.91 %), monoterpene (3.94 %) and fatty acid ester (86.93 %). Out of the seven constituents analysed in the leaf extract of *S. alata*, four are terpene compounds. Hundreds of essential oils were used as perfumes, flavourings and medicines for centuries before Chemistry was capable of studying the mixtures¹⁷.

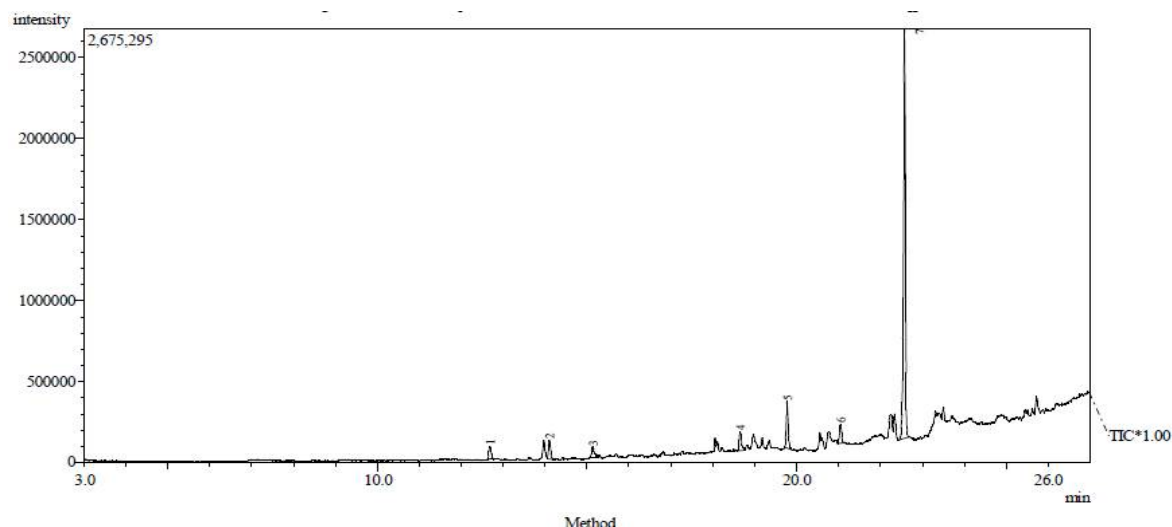


Fig. 1: GC/MS chromatogram of essential oil of *S. Alata* Linn

Table 1: Phytochemicals identified from the GC/MS analysis of the leaf essential oil of *S. alata* Linn

| Chromatogram peak | Compound name | Molecular formula | Molecular weight | Retention time(min) | Peak area (%) | Nature of compound |
|-------------------|---|--|------------------|---------------------|---------------|---------------------|
| 1 | (6z)-7,11-dimethyl-3-methylidenedodeca-1,6,10-triene | C ₁₅ H ₂₄ | 204 | 12.683 | 2.42 | sesquiterpene |
| 2 | 4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene | C ₁₅ H ₂₄ | 204 | 14.102 | 3.80 | Sesquiterpene |
| 3 | 4,4,7a-trimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2(4H)-one | C ₁₁ H ₁₆ O ₂ | 180 | 15.138 | 2.91 | monoterpene lactone |
| 4 | 3,7-dimethylocta-1,6-diene | C ₁₀ H ₁₈ | 138 | 18.653 | 3.94 | Monoterpene |
| 5 | hexadecanoic acid methyl ester | C ₁₇ H ₃₄ O ₂ | 270 | 19.774 | 8.59 | Fatty acid ester |
| 6 | hexadecanoic acid | C ₁₆ H ₃₂ O ₂ | 256 | 21.051 | 3.31 | Fatty acid |
| 7 | octadecanoic acid methyl ester | C ₁₉ H ₃₈ O ₂ | 298 | 22.578 | 75.03 | Fatty acid ester |

Terpenes are a diverse family of compounds with carbon skeletons composed of five-carbon isopentyl (isoprene) units. Terpenes are commonly isolated from the essential oils of plants. They often have pleasant tastes or aromas, and they are widely used as flavourings, deodorants and medicines¹⁷. Terpenes are basic hydrocarbons, whereas terpenoids contain extra functional groups that could be comprised of a range of chemical elements. However, it is common for the term 'terpene' to also include terpenoids in many existing writings¹⁸. Compound **3** is a terpenoid. Terpenes are major constituents of plant resin and essential oils extracted from such plants¹⁸. Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites¹⁹. In nature, essential oils play an important role in the protection of plants as antibacterial, antivirals, antifungals, insecticides and also against herbivores by reducing their appetite for such plants. They may also attract some insects, thereby favouring the dispersion of pollens and seeds, or repel other undesirable insects [19]. Medical professionals are more interested in the medicinal properties of essential oils. Many oils show antibacterial, fungicidal, relaxant, stimulating, antidepressant effect and can be very effective therapeutic agent. Essential oils are known for their therapeutic properties hence, used in the treatment of various infections caused by both pathogenic and non-pathogenic diseases. Pathogenic diseases caused by bacterial, virus, and the fungi can be treated with essential oils²⁰. Compounds 1, 2 and 4 are hydrocarbons. The majority of essential oils fall into this category; these contain molecules of hydrogen and carbon only and are classified into terpenes. Compounds 1 and 2 are

sesquiterpenes while compound 4 is a monoterpene. These compounds have been associated with various therapeutic activities which include being used as stimulants, antiviral, antitumor, decongestants, and antibacterial as well as hepatoprotective agents^{21,22,23}. Compound 3 is a lactone essential oil. Lactones are of relatively high molecular weight and are usually found in pressed oils. They may be used for antipyretic, sedative and hypotensive purposes, but their contraindication is allergy, especially such involving the skin. They are also used as antimicrobial, antiviral and analgesic agents^{21,22,24}. Biologically, the monoterpenes have been found to possess a variety of biological effects, including antibacterial, sedative, antitumor, cytotoxic, anti-inflammatory, insecticidal, molluscicidal and others. They have historically been important ingredients individually or as constituents of volatile (essential) oils in medicinal and economic products including cosmetics and other fragrant products²⁵. A number of dietary monoterpenes have antitumor activity, exhibiting not only the ability to prevent the formation or progression of cancer, but the ability to regress existing malignant tumors^{26,27}. Many plant sesquiterpenes have been shown to be effective against the causal agent of tuberculosis²⁶. Compound 1 is also called β -farnesene. The term farnesene refers to a set of six closely related chemical compounds which all are sesquiterpenes. α -Farnesene and β -farnesene are isomers, differing by the location of one double bond. α -Farnesene is 3,7,11-trimethyl-1,3,6,10-dodecatetraene and β -farnesene is 7,11-dimethyl-3-methylene-1,6,10-dodecatriene. The alpha form can exist as four stereoisomers that differ about the geometry of two of its three internal double

bonds (the stereoisomers of the third internal double bond are identical). The beta isomer exists as two stereoisomers about the geometry of its central double bond²⁸. Compound 2 is otherwise known as alpha-selinene which belongs to a group of closely related isomeric chemical compounds which are classified as sesquiterpenes. The

selinenes all have the molecular formula $C_{15}H_{24}$ and they have been isolated from a variety of plant sources. α -Selinene and β -selinene are the most common and are two of the principal components of the oil from celery seeds²⁹. γ -Selinene and δ -selinene are less common³⁰.

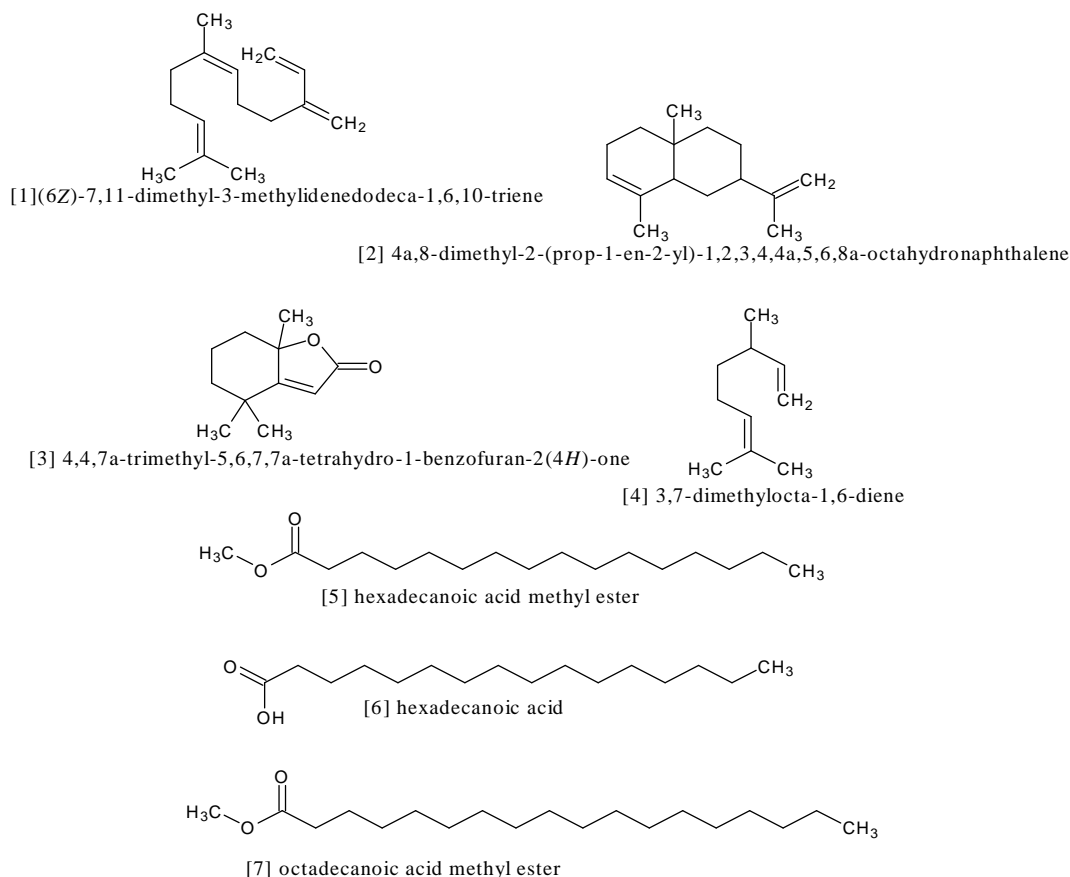


Fig. 2: Structures of phytochemicals from the leaf essential oil of *S. alata* Linn.

Table 2: Antimicrobial activity of the leaf extract of *S. alata* Linn

| Test Microorganism | Concentration (%) | | | | |
|-------------------------------|-------------------|-------|-------|-------|---------|
| | 25 | 50 | 75 | 100 | MIC (%) |
| <i>Staphylococcus aureus</i> | 9.55 | 12.63 | 16.35 | 19.33 | 25 |
| <i>Streptococcus faecalis</i> | 8.85 | 12.23 | 15.57 | 17.33 | 25 |
| <i>Escherichia coli</i> | 8.81 | 11.63 | 13.33 | 15.97 | 25 |
| <i>Proteus mirabilis</i> | 6.83 | 9.91 | 13.63 | 16.22 | 25 |

Figures are in mm and include the diameter of the paper disc (5 mm).

Data are means of triplicate determinations.

MIC = Minimum Inhibitory Concentration

Gentamycin which was used as a standard antimicrobial agent showed the highest inhibitory effect against all the microorganisms with inhibition zone of 37 mm for *S. aureus*, 43 mm for *S. faecalis*, 39 mm for *E. coli* and 35 mm for *P. mirabilis*, all at 100 % concentration.

The extract showed marked antibacterial activity against the pathogens and this could be the reason why the plant is used in dermal infections. So far, there is no study that can give a clear idea and be accurate on the mode of action of the essential oils. Given the

complexity of their chemical composition, everything suggests that this mode of action is complex, and it is difficult to identify the molecular pathway of action. It is very likely that each of the constituents of the essential oils has its own mechanism of action²¹. Because of the variability of amounts and profiles of the components of essential oils, it is likely that their antimicrobial activity is not due to a single mechanism, but to several sites of action at the cellular level. Then, different modes of action are involved in the antimicrobial activity of essential oils. One of the possibilities for action is the generation of irreversible damage to the membrane of bacterial cells, that induce material losses (cytoplasmic), leakage of ions, loss of energy substrate (glucose, ATP), leading directly to the lysis of bacteria (cytolysis) and therefore to its death. Another possibility of action is inhibition of production of amylase and protease which stop the toxin production, electron flow and result in coagulation of the cell content^{21,31,32,33,34}.

CONCLUSION

The GC-MS results of the isopropanolic leaf extract of *S. alata* have revealed some of the chemical components of the leaves of the plant. The strong antimicrobial activities exhibited by the extract are attributed to the presence of hydrocarbon sesquiterpenes and monoterpene as well as the monoterpene lactone by way of their synergistic effects. These investigations provide supporting evidence to the use of the leaves of *S. alata* in herbal medicine in Nigeria for the treatment of dermal infections, syphilis, gonorrhoea, heart failure, abdominal pains, oedema, dysentery and weakness. Some of the numerous medicinal importance of monoterpenes and sesquiterpenes have been highlighted which show that the plant could possess antioxidant, anti-inflammatory, antiviral, antitumor, sedative, insecticidal, anti-tuberculosis and molluscicidal properties. *S. alata* is indeed an asset.

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