Chemical constituents of leaves of *Senna singueana* (Del.) Lock

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**Abstract**

We report the preliminary phytochemical screening and GC-MS analysis of leaves of *Senna singueana* (Del.) Lock. This plant is endemic to East and West Africa, in particular Ethiopia and Cote D’Ivoire. The dry powdered leaf of *Senna singueana* was extracted with ethanol to give the crude extract. The chemical constituents of the crude extracts of the leaves of *Senna singueana* (Del.) Lock collected from Kashere, Gombe State – Nigeria were analyzed using GCMS spectrometry and preliminary phytochemical screening. The mass spectra of the constituents present in the extracts were interpreted by the comparison with data library of the National Institute of Standard and Technology (NIST) having over 62,000 patterns. The GCMS results indicated the presence of eighteen compounds including, 3-tridecane, 1,3-benzenediol, 3-O-methyl-D-glucose, (Z)-13-docosenoic acid, Z-7-tetradecanal and 2,6-dimethyl-1,7-octadien-3-ol which are in good agreement with the results of the phytochemical investigation of the ethanolic extract of the leaves, indicating the presence of steroids, alkaloids, saponins, phenols and reducing sugars. The presence of these phytoconstituents may be responsible for the pharmacological properties of *Senna singueana* as they are recommended as a plant of phytotherapeutically significant.

**Keywords:** Ethanolic extract, GC-MS analysis, *Senna singueana*, phytochemical screening

**Introduction**

The genus *Senna* with about 600 species is one of the largest genera belonging to the Fabaceae and formally included in the genus *Cassia* (Kolawole, 2017) [11, 13]. It comprises of shrubs, trees and is widely spread in the tropics (Mabberley, 2008) [16]. *Senna singueana* is a tropical plant commonly found in the East and West Africa belonging to the family Fabaceae, sub-family Caesalpinioideae, tribe Cassiae (Hibsen et al., 2016) [18] and subtribe Cassinae (Kolawole and Abdulrahaman, 2019) [12]. It is called Rumfu in Hausa language (Gibile, 2018; Kolawole, 2017) [11, 13]. Several phytochemicals have previously been reported from the genus *Senna* including flavonoids, anthraquinones, stilbenes, terpenes, alkaloids, and proanthocyanidins (Mondal, 2014). However, very few studies have been conducted on *Senna singueana*. The root and stem bark extract of *Senna singueana* have been used for treatment of many ailments and diseases across many African countries ranging from skin cancer in Ethiopia (Hibsen, 2012) [7], liver diseases in Egypt (Sibeh et al., 2017), treatment of diabetes in Nigeria (Mukhtar et al., 2020), malaria in Ethiopia (Hibsen, 2012) [7] and as a pain killer in Malawi (Kariuki et al., 2012) [9]. Other traditional uses of the plant are in the treatment of convulsion, inflammatory conditions, gonorrhea, constipation and heartburn (Ezuruike and Prieto, 2014) [2]. GC-MS method of analysis is an interesting tool for testing amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry, environmental and forensic applications (Uma et al., 2009) [13]. GC-MS method of analysis also combines two analytical techniques to a single method of analysing mixtures of chemical compounds (Gomathi et al., 2015). Gas chromatography separates the components of the mixture and mass spectrometry analyses of each of the individual components. In the continuation of our study of the Nigerian medicinal plants as a source of antimicrobial and anti-infective agents, we decided to conduct preliminary phytochemical GCMS analysis as a prelude to further investigation on the search for active constituents of the plant. The preliminary findings are reported in this study.

**Materials and Methods**

**Plant material**

The leaf of *Senna singueana* (Rumfu) was collected from the forest of Kashere, Akko LGA of Gombe State – Nigeria in August 2017, by Dr Kolawole O. Saheed.
It was authenticated at the Federal University of Kashere (FUKH), and a voucher specimen (No: FUKH 0202) was deposited at the University herbarium. The collected leaf was air-dried and powdered using a mechanical grinder.

**Extraction**
The air-dried and powdered leaf of *Senna singueana* (1kg) were macerated 2X using ethanol for 48 hours and 24 hours, respectively. It was then evaporated to afford the crude extract (50.60g).

**Phytochemical analysis**
The crude extract was screened for phytochemicals according to the standard methods Trease and Evans (2000) and Kolawole et al. (2017) [11, 13] to determine the classes of phytochemicals present in the crude extract.

**GC-MS Analysis**
The GCMS spectra were recorded on a GC-MS equipment (Shimadzu, Japan) GCMS QP 2010 PLUS fused with a GC Column (AOC 2i) coated with polymethyl silicon with a dimension of 0.25 mm X 30 mm. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as one ml/min. The ionization voltage was 70eV.

**Identification of phytocompounds**
Interpretation on Mass-Spectrum GC-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained and presented in Table 2.

**Results and Discussion**
The results of the phytochemical investigations of the crude ethanol extract of the leaf of *S. singueana* indicated the presence of a wide range of classes of natural products which includes, steroids, alkaloids, saponins, phenols and reducing sugar (Table 1), but no tannins, glycosides, resins and flavonoids. GC-MS is the best technique used to identify the constituents of volatile matter, long chain, branched chain hydrocarbon, alcoholic acids and others. Peak area, retention times and molecular formula are used for the confirmation of phytochemical constituents. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Table 2. GC-MS analysis of ethanolic extract of *Senna singueana* leaves revealed the existence of (Z)-13-docosenoic acid (20.195%); Z-7-tetradecanal (25.733%); 1,5-cyclododecadiene, (Z,Z)- (23.567%); squalene (26.582%); 3-O-methyl-D-glucose (15.713%); hexadecanoic acid, 2,3-dihydroxypropyl ester (24.104%) (Table 2).

It is of very importance to identify the possible roles of these constituents compound in the curative claims attributed to leaves of *Senna singueana* by Traditional Medical Practitioners. Our study on active constituents of Ethanolic extract of *Senna singueana* revealed that the plants contained a wide range of GCMS chemical phytocompounds which may have contributed to its therapeutic value.

Tridecane is a flammable colourless liquid. In industry, they have no specific value aside from being components of various fuels and solvents. 1-Butanol-3-methyl acetate, isoamyl acetate or isopentyl acetate, belongs to the class of organic compounds known as carboxylic acid esters. Isoamyl acetate is the ester formed from isoamyl alcohol and acetic acid. It is a colourless liquid that is only slightly soluble in water, but very soluble in most organic solvents. Isoamyl acetate has a strong odour (just like Juicy Fruit or a pear drop) which is also described as similar to both banana and pear. In addition to its role as a flavouring or aroma agent in foods and perfumes, isoamyl acetate is released by a honey bee's sting where it serves as a pheromone to attract other bees and provoke them to sting.

Also reported in the ethanolic extract of of leaves of *Senna singueana* is the triterpene, squalene (Figure 2r). It serves as a precursor for the production of secondary metabolites like vitamins, hormones and sterols (Lozano-Grande et al., 2018) [15]. It is a source of carbon for microorganisms in aerobic and anaerobic fermentation (Ghimire et al., 2016) [5]. It had been reported in previous studies to be present in several plant parts such as seed oil of *Carica papaya*, leaf of *Bidens pilosa*, seed oil of *Glycine max*, seed oil of *Cocos nucifera*, olive oil, rice, grape seed oil, peanut, corn and amaranth (Duke, 1992; Lozano-Grande et al., 2018) [15, 1]. It has therapeutic potentials such as antibacterial, antifungal, antitumor, immunostimulant, cardioprotector, antioxidant, chemopreventive and lipoxigenase, anticancer as well as detoxifying (Smith, 2000; Liu et al., 2009; Farvin et al. 2006; Lozano-Grande et al., 2018) [19, 14, 3, 15]. It also has relevance in the cosmetic industries for the production of emollients and moisturizers (Kelly, 1999; Lozano-Grande et al., 2018) [10, 15].

Medicinal plants are used in traditional treatment to cure diseases of humans (Hariprasad and Ramakrishnan, 2011) [6]. Various scientific studies reported the analgesic, antiviral; antimarialar, antibacterial and antifeertility activity (Vishnukanta, 2008; Sen and Batra, 2011; Gani et al., 2019) [22, 18, 4]. Plants based phytocompounds have enormous therapeutic potential as they can serve the purpose with lesser side effects.

**Conclusion**
GC-MS analysis of the ethanolic extract of leaves of *Senna singueana* reveals the presence of medicinally highly complex profile containing approximately 18 components. From this study it can be concluded that the *Senna singueana* may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds.
Fig 1: GC-MS Chromatogram of ethanolic leaf extract of *Senna singueana*

Table 1: Phytochemical analysis of leaves of *Senna singueana*

| Phytochemical       | Inferences |
|---------------------|------------|
| Tannins             | -          |
| Steroids            | +          |
| Alkaloids           | +          |
| Saponins            | +          |
| Glycosides          | -          |
| Resins              | -          |
| Phenols             | +          |
| Reducing sugars     | +          |
| Flavonoids          | -          |

Key: present= +; Absent= -

Table 2: Some phytocomponents identified from ethanolic extracts of *Senna singueana* leaves

| No | RT (Min) | Name of Compound                              | Molecular formula | Molecular Weight | Peak Area % |
|----|----------|-----------------------------------------------|-------------------|------------------|-------------|
| 1  | 3.894    | 3-Tridecene                                    | C₁₃H₂₆O₂           | 182              | 0.91        |
| 2  | 6.184    | 1-Butanol, 3-metyl, acetate                    | C₇H₁₄O₂            | 130              | 2.61        |
| 3  | 7.700    | 2,3-Dihydrobenzofuran                          | C₉H₁₀O            | 120              | 1.61        |
| 4  | 9.287    | 1,3-Benzenediol                                | C₇H₁₀O₂           | 110              | 19.02       |
| 5  | 13.051   | alpha-D-Glucopyranoside, methyl                | C₇H₁₀O₂           | 194              | 7.55        |
| 6  | 15.713   | 3-O-Methyl-d-glucose                           | C₉H₁₄O₂           | 194              | 14.50       |
| 7  | 17.069   | n-Nonanoic acid                                | C₉H₁₈O          | 158              | 6.44        |
| 8  | 18.925   | 7,10-Hexadecadienoic acid, methyl ester       | C₁₁H₂₂O₂          | 266              | 1.41        |
| 9  | 18.982   | Methyl 11-(3-Pentyl-2-oxiranyl) undecanoate    | C₁₀H₁₆O₂          | 312              | 4.09        |
| 10 | 19.624   | 2,6-Dimethyl-1,7-octadien-3-ol                 | C₁₀H₁₆O          | 210              | 4.28        |
| 11 | 20.195   | (Z)-13-Docosenoic acid                         | C₁₂H₂₂O₂          | 338              | 24.85       |
| 12 | 21.699   | 1,4-Diethyloctyl trifluoroacetate              | C₁₄H₂₆F₂O₂        | 282              | 0.72        |
| 13 | 23.111   | Z-7-Tetradecanal                               | C₁₄H₂₈O          | 210              | 0.29        |
| 14 | 23.567   | 1,5-Cyclododecadiene, (Z,Z)-                   | C₁₂H₂₀O          | 164              | 3.12        |
| 15 | 23.782   | Diureidoacetic acid                            | C₁₂H₁₉NO₄         | 176              | 0.61        |
| 16 | 24.104   | Hexadecanoic acid, 2,3-dihydroxypropyl ester   | C₁₀H₁₆O₄          | 330              | 2.10        |
| 17 | 25.733   | Z-7-Tetradecanal                               | C₁₄H₂₈O          | 210              | 8.12        |
| 18 | 26.582   | Squalene                                       | C₃₀H₅₀            | 410              | 0.78        |
a. 3- Tridecane
b. 1-Butanol, 3-methyl, acetate
c. 2,3-Dihydrobenzofuran
d. 1,3- Benzenediol
e. alpha-D-Glucopyranoside, methyl
f. 3-O-Methyl-d-glucose
g. n-Nonanoic acid
h. 7,10-Hexadecadienoic acid, methyl ester
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