Evaluation of Phytochemical Compounds in *Senna siamea stembark* Powders for Insect Pest Control

Kashere, M. A[1]., Sabo,M[2]., Tijjani, A[2]. and Aliyu, M[2].,

[1]Department of Agricultural Technology, Federal College of Horticulture P.M.B 108, DadinKowa, Gombe, Gombe State, Nigeria.

[2]Department of Crop Production, Faculty of Agriculture and Agricultural Technology, AbubakarTafawaBalewa University P.M.B. 0248, Bauchi, Nigeria.

Corresponding Author: Muhammad Abdullahi KASHERE, Department of Agricultural Technology, Federal College of Horticulture P.M.B 108, DadinKowa, Gombe, Gombe State, Nigeria. **+2349056666317; mkashere@gmail.com.**

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ABSTRACT

A laboratory screening was conducted to evaluate the phytochemical compounds in Senna siamea stembark powders and their insecticidal activity on Callosobruchus subinnotatus PIC on stored Bambara groundnut through untargeted Gas Chromatography – Mass Spectrometry (GC-MS) using three different extraction solvents (ethanol, methanol and distilled water). To mention but few, the ethanol extracts revealed Octanoic acid, 4,6-dimethyl-, methyl ester, (4S,6S)-(+)- ($C_{11}H_{22}O_2$); cis-Vaccenic acid ($C_{19}H_{36}O_2$); Z,Z-10,12-Hexadecadien-1-ol acetate ($C_{18}H_{32}O_2$); 2-Methyl-Z,Z-3,13-octadecadienol ($C_{19}H_{36}O$). Methanol extracts showed Oleic Acid ($C_{18}H_{34}O_2$); Cyclononasiloxane, octadecamethyl- ($C_{18}H_{54}O_9Si_9$); Cyclodecasiloxane, eicosamethyl- ($C_{20}H_{60}O_{10}Si_{10}$); 2-Methyl-Z,Z-3,13-octadecadienol ($C_{19}H_{36}O$). Distilled water extraction revealed cis-Vaccenic acid ($C_{19}H_{36}O_2$); 1,2-Benzisothiazole,3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide ($C_{13}H_{16}N_2O_2S$); Oleic Acid ($C_{18}H_{34}O_2$); 11-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)as compounds with the highest percentage area. Phytochemicals have been used for many years to control insect pest damage in agricultural crops. Pyretre, Nicotine, Rotenone and tobacco have long been used as control agents against insects in some regions of sub-Saharan Africa. Unlike synthetic chemical insecticides that kill both pests and non-target organisms, botanicals pesticides are relatively target specific. They are also biodegradable, environmentally friendly, and can also be used in insecticide resistance management programs. Hence, could serve as good alternatives to chemical insecticides. Further research on the bio insecticidal activity of these compounds is highly advocated.

Key words: *Phytochemical; evaluation; botanicals; pesticides; Callosobruchus subinnotatus PIC.*

Introduction

The genus *Senna,* (*Senna siamea* (Lam.) H.S. Irwin & Barneby) belongs to the family Fabaceae; subfamily Caesalpinioideae, consists of about 350 tropical and warm temperate species of trees, shrubs and herbs. In West Africa, the genus contains about 22 indigenous species apart from those introduced (Hutchinson and Daziel, 1958). Burkill (1995) reported about 19 species in West African floristic region with the whole 19 species in Nigeria (Soladoye and Lewis, 2003) and at least 8 species in South Western Nigeria especially in Oyo and Ogun States. The genus was divided into six sections: Psilorhegma, Chamaefistula, Senna, Peiranisia, Paradyction and Astroites (Irwin and Barneby, 1982; Randell and Barlow, 1998). These species (*Senna siamea)* were formerly placed in *Cassia* subgenus *Senna* (Mabberley, 1997), before it was transferred, along with a number of other *Cassia* species, to the new genus *Senna*. The synonym *Cassia siamea* is, however, still widely used today and this should be noted where searching for information on the species. A number of authors isolated and identified several compounds from different *Cassia* species such as anthraquinones, anthracenes, polyphenols, fatty acids, sterols, polysaccharides and some other miscellaneous compounds from different *Cassia* species (Caro, *et al.* 2012). More than 500 different active as well as non-active phytoconstituents have been reported from the genus. The primary chemical constituents of *Cassia* include cinnamaldehyde, gum, tannis, mannitol, coumarins, and essential oils (aldehydes, eugenol and pinene); it also contains sugars, resins, and mucilage, among other constituents (Singh, and Khan, 1990). The presence of Anthraquinones, alkaloids, glycosides, coumarins, chromones, terpenenoids, tannin, sterols and polyphenols have been reported in *C. siamea* (Dilip, *et al.* 2017). Isolation of anthraquinone, 1-hydroxy-5-methoxy-2-methyl anthraquinone and its glycoside, 5-methoxy-2-methyl anthraquinone-1-O-α-L-rhamnoside along with chrysophanol,emodin and β-sitosterol from the stem of *Cassia* species has been reported (Anonymous). The stem also contains d-mannitol, myricyl alcohol, β-sitosterol, glucose, tigonelline, 1-stachydnine and choline. The stem-bark yields ethyl arachidate and behenic acids, marginic and palmitic acids, euphol, aurapterol, basseol, rhein, 3, 5, 8, 3′4′5′- hexahydroxy flavones (Kapoor, *et al.* 1980).The insecticide activity of *Cassia siamea* extracts and pure compounds has been reported recently (Kamara, *et al.*, 2011; Mamadou, *et al.*, 2014). Fresh leaves from *S. siamea* is used for repelling or killing insects such as termites, bed bugs and mosquitoes (Jimoh, *et al.* (2013). In India, *Cassia* species is used as a natural pesticide in organic farms (Shivjeet, *et al*. 2013). Many plants from the cassia genus; *C. didymobotrya*, has been reported to exhibit larvicidal activity against *C. quinquefasciatus* in Ethiopia (Nagappan, 2012), *Cassia fistula* against *Culex tritaeniorhynchus* in India (Govindarajan, *et al.* 2011), *Cassia nigricans* against mosquito and white flies in West Africa (Georges, *et al.* 2008).

Control of *C.subinnotaus* in Bamabara groundnut is largely depends and conducted using conventional synthetic insecticides in the study area, but resistance to organophosphates and other class of the synthetic insecticides has been observed by local farmers. The study was aimed to determine the phytochemical compounds in *Senna siamea* stembark powders found in Gombe State and their insecticidal potentials on *C.subinnotaus* in stored Bamabara groundnut.

MATERIALS AND METHODS:

Experiment Site

The experiment was conducted at the Federal University of Kashere (FUK), Gombe State Nigeria.

Collection of plant materials

Fresh plant materials of *Senna siamea* stembark were locally collected in Gombe and suburb subsequently, identified at the herbarium of Biological Sciences Department (specimen voucher number 090), Faculty of Science, Federal University of Kashere (FUK), Gombe State, Nigeria.

Preparation of plant materials

All the plant materials collected from *Senna siamea* (stembark) were shade dried to a crispy dry condition and thereafter was grounded using pestle and mortar, blended with an electric blender and then sieved through a mesh size of 600µm to obtain fine powders.

Preparation of crude extracts of the Plant Materials

A weight of 1.8 kg was obtained for each of the powdered plant parts. A 600g portions of the powdered leaves and stembark were soaked separately in 1.8 litres of 70% aqueous ethanol, 1.8 litres of 70% aqueous methanol and 1.8 litres of distilled water for 24 hours. After 24hours, the extract was sieved with a muslin cloth and this was stored in a refrigerator when not in use.

Phytochemical Screening of the crude extract

The processed crude extracts were aseptically carried using Laboratory sample bottles (to avoid contamination on transit) to the National Research Institute for Chemical Technology (NARICT) Basawa, Zaria, Kaduna State where laboratory tests was conducted on the crude extracts of the powdered specimens using standard procedures (Untargeted Gas Chromatography – Mass Spectrometry (GC-MS)).

GC - MS Experimental Conditions

The analysis was performed using Agilent Gas chromatography couple to the mass spectrometer system (model GC Agilent S/N 7890A and 5975A). HP 5ms 5% phenyl Methyl) siloxane Capillary Colum (30M x 250m) was used under the following conditions: Oven temperature 70^{o}C for 1 min, then increase to 280^{o}C by 10^{o}C/min for 10 min and Injector temperature of 260^{o}C. Helium gas was used as the carrier gas with flow rate of 1.9m/min, the volume of the injected sample was 1uL of diluted extract in ethanol, methanol and distilled water. Split injection techniques was used during sample injection with ionization energy 70ev in the electron ionization (EI) mode, ion source temperature 230^{o}C scan mass range of M/Z 50-500.

Identification of constituents

The constituents of the plant material were identified base on the result obtained from the Library search and mass spectra of most of the compound with data generated under identical experimental conditions by applying a search algorithm considering the retention index as well as mass spectra similar with those of authentic compounds available in NIST 2011and NIST 2014 Library.

RESULTS

Result of the untargeted Gas Chromatography-Mass Spectrometry (GC-MS) used for screening of the phytochemical constituents of the plant material was presented in (Table 1). The result revealed the presence of 23, 30 and 27 bio-active compounds in Ethanol, Methanol and Distilled water extracts, respectively. The study report considered only compounds with the highest peak area percentage from each of the extraction solvents used. However, only the first four compounds with the highest percentage area (peaks) and their chromatogram (Figure 1 - 3) was reported out of the numerous compounds screened in the various extraction solvents used.

Table 1: GC-MS analytical report of ethanolic, methanolic and distilled water extracts of *Senna siamea*stembark

Extraction solvent	Peak	Compounds	Molecular Formula	Molecular Weight	Retention Time (Min)	Area (%)
Ethanol	1	Octanoic acid, 4,6-dimethyl-, methyl ester, (4S,6S)-(+)-	$C_{11}H_{22}O_2$	186	14.519	17.84
	2	cis-Vaccenic acid	$C_{19}H_{36}O_2$	296	15.938	16.44
	3	Z,Z-10,12-Hexadecadien-1-ol acetate	$C_{18}H_{32}O_2$	280	17.088	13.18
	4	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280	17.678	12.88
Methanol	1	Oleic Acid	$C_{18}H_{34}O_2$	282	17.088	15.72
	2	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	14.268	13.72
	3	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	740	18.359	12.29
	4	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280	15.732	9.41
Distilled water	1	cis-Vaccenic acid	$C_{19}H_{36}O_2$	296	15.498	30.15
	2	1,2-Benzisothiazole,3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	$C_{13}H_{16}N_2O_2S$	246	18.153	6.67
	3	Oleic Acid	$C_{18}H_{34}O_2$	282	17.695	6.55
	4	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	14.525	5.41

Figure 1 (1a – 4a): GC-MS chromatogram of ethanolic extract of *S.siamea*stembark

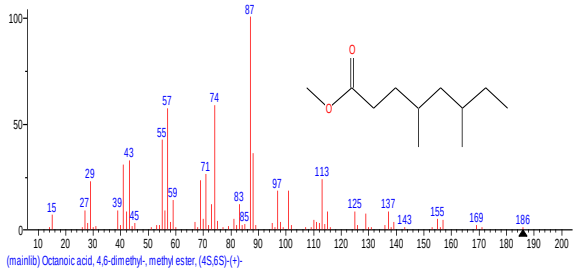

Figure 1a.

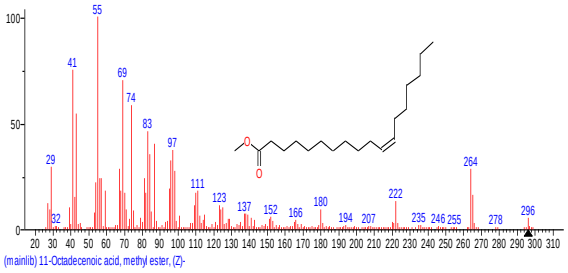

Figure 2a.

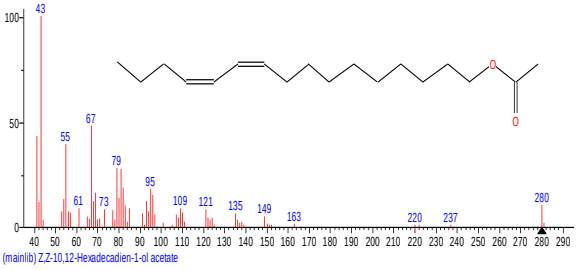

Figure 3a.

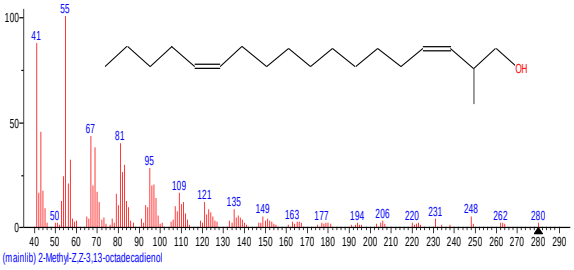

Figure 4a

Figure 2 (1b – 4b): GC-MS chromatogram of methanolic extract of *S. siamea*stembark

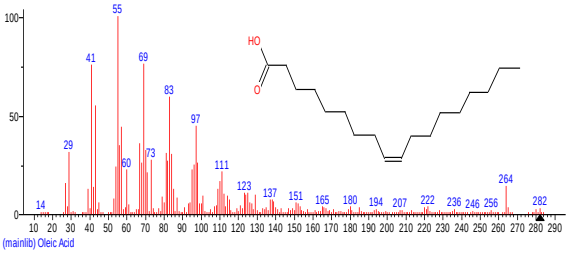

Figure 1b.

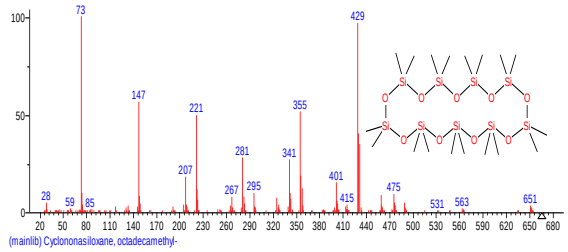

Figure 2b.

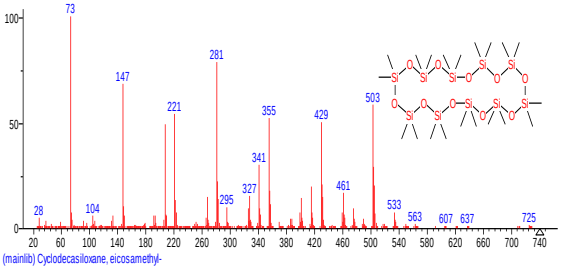

Figure 3b.

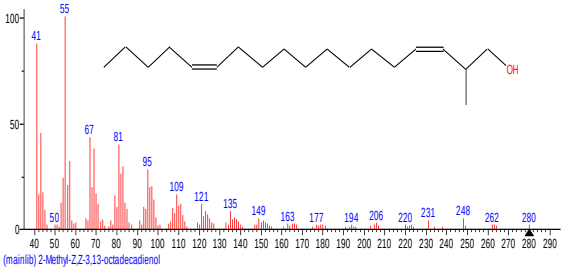

Figure 4b

Figure 3 (1c – 4c): GC-MS chromatogram of distilled water extract of *S. siamea* stembark

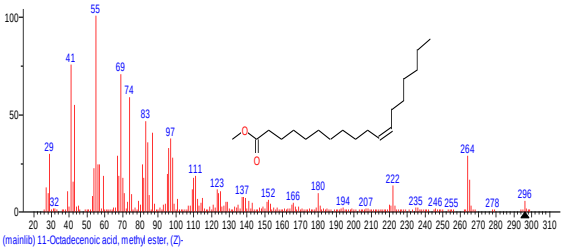

Figure 1c.

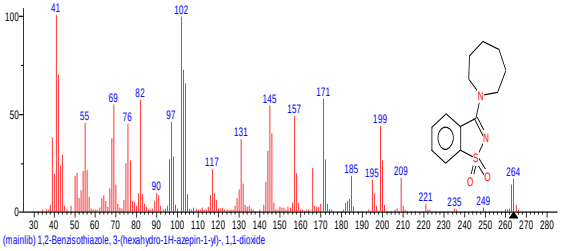

Figure 2c.

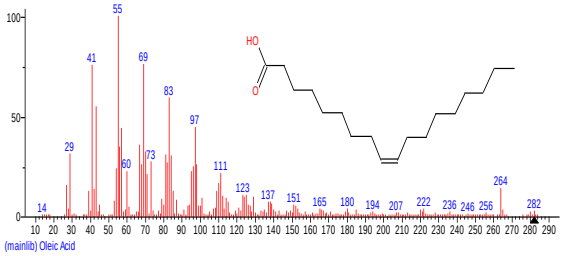

Figure 3c.

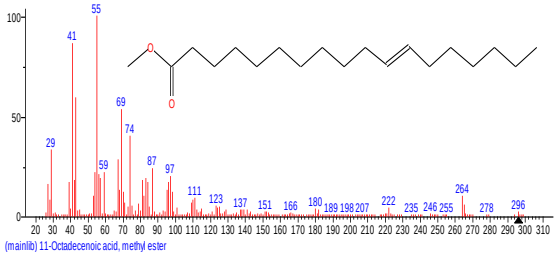

Figure 4c.

DISCUSSION

The result presented in (Table 1) was limited to only first four compounds with the highest percentage area (peaks) and their chromatogram (Figure 1 – 4) out of the numerous compounds screened in the various extracts. The ethanol extractsof *S.siamea* stembark Powder revealed Octanoic acid, 4,6-dimethyl-, methyl ester, (4S,6S)-(+)- ($C_{11}H_{22}O_2$); cis-Vaccenic acid ($C_{19}H_{36}O_2$); Z,Z-10,12-Hexadecadien-1-ol acetate ($C_{18}H_{32}O_2$) and 2-Methyl-Z,Z-3,13-octadecadienol ($C_{19}H_{36}O$). A number of authors isolated and identified several compounds from different *Cassia* species such as anthraquinones, anthracenes, polyphenols, fatty acids, sterols, polysaccharides and some other miscellaneous compounds from different *Cassia* species (Caro, *et al.* 2012). Hafez *et al.* (2019) in their review, reported anthraquinones, anthracenes, Phenolic compounds and their derivatives and some miscellaneous compounds have been isolated from different *Cassia* species. Onoarigo, *et al.* (2017). The isolation of anthraquinone, 1-hydroxy-5-methoxy-2-methyl anthraquinone and its glycoside, 5-methoxy-2-methyl anthraquinone-1-O-α-L-rhamnoside along with chrysophanol, emodin and β-sitosterol from the stem of *Cassia* species Linn. is reported (Anonymous).The methanol extracts of *S.siamea* stembark Powder revealed Oleic Acid ($C_{18}H_{34}O_2$); Cyclononasiloxane, octadecamethyl- ($C_{18}H_{54}O_9Si_9$); Cyclodecasiloxane, eicosamethyl- ($C_{20}H_{60}O_{10}Si_{10}$) and 2-Methyl-Z,Z-3,13-octadecadienol ($C_{19}H_{36}O$). In a different studies carried out by Cyril, (2020), the GC-MS chromatogram of the methanolic extract of *S.siamea* stembark analysis showed the presence of 23 different phytocomponents. These compounds belongs to different chemical classes and most of them are reported to exhibit important biological activities. Anthraquinones such as cassianin, siameanin and siameadin has been isolated from the trunk bark of *C. siamea* (Chaterjee, *et al.*1925). The stem-bark yields ethyl arachidate and behenic acids, marginic and palmitic acids, euphol, aurapterol, basseol, rhein, 3, 5, 8, 3′4′5′-hexahydroxy flavones (Kapoor, *et al.* 1980). The distilled water extracts of *S.siamea* stembark Powder revealed cis-Vaccenic acid ($C_{19}H_{36}O_2$); 1,2-Benzisothiazole,3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide ($C_{13}H_{16}N_2O_2S$); Oleic Acid ($C_{18}H_{34}O_2$) and 11-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$). The use of an alternative solvent, such as water, has increased due to environmental, health and safety awareness; moreover, the cost and economics are also a concern (Wang and Weller 2006). Essien, *et al.* (2011), using GC-MS analysis, isolated oils from hydrodistillation of *S. alata*, *S. hirsuta*, and *S. occidentalis* and reported the following compounds viz., ar-turmerone, *β*-caryophyllene, (E)-phytol, and 6,10,14-trimethyl-2-pentadecanone. (E)-Phytol and pentadecanal were the main components of *S. hirsuta* while *S. occidentalis* had (E)-phytol, hexadecanoic acid, and 6,10,14-trimethyl-2-pentadecanone. The insecticide activity of *Cassia siamea* extracts and pure compounds has been reported recently (Kamara, *et al.*, 2011; Mamadou, *et al.*, 2014). Fresh leaves from *S. siamea* is used for repelling or killing insects such as termites, bed bugs and mosquitoes (Jimoh *et al.* (2013).In a study

conducted by Tapondjou *et al.* (2002), it was reported that the powder prepared from dry leaves of *C. ambrosioides* at a dosage of 0.4% killed more than 60% of the bruchids, *C. chinensis, C. maculatus,* and *Acanthoscelides obtectus* within 2 days, while a dosage of 6.4% induced total mortality of *Sitophilus granarius, Sitophilus zeamais* and *Prostephanus truncatus* within the same exposure period.In a related study, Delobel and Malonga, (1987) found that the dry powdered leaves of *C. ambrosioides* at a dosage of 1:40 (w/w) caused 90% mortality of *Caryedon serratus* (Olivier) adults, a bruchid pest of groundnuts, within 13 days. In many parts of the world, locally available plants are currently in wide use to protect stored products against damage caused by insect infestation (Khater, 2012; Hassanalli and Lwande, 1989; Tripathi, *et al.* 2009). Indian farmers used neem leaves and seed for the control of stored grain pests (Ahmed and Koppel, 1985). In eastern Africa, leaves of the wild shrub *Ocimum suave* and the cloves of *Eugenia aromatic* are traditionally used as stored grain protectants (Powel, 1989). In Rwanda, farmers store edible beans in a traditional closed structure (imboho) and whole leaves of *Ocimum canum* are usually added to the stored food stuff to prevent insect damage within these structures (Weaver, *et al.* 1991).

Conclusion

The nature of the active compound of *S.siamea* stembark**,** which could possibly be the one responsible for its insecticidal properties, is of paramount importance as the subject of further investigation by researchers. Its mode of action, persistence and spectrum of activity against other stored products insects, as well as the optimal size of particles and level of application necessary to afford complete protection of stored grain, is highly advocated.

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