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Chemical Composition of Three Different Fractions Obtained from the Leaves of *Cassia occidentalis*

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Authors' contributions

This work was carried out in collaboration between both authors. Author COO designed the study, wrote the protocol, did the literature searches and performed the statistical analysis. Author PSU wrote the first draft of the manuscript and managed the analysis of the study. Both authors read and approved the final manuscript.

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ABSTRACT

The chemical composition of three different fractions (dichloromethane, methanol, benzene) obtained from the leaves of *Cassia occidentalis* via column chromatography was studied. The aim of the research is to identify the fraction with the highest composition of insecticidal compounds which may be responsible for the insecticidal activity of the plant. The leaves were dried and milled into fine powder, they were first extracted by maceration in various solvents and thereafter the resulting extracts were purified using column chromatography. Fractions obtained from the columns were analysed using Gas Chromatography-Mass Spectrometry (GCMS). The GC-MS results revealed a total of nineteen compounds, prominent among them were hexadecane (45.10%) and heptadecane 8-methyl (13.61%) in the dichloromethane fraction. In the methanol fraction we had; pentadecanoic acid 14-methyl ester (15.50%) 9-octadecanoic acid (48.76%) and methyl stearate (27.6%). The benzene fraction contained; decane (4.70%); hexadecane (21.60%) and nonadecane (11.65%). The presence of decane, methyl stearate, hexadecane, 9-octadecanoic acic methyl ester, pentadecanoic acid -14 methyl ester, heptadecane 8-methyl and nonadecane in the plant extracts may be responsible for the insecticidal properties of *Cassia occidentalis* and should be of

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considerable interest to the development of novel natural insecticdes from this plant. All three fractions contained important insecticidal compounds, thus any of the solvents could be utilised in the formulation of potential biopesticides from *C. occidentalis.*

Keywords: Cassia occidentalis; composition; chromatography; dichloro-methane; insecticides.

1. INTRODUCTION

The importance of plants to all living organisms on the surface of the earth cannot be over-emphasized. Plants contain a wide range of secondary metabolites including; alkaloids, unsaturated fatty acids, flavonoids, Phenols, tannins and terpenes that can be exploited for medicinal, insecticidal and pharmaceutical purposes [1,2]. Many insect pests and vectors have continued to develop resistance due to the repeated use of synthetic products [3], hence the need for the development of biologically active natural alternatives that could act as insecticidal agents and also reduce the risk of exposure of humans to synthetic pesticides most of which are toxic [4].

Cassia occidentalis is basically a flowering plant belonging to the legume family [5]. It is an erect, foetid, annual shrub, sixty to one hundred and fifty centimeters (60-150 cm) in height [6]. It belongs to the kingdom; *plantae*, subkingdom; *tracheobionta*, class; *magnolipiopsida*, subclass; *Rosidae*, order; *fabales*, family; *fabaceae*, genus; *cassia*, specie; *occidentalis.* It is commonly found by the road sides, ditches and waste dumping sites [7]. It bears pink, yellow or white flowers and has its origin in North Africa, the Middle East and some parts of Asia [5]. It has a characteristic odour and acrid taste. Its flowers are yellow, white or pink. The leaves have a very stinking odour and possess 3-5 pairs [8]. In English, *Cassia occidentalis* is called septic weed, coffee senna, negro coffee or stinking weed [9]. It flowers throughout the year under favourable conditions [10]. As a result of toxicity to non-target species and the environment at large of most synthetic pesticides currently used in pest management, natural pesticides are currently under investigation especially with respect to their action on non-target species and the ecosystem.

Reports have it that *Cassia occidentalis* could be employed as a potent insecticidal agent, to replace the more toxic synthetic chemicals currently being used in most parts of the world [11]. This study was designed to determine the chemical composition of three different fractions (dichloromethane, methanol and benzene) obtained from the leaves of *Cassia occidentalis* through Gas Chromatography-Mass Spectrometry (GC-MS) with the view to identify which of the fractions is rich in insecticidal compounds and could therefore be utilized in the formulation of biopesticides.

2. MATERIALS AND METHODS

Equipments: Some of the equipments used include: an electric blender: Akai Tokyo Japan, modeL no: BD0011DA-1033 M, made in PRC, weighing balance- Symmetry Colle-pammer Instrument Co USA, Gas chromatography-Mass spectrometry analyser (GCMS-QP2010 PLUS SHIMADZU, JAPAN). Chromatography Column, Ace glass incorporated, Vineland New Jersey, USA, Rotary evaporator- Fisher Scientific USA.

2.1 Reagents and Chemicals

All reagents and chemicals used including: dichloromethane, methanol, benzene, alumina gel and silica gel were of analytical grade and manufactured by BDH Chemicals Ltd Poole England.

2.2 Collection, Identification and Preparation of Plant Materials

The plant leaves were harvested in the month of October, 2018 from Ikot Ewa, Akpabuyo in Cross River State Nigeria. The identification was done by a Botanist in the Department of Biological Science, University of Calabar and deposited at the herbarium unit with Voucher number: Herb/Bot/Ucc/095A. Leaves were air-dried for two weeks and thereafter ground manually into a fine powder. One hundred grams of powder was macerated in 500 mls of the various solvents (dichloromethane, methanol and benene) for 48 hours. The filterate was obtained after 24 hours using a filter paper (Whattman) and the solvents evaporated using a rotary evaporator. A gel like substance was obtained for all three solvents ranging in colour from green to brown. The extracts obtained were purified within 24 hours using column chromatography technique.

2.3 Column Chromatography Procedure

A piece of cotton wool was used to block the outlet of the chromatogram, in order to prevent the leakage of silica and alumina gels which were used in packing the column. The silica and alumina gels were packed in the ratio of 3:2 in the column. As the gels were loaded into the column, the column was tapped at intervals to allow the particles (silica and alumina) settle properly and to fill up all empty spaces within the column in order to avoid cracks. After the column was completely loaded, dichloromethane was flushed down the column until it was eluted from the outlet to be sure it was working properly and to avoid dryness. The extract obtained from maceration was poured into the column and eluted with dichloromethane. The entire process lasted for 12 hours with eluates collected between the 5th and 12th hour. The eluate was collected in a clean test tube. The procedure was repeated using methanol and benzene as eluents. Eluates from dichloromethane and methanol were greenish-brown in colour while that from benzene was dark brown in colour. Fractions obtained from the columns were collected in clean glass bottles, capped and stored at 4°C for GC-MS analysis. Fractions were later analysed using Gas Chromatography Mass Spectrometry (GC-MS) technique.

2.4 GC-MS Analysis of Fractions

GC-MS analysis of the different fractions extracted from *Cassia ocidentalis* was carried out at the Federal University of Technology Akure (FUTA). The NIST 2011 Mass Spectral Library was used as Reference Library. The Perkin-Elmer GC Clarus 500 system (Perkin-Elmer Scientific Co. Norwalk, CT06859 and USA), interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite- 1,5 fused silica capillary column measuring 30 mm X 0.25 mm with a film thickness of 0.25 mm composed of 100% dimethyl polysiloxane was used. For GC-MS detection, an electron ionization system with ionizing energy of 70eV was used. The carrier gas used was Helium (99.999%) at a constant flow rate of 0.5 ml/min. About 1µ sample injection volume was utilized (Split ratio of 10:1); the inlet/injection temperature was maintained at 250°C, ion source temperature 300°C. Oven temperature was programmed initially at 60°C for 2 min, then increased to 120°C, then programmed to 280°C at a rate of 20°C for 5 mins. Total run time was 77 min. The MS transfer line was maintained at a temperature of 300°C for 5 mins. The Mass Spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 415.00KD.

2.5 Identification of Components

Identification was based on the molecular structure, molecular weight and calculated fragments. The name of the compound, molecular weight, molecular formular, peak area% (concentration) and retention time of the components of the test materials are outlined in the result section. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library version 2011 software, having more than 62,000 patterns [12].

2.6 Statistical Analysis

Statistical analysis was perforemed using Agilent G1701EA MSD Productivity Chemstation Software (second edition) [13].

3. RESULTS

Hexadecane is the major constituent of the dichloromethane and benzene fractions. The dichloromethane fraction also contained in addition; heptadecane 8-methyl, while the benzene fraction contained decane and nonadecane in appreciable concentrations. In the methanol fraction, 9-octadecanoic acid (2) methyl ester was the most predominant constituent; it also contained methyl stearate and pentadecanoic acid 14-methyl ester in significant concentrations.

4. DISCUSSION

So many plants have been studied as potential pest control agents, as they contain potent bioactive principles that may act individually or in synergy [14]. Many of these plant derived pest control agents could be developed and formulated into suitable products for integrated pest management due to their selective toxicity to pests, high effectiveness and safety to the environment [15]. It has been reported that *Cassia occidentalis* oil has larvicidal potential against the malaria vector *Anopheles stephensi* [16]. Also, *Cassia occidentalis* has been reported to prohibit wood damage by termites causing mortality of worker termites within the shortest period of application [17]. Reports have it that the Crude leaf extract of *Cassia occidentalis* showed growth regulating and adulticidal ability in *Anopheles stephensi* [18].

Table 1. Results showing the major constituents of the three fractions from *C. occidentalis*

Eluent	Name of compound	Molecular formula	Molecular weight (g/mol)	Retention time (RT)	Percentage peak area (% PK)
Dichloromethane	*Hexadecane	$C_{16}H_{34}$	226.448	3.615	17.12
	*Hexadecane	$C_{16}H_{34}$	226.448	8.547	27.98
	*Heptadecane 8-methyl	$C_{18}H_{36}$	254.502	10.830	13.61
Methanol fraction	*Pentadecanoic acid 14-methyl ester	$C_{17}H_{34}O_2$	270.457	22.74	15.50
	*9-octadecanoic acid (z) methyl ester	$C_{19}H_{36}O_2$	296.4879	15.013	48.76
	*Methyl stearate	$C_{19}H_{38}0_2$	298.5038	15.233	27.69
Benzene fraction	*Decane	$C_{10}H_{22}$	142.286	4.302	4.70
	*Hexadecane	$C_{16}H_{34}$	226.448	14.383	21.60
	*Nonadecane	$C_{16}H_{34}$	268.529	16.724	11.65

The potency of *Cassia occidentalis* fractions as an insecticidal agent may be due to the presence of the following compounds observed in the fractions from GCMS analysis; decane, methyl stearate, hexadecane, heptadecane 8 –methyl; 9-octadecanoic acid methyl ester and pentadecanoic acid 14 methyl ester. Decane which is an alkane hydrocarbon is a non-polar solvent and is readily combustible [19]. Decane has been identified as an organochlorine pesticidal agent present in mirex [20]. Therefore, *Cassia occidentalis* fractions may belong to the organochlorine class of insecticides. Decane which was discovered in the benzene fraction of *C. occidentalis* at a concentration of 4.70%, has been implicated as an insecticidal compound by the reports of several authors; Ahmet *et al* [21] found 5.8% of decane in the extract of *Achillea wilhelmsii* which exhibited toxic effects against some weeds and pests of Agriculture. Also the insecticidal activity of essential oil of *Hypericum perforatum* has been linked to its main component: decane (59.58%) [22]. In another study, decane (15.10%) was found in hexane oil extracts obtained from the leaves of *Cassia occidentalis;* a plant reported to possess insecticidal efficacy against insect pests and vectors [11]. Decane (3.8%) has also been reported to be present in the essential oil of *Teucrium polium* which is used in the management of red flour beetle [23]. Decane has also been discovered in the hexane extracts of *Trichilia gilgiana* used in pest management in Central Africa Republic [24]. Decane may therefore play a very crucial role in the insecticidal efficacy of *C. occidentalis.*

Another compound of interest discovered in this study is hexadecane. Hexadecane has also been reported to be present in hexane extracts of *Trichilia gilgiana* used in the management of pests in Central Africa [24]. This compound was discovered to be the major constituent of the dichloromethane and benzene fractions in this study at concentrations of 45.11% and 21.60% respectively. It is thus likely to contribute to the insecticidal ability of the plant.

Heptadecane 8 methyl was discovered in the dichloromethane fraction of *C. occidentalis.* Even though there is currently no data on the insecticidal properties of heptadecane 8 methyl, heptadecane has been found present in essential oil of *Teucrium polium* used in the management of red floor beetle [23]. Also 1-heptadecane, 8-heptadecane has been reported in crude extracts of algal and cyano bacteria which are active against the cotton leafworn *Spodoptera littoralis* [25].

Another compound of interest; 9- octadecanoic acid methyl esther which was found in the methanol fraction of *C. occidentalis* was reported present in the crude extract of white Sapote *Casimiroa edulis* leaf extract at a concentration of 3.69%, it was observed that the extract and its fractions were insecticidal against *Spodoptera littoralis* Larvae [26]. Also, 9-octadecanoic acid methyl ester was found in steam distilled essential oil from *C. occidentalis* at a concentration of 22.34% [27].

Pentadecanoic acid, 14 methyl ester, another compound found in the methanol fraction of *C. occidentalis,* has also been reported to be present in oils from *Euphorbia milii* and may be partly responsible for the insecticidal efficacy of *E. milii* [11].

Nonadecane found in the benzene fraction at 11.65% concentration has also been reported present in *Achillea biserrata* extract which has been recommended as a natural alternative biopesticide [21]. Nonadecane has also been reported present in essential oil of *Teucrium polium* L. used in the management of red flour beetle [23].

Methyl stearate, another compound found in the methanol fraction has also been reported in hexane extract of *Phyllantus amarus*; a plant reported to possesss insecticidal properties against insect pests [28]. In another study, 10.1% of methyl stearate was discovered in essential oil obtained from *C.occidentalis* via steam distillation [27]. Methyl stearate, in concentrations as low as 0.7% (V/V), has also been observed to be fungistatic against a wide range of molds and yeast [29].

5. CONCLUSION

From our results, all three fractions contained appreciable concentrations of insecticidal compounds. However, the insecticidal activity of heptadecane 8-methyl which was found in high concentration in the dichloromethane fraction have not been previously reported. Hexadecane was also observed to be more predominant in the fractions than the other compounds as it was present in dichloromethane and benzezne fractions in high concentrations. All the fractions contained potent insecticidal compounds. Any of the three solvents may therefore be used in the formulation of potent bio-insecticides. Further research is required to investigate the insecticidal efficacy of heptadecane 8-methyl.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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