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EVALUATION OF EUPHORBIA POISSONII PAX FOR THE FORMULATION OF AN INCAPACITATING AGENT

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ABSTRACT

This research was designed to explore the biological effects of compounds isolated from Euphorbia poissoni pax for the formulation of an Incapacitating Agent against Albino rat; which will serve as an indigenous weapon for the Nigerian defence system and also as an insecticide. Chloroform extracts of Euphorbia poissoni pax was subjected to preliminary phytochemical screening to ascertain the presence of phenolic compounds, alkaloids, saponins, and terpenoids. Fourier-Transform Infrared (FT-IR) spectroscopy and Gas Chromatography-Mass Spectrometry (GC-MS) analysis indicated the presences of some chemical compounds including (4-(4-Hydroxyphenyl)-4-methyl-2-pentanone) trimethylsilyl Ether, n-Hexadecanoic acid, Carbonic acid(1R)-(-)-menthyl nonyl ester, 13-Octadecenoic acid methyl ester, 1,3,5-triazine, 2-chloro-4,6-bis(mthylthio) and Phthalic acid, 6-ethyloct-3-yl-2-ethylhexyl ester. In the chloroform extract. The LD_{50} of the plant extract was calculated to be 1733.33 mg/kg, which is within the OECD (Organization of Economic Cooperation and Development) guidelines. The extract exerted a potent biological activity at 1000 mg/kg on applying under pressure on the rat skin and incapacitated the rat for 2 minutes. The extract showed an effective insecticidal effect against cockroaches and beans weevils.

Keywords: Phytochemical, incapacitating, agent, insecticide, Extract.

INTRODUCTION

Euphorbia poissoni pax is commonly found in the Northern part of the country, *Euphorbia poissoni pax* is known to produce severe inflammation of skin, the plant is a highly irritant and toxic succulent member of the large and varied spurge family of plants. It is native to northern Nigeria, the Hausa name is Tinya, and Oro adete in Yoruba, the local farmers extract its latex for use as a pesticide. Its powerfully irritant and pain-producing nature mandates (Tchinda, 2008).

The plant prefers full sunlight, does not tolerate wet roots and requires good drainage or careful watering. The sap is highly irritant and toxic; it is extracted and used as a pesticide in local farms. One of the chemicals in the sap (resinifera toxin) is being researched as a possible treatment for chronic pain (Tchinda, 2008).

Throughout West Africa the latex is used as fish poison. A piece of its stem is mixed with the seeds of Strophanthus to prepare arrow poison. In Nigeria the latex is said to be added sometimes to tobacco snuff to increase its pungency. The Hausa people use the latex on cereals to entrap guinea fowl. The latex is also used as bait to kill rodents and birds, and is used for removing hairs from hides. The latex is applied as glue on branches to capture birds. The latex is highly poisonous when added to food, drinking water and kola nuts, and in Nigeria homicides are sometimes committed through *Euphorbia poissonii* poisoning (Neuwinger, 2000).

In West Africa, it is sometimes planted in gardens as an ornamental plant or as a hedge around fields and graveyards. In Europe and the United States of America, it is kept as a pot plant in succulent collections. The flowers are much visited by bees and other insects (Eggli, 2002).

MATERIALS AND METHODS

Sample Collection

The *Euphorbia poissonii pax* stem was freshly harvested from Ekiti Local Government Area of Kwara State North Central Nigeria. The plant stem was identified and authenticated in the Herbarium Unit of the Department of Biological Science, Ahmadu Bello University, Zaria, Kaduna State, with voucher number ABU0298.

Sample Preparation

Euphorbia poissonii pax was harvested, carefully chopped into smaller pieces using cutlass and air dried for about three weeks. It was subsequently ground into powder at the Kaduna polytechnic Mining laboratory with MACSA 300 Overhung Hammermill, and stored in polythene bags.

Experimental Animal

Mature and healthy wistar rats of both sexes weighing between 173-150 g were obtained from the Animal House of the Kaduna State University, Kaduna. Using the method described by Angalabiri-Owe and Isirima, (2014), The mice were kept in plastic cages with perforated net-cover that allowed the inflow of air, with softwood shavings (sawdust) as beddings. The animals were housed in Kaduna State University animal house. The cages were cleaned providing feed and water daily. Mice had free access to pellet diet and clean drinking water, the saw dust were changed twice a week. The animals were acclimatized to pharmacology laboratory conditions for three weeks prior to the commencement of the experiment (Angalabiri-Owe and Isirima, 2014)

Extraction and Isolation of Plant Material

The extraction method adoption for this work is maceration. The labeled extraction bottles were filled with 1200g powdered sample and extracted with four and half liters of $CHCl_3$, for 5 days. The extracts were filtered and concentrated until almost all the solvent have been removed to get a dry yellowish gummy extract of the plant. (Abdullahi and Mainul, 2020)

Percentage Yield of Extract

The chloroform extract of the plant yielded yellowish solid gummy with a percentage yield of 7.1 %, accounting for 1200g of the dried pounded Euphorbia poissonii pax extracted.

Separation of plant constituents

Using Devika and Koilpillai (2015) method, Thin layer Chromatography was used to determine the suitable solvent ratio for the column chromatography of the $CHCl_3$ crude extract, in which 1:1:1 of ethyl acetate, chloroform, and methanol, gave the best result and was chosen. The $CHCl_3$ extract was chromatographed over silica gel column chromatography and eluted using solvent mixtures 1:1:1 of ethyl acetate, chloroform, and methanol to run the column. Various fractions were collected separately (Naga *et al*., 2013).

Phytochemical Analysis

Phytochemical analysis of plants extracts was carried out using standard phytochemical methods as described by Trease and Evans (1989), Sofowora (1993) and Das *et al.,* 2014. One gram of the crude extracts of the plant was dissolved in 10 ml distilled ethanol. The solution was divided into various test tubes to test for the presence of the following phytochemicals:

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Test for alkaloids

One cm^3 ($1cm^3$) of 0.1M Hydrochloric acid (HCl) were added to the test fraction in a test tube. The solution was treated with Mayer (Mercuric Chloride 1.36 g, Potassium Iodide 5 g, and Water 100 ml), Dragendorff (Bismuth Nitrate 8 g, Nitric Acid 20.5 g, Potassium Iodide 27.2 g, and Water 100 ml) and Wagner (Iodine 1.3 g, Potassium Iodide 2 g and Water 100 ml) reagent separately. A creamy-white (Mayer), reddish-brown (Wagner), and orange-brown (Dragendorff) precipitate was observed.

Test for phenolic compounds

The sample was dissolved in ethanol, and a few drops of dilute ferric chloride solution were added. The formation of a yellow coloration indicated the presence of phenolic compounds.

Test for saponins

Three drops of oil were added to the test extract solution and the mixture was shaken vigorously. A stable emulsion formed indicates the presence of saponins.

Test for Terpenoids

Three drops (2 ml) of chloroform was added to the test extract solution and evaporated on the water bath, it was boiled with 3 ml of concentrated H_2S0_4. A grey color formed shows the presence of terpenoids.

Table 1: Phytochemical from crude extract of Euphorbia poissoni pax.

Phytochemical components	*Euphorbia poissoni pax*. Extract
Alkaloids	+
Saponins	+
Phenolics	+
Terpenoids	+

Keys
+: Present

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The fractions from column chromatographic separations was poured into the sample bottles then analyzed on Agilent 5977 GC/MS equipped with a Mass Hunter detector and an Auto-sampler to identify the compound present in the extracts, by comparing the characteristic of the extracts from Mass Spectrometry (MS) fragmentation pattern of the isolates with Mass Spectrometry (MS) fragmentation patterns proposed by NIST Library search for Mass Spectrometry (MS) fragments, to elucidate the structure of the constituent responsible for the Biological effect of the plant.

The result of Gas Chromatography- Mass Spectrometry is presented in the tables and figures below;

Table 2: The compounds from Gas Chromatography- Mass Spectrometry

S/N	Compounds	Area%	M. W. (g/mol)	M. F.	Biological activity	References
1	n-Hexadecanoic acid	6.56	256.42	$C_{16}H_{32}O_2$	antibacterial, antioxidant, insecticidal and cytotoxic	Vasudevan *et al.*, 2012
2	Carbonic acid, (1R)-(-)-menthyl nonyl ester	3.58	326.5	$C_{20}H_{38}O_3$	cytotoxic, antibacterial, Antifungal,	Jaradat *et al.*, 2021

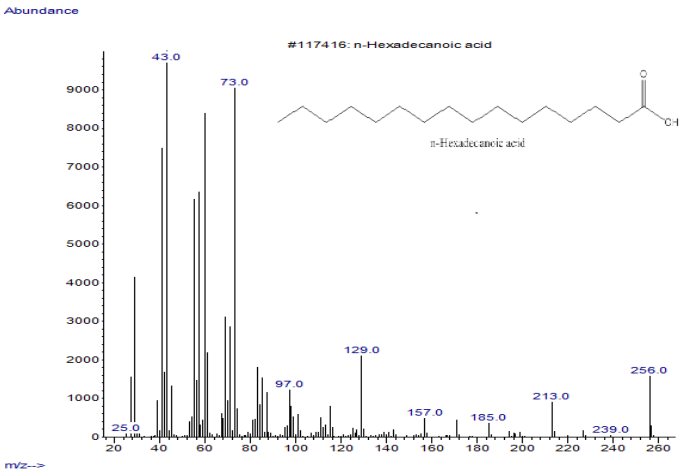
3	Phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester	20.23	418.6	$C_{26}H_{42}O_4$	antioxidant and cytotoxic activities	Karmakar *et al.*, 2019
4	1,3,5-Triazine, 2-chloro-4,6-bis(methylthio)-	0.65	207.7	$C_5H_6ClN_3S_2$	Antioxidant, antimicrobial, cytotoxicity, anti-HIV	Ferdinand *et al.*, 2020
5	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	5.26	264.43	$C_{15}H_{24}O_2Si$	Antioxidant, Cytotoxic, and Antimicrobial Activities	Zhou *et al.*, 2019
6.	13-Octadecanoic acid, mehyl ester	7.74	296.5	$C_{19}H_{36}O_2$	Anti-inflammatory, insectifuge, dermatitigenic, antimicrobial	Siswadi and Saragih, 2020

Result for Gas Chromatography-Mass Spectrometry (GC-MS) Analysis.

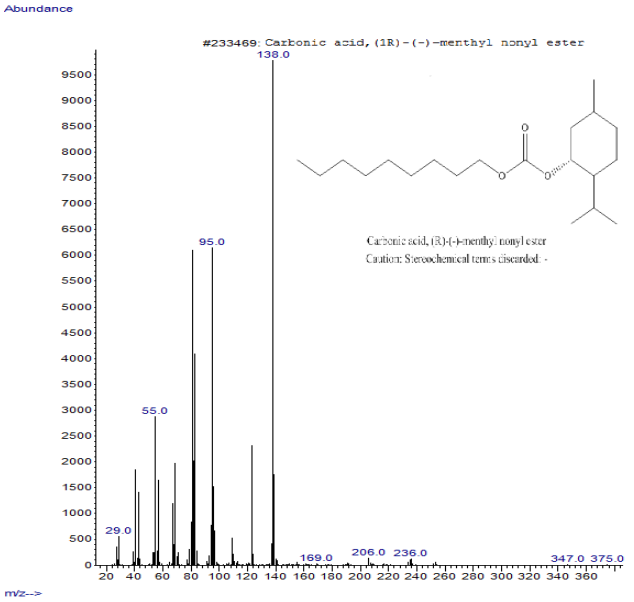
Gas Chromatography-Mass Spectrometry (GC-MS) analysis of fraction A revealed the presences of components like n-Hexadecanoic acid which is known to possess insecticidal and cytotoxic activities accounding (Vasudevan *et al*., 2012), Carbonic acid, (1R)-(-)-menthyl nonyl ester known to possess cytotoxic activity (Jaradat *et al*., 2021), and 13-Octadecenoic acid methyl ester known to possess insectifuge, and dermatitigenic activities (Siswadi and Saragih, 2020). Fraction C revealed the presence, Phthalic acid-6-ethyloct-3-yl-2-ethylhexyl ester, and 1,3,5-Triazine 2-chloro-4,6-bis(methylthio)- are known to possess hemolytic, cytotoxic and also insecticidal activities (Marinelli *et al*., 2018, Anjukrishna *et al*., 2015, Karmakar et al., 2019, Ferdinand et al., 2020).

Fraction E revealed the presences of 4-(4-Hydroxyphenyl)-4-methyl-2-pentanone Trimethylsilyl Ether which is known to possess cytotoxic activity (Zhou *et al*., 2019).

GCMS Spectrums of the Bioactive Compounds



Spectrum of n-Hexadecanoic acid



Spectrum of Carbonic acid, (R)-(-)-menthyl nonyl ester

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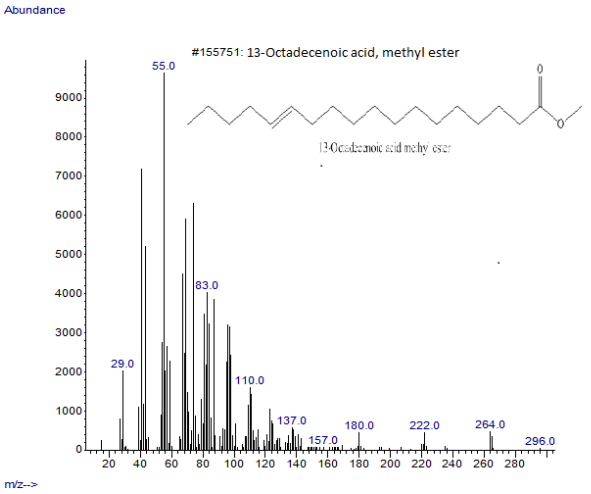
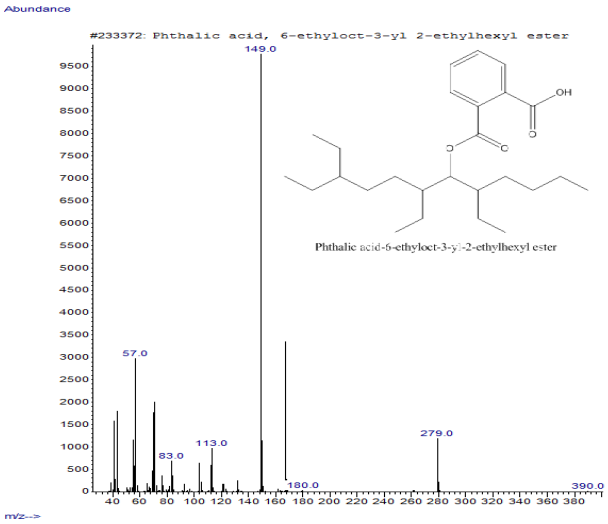
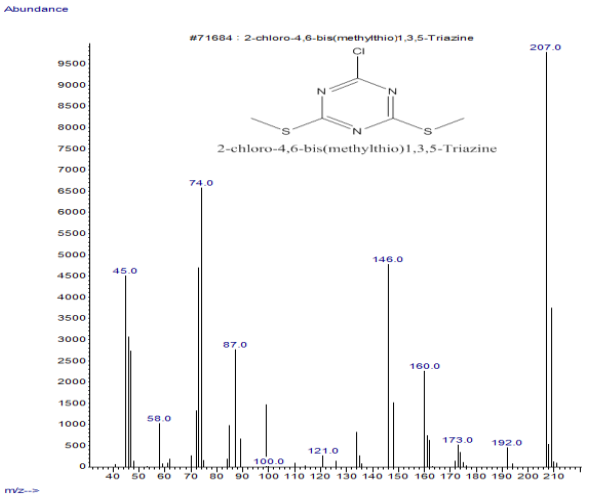


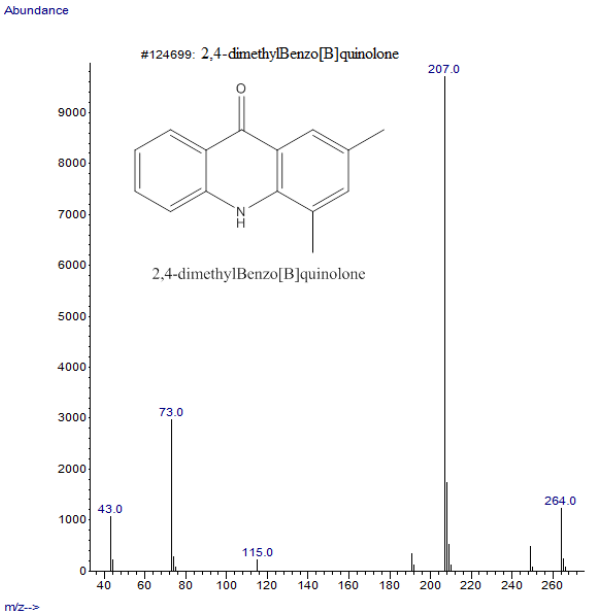
Spectrum of 13-Octadecenoic acid methyl ester



Spectrum of Phthalic acid-6-ethyloct-3-yl-2-ethylhexyl ester



Spectrum of 2-chloro-4,6-bis(methylthio) 1,3,5-Triazine



Spectrum of 2,4-dimethylBenzo[B]quinolone

Preparation of the Doses

The plants extracts were used in the preparation by dissolving 500 mg of the crude extract in 100 ml of distilled water and stored in the test-tube. 1 mg/kg, 1.2 mg/kg, 1.4 mg/kg, 1.6 mg/kg, 1.8 mg/kg and 2 mg/kg were prepared from the stock solution by serial dilution and administered to the animals (Angalabiri-Owe and Isirima, 2014).

Karbers method for determining LD_{50}

The procedure by Angalabiri and Isirima, (2014) called Karbers method was adopted, the Rats of both sexes were divided into six groups (A-F) of six animals each. The groups were given different doses of the chloroform extract orally as follows: Group A: 1000 mg/kg, Group B: 1200 mg/kg, Group C: 1400 mg/kg, Group D: 1600 mg/kg, Group E: 1800 mg/kg, Group F: 2000 mg/kg. The animals were then observed for 24 h for signs and symptoms of toxicity and death. The LD50 of the extract was calculated using the arithmetic method;

The LD50 was caculated using the formula:
LD_{50} =LDy – Σ (Dd x md)/N

Where LDy =Highest dose (LD_{100})

N =Number of animals per group

Dd =Dose difference

Md = Average dead

LD_{50} =Dose that killed 50% of test animals

LD_{100} = Dose that killed 100% (all) the test animals.

Results of the KARBERS method for the determination of the lethal dose

Table 3; Karber's method result

Dose (mg/kg)	No. of death	Average of Death	Dose diff.	Average of Death x Dose diff.
1000	0	0	0	0
1200	1	0.5	200	100
1400	2	1	200	200
1600	3	1.5	200	300
1800	4	2	200	400
2000	6	3	200	600
∑				∑=1600

KARBER'S Arithmetic method

LD_{50}=LDy – Σ (Dd x md)/N

Where LDy =Highest dose (LD100)

N =Number of animals per group

Dd =Dose difference

Md = Average dead

LD50 =Dose that killed 50% of test animals

LD_{100} = Dose that killed 100% (all) the test animals

=2000-(1600/6)

LD50= 1733.33 mg/kg

Result of LD50 Calculation

The LD50 of the extract was determined using the arithmetic method of Karber as modified by Angalabiri and Isirima, (2014), in which none of the animals in Group A showed any clinical or behavioral changes throughout the observation period after injecting. However, depression, weakness and loss of appetite in the first 5 hours was observed in Groups C, D, E and F animals that were treated with the higher doses of the extract. All animals in Group A were active all through the study. In Group B two of the animals suffered weakness and loss of appetite and one eventually died after 48 hours, this shows that lethal dose (LD_{50}) of the crude extract of Rhizophora racemosa in rats was 1733.33 mg/kg, which is still okay by OECD guideline.

The Mouse Ear model for assessment of the biological effect on the skin

The extract exerted a potent but not permanent biological activity on applying under pressure on the rat skin, they were in discomfort for 20 seconds after which they return back to their normal state.

CONCLUSION

The present study shows the presence of some bioactive compounds, such as Trimethyl[4-(2-methyl-4-oxo-2-pentyl) phenoxy]silane, 2,4-dimethylBenzo[B] quinoline, Hexamethylcyclotrisiloxane, (5-Isopropyl-2-methylphenoxy)trimethylsilyl Ether, Phthalic acid 6-ethyloct-3-yl 2-ethylhexyl ester, 2-chloro-4,6-bis(methylthio) 1,3,5-Triazine, n-Hexadecanoic acid, Carbonic acid(1R)-(-)-menthyl nonyl ester, and 13-Octadecenoic acid methyl ester. The extract exerted a potent though not permanent biological activity at 1000 mg/kg on application under pressure on the rat skin, The LD_{50} of the plant extract was calculated to be 1733.33 mg/kg.

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Recommendation

Further studies should be carried out using the wet plant as the sample, and other spectroscopic methods such as Nuclear magnetic resonance (NMR) which will help to elucidate the structure of the compound responsible for incapacitating agent.

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Conflict of Interest

The authors declares that there is no conflict of interest.

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