J. Res. Sci. Mgt. Vol 8No. 1, July (2012)
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Journal of Research in Science and Management

PHYTOCHEMICAL SCREENING AND PESTICIDAL EFFECTS OF MISTLETOES (*Viscum album*) GROWING ON COCOA TREE IN NIGERIA

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ABSTRACT

A number of Nigeria higher plants are traditionally noted for their pesticidal, antimicrobial, nematicidal and fungicidal properties. Some of these have been biologically and phytochemically screened for their activities and chemical constitution. In most cases, these activities have been associated with specific compounds or classes of compounds that are present in such plants. The quantitative phytochemical screening of 60% methanolic extract of *Viscum album* from cocoa indicated the percentage presence of tannins(4.75%), saponins(1.45%), alkaloids(34.7%), phenols(3.75%) and flavonoids(0.55%). The result showed that anthraquinones was absent, alkaloid content was the highest (34.7%) and flavonoids was the least (0.55%). The pure extracts obtained from column chromatography (CC) and thin layer chromatography (TLC) was subjected to infrared spectrophotometry analysis. The range of spectrum was between 500 cm^{-1} at low frequency and 4000 cm^{-1} at high frequency. The peaks obtained indicated the presence of hydroxyl groups alkene, amine, amides including benzamide, ketones including benzylethanone and o – hydroxylbenzanone. Mistletoes extract was found to exhibit significant insecticidal activity against the garden insect *Zonocerus variegatus* at concentration range between 0.05 g/ml and 0.10 g/ml per hour of treatments.

INTRODUCTION

The major local industries in Nigeria are gradually increasing their capacity to generate intermediate industrial materials from local agricultural products. This means therefore that the agricultural sector must achieve an output far beyond what is normally required directly as food. Adoption of any plant disease control measure depends greatly on the level of expertise and social-economic situation of the farmer. The use of synthetic pesticides have been most widely adopted (Onifade, 2000). However the prohibitive costs and toxic side effects associated with synthetic pesticides has necessitated the search for pesticides of plant origin which are not only cheap and readily available but also environmentally friendly (Onifade, 2010).

Mistletoes are hemi-plants parasite of different species. A number of biological effects, such as anticancer, antimycobacterial, antiviral, apoptosis inducing and immunomodulatory activities have been reported for mistletoes (Onay-Ucar et al; 2006). It has also been reported to be effective in the management of chronic metabolic disorder such as diabetes (Obatomi et al; 1994). Ademiluyi and Oboh (2010) reported the antioxidant properties in mistletoes growing on cocoa and cashew trees in Nigeria. They found out that

mistletoes growing on cocoa had more anti oxidant properties than from cashew tree. The present study therefore identifies the phytochemicals and pesticidal ingredient that may be present in *Viscum album* extracts with a view to assessing their potential use in agriculture and related fields.

MATERIALS AND METHODS

Preparation of Extracts

Young leaves of *V. album* were harvested from Olotu camp in Akure, Nigeria and were identified and authenticated in the Department of Crop, Soil and Pest Management, Federal University of Technology Akure. The leaves were washed with sterile distilled water to remove dirt and air dried at 30°C ± 2°C to a constant weight. Thereafter it was pulverized and kept in an airtight jar before analysis.

Test for Alkaloids

Two grams of fine powdered sample were extracted in 20ml of 95% ethanol, boiled in water bath (100°C) for 5min. and filtered hot. Ethanolic and aqueous extracts of filtrate were spotted on Thin Layer Chromatography (TLC) plates and sprayed with Dragendroff's reagents. Orange-red colour indicated the presence of alkaloids (Trease and Evans, 1989). The percentage total alkaloids = % Nitrogen from Kjeldahl Distillation method x 3.26.

Test for Flavonoids

For flavonoids test, 0.5g of powdered plant material was warmed with 10ml of distilled water at 100°C for two minutes. A portion of the extract (1ml) was dissolved in dilute sodium hydroxide solution. A yellow solution that decolorises in hdrorochloric acid confirmed the presence of flavonoids (Trease and Evans, 1989).

$$\text{The percentage flavonoids} = \frac{\text{Absorbance x Average gradient x Dil. Factor}}{10{,}000}$$

Test for Phenol

Two grams of fine powdered sample were mixed with 20 ml of 95% ethanol, boiled in water bath at 100°C for 5min. and filtered hot. Distilled water (20 ml) were added and the ethanol was evaporated at a reduced pressure in water bath. The resultant aqueous concentrate was made up to 20 ml by distilled water. Five drops of freshly prepared mixture of 1% ferric chloride and 1% potassium ferricyanide were added to 5 ml portion of the extract. A violet, green, blue, wine, red, purple or black colour that developed immediately indicated a positive test for phenol (Sofowora, 1993).

$$\%\text{ total phenol} = \frac{\text{Absorbance x Ave. gradient x Dilution factor}}{\text{Weight of substance x } 10{,}000}$$

Test for Saponins

Two grams of sample powdered were mixed with 20 ml of distilled water. The mixture was shaken vigorously and warmed in a water bath to 100°C. Frothing that persisted for 3min indicated the presence of saponins.

$$\%\text{ saponins} = \frac{\text{Abs. std.} - \text{Abs. Sample}}{\text{Weight of sample}} \times \frac{\text{Dil. Factor x Ave. gradient}}{10{,}000}$$

Test for Tannins

Two grams of sample powder were extracted with 20 ml acidified and non-acidified methanol (conc. HCl: Methanol, 1:100 (v/v) (Scneider, 1995), refluxed for 15min and allowed

to further extract over night. Distilled water (5 ml) were added to 10 ml portion of the filtrate extract, subsequently 5 drops of freshly prepared 1% ferric chloride were added. The presence of blue-green or green precipitate was taken as presence of condensed tannins while greenish-black for catechol tannin and blue-black for garlic tannin.

$$\% \text{ tannin} = \frac{\text{Absorbance} \times \text{Ave. gradient} \times \text{Dilution Factor}}{10{,}000}$$

Test for Anthraquinone

Ten mililitre of conc. H_2SO_4 were added to 2g of sample powder, boiled for 15min and 20 ml of benzene were added and filtered hot. Ammonia (10 ml of 10%) solution were added to 10 ml of cooled filtrate extract. A pink, red or violet colour in the lower ammoniacal phase indicated presence of anthraqunones (Sofowora, 1993).

Infrared (IR) analysis of *V. album* Leaves

For IR analysis, 0.1g of the powdered sample was used for the analysis. Potassium bromide (KBr) disc was prepared by grinding 0.1g or the sample with KBr and compressing the whole into transparent disc. The disc was thereafter scanned in a Buck Scientific 500M IR machine. The IR spectrum was printed out with the aid of the machine printer (William, 1987). The IR spectrum of the plant material was compared with those of some named compounds in the process of identification of active ingredients.

Results and Discussion

Table 1 shows the active chemical ingredients in *V. album* leaves. The result of the analysis shows the presence of alkaloids, flavonoids, phenol, saponnis and tannins but anthraquinones was absent.

Table 1. Active Chemical Ingredient in *V. album* leaves.

Sample	Chemical ingredients					
V. album	Alkaloids	Anthraquinones	Flavonoids	Phenol	Saponins	Tannins
Sample	+	-	+	+	+	+

+ indicates presence, - indicates absence

Table 2 shows the quantitative determinations of the phytochemicals that are present in *V. album* extracts. It is indicated that alkaloid was the highest (34.70%) and flavonoids was the least (0.55%).

Sample	Percentage Phytochemical ingredients				
V. album	Alkaloids	Flavonoids	Phenol	Saponins	Tannins
Sample	**34.70**	**0.55**	**3.75**	**1.45**	**4.75**

Figure 1 shows the percentage of insect dead or moribund per hour of treatment with the crude and purified extracts. It is observed that purified extract has more insecticidal effect on *Z. variegatus*. The insecticidal effect varies directly as the concentration of the extract.

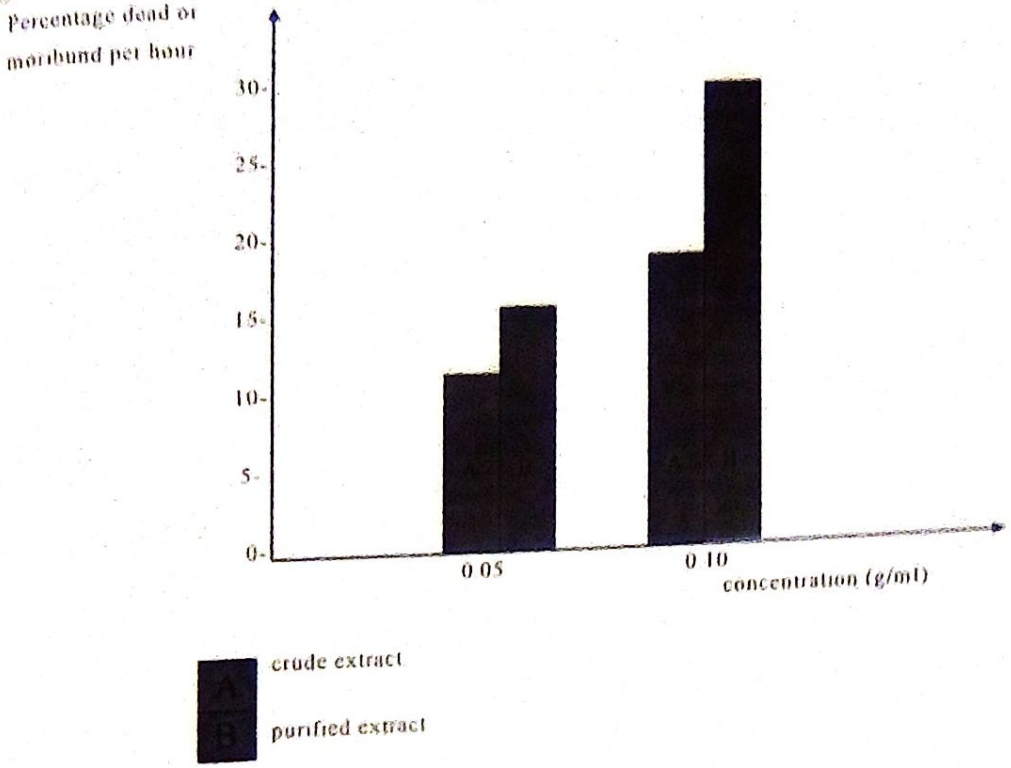

Figure 1: A bar chart showing the percentage of insect dead per hour of treatment with extract.

The result of infrared analysis presented in Figure 2 shows that *V. album* leaves contain hydroxyl groups, alkene, amine, amides, including benzamide, ketones including benzylethanone and O – hydroxyl benzanone.

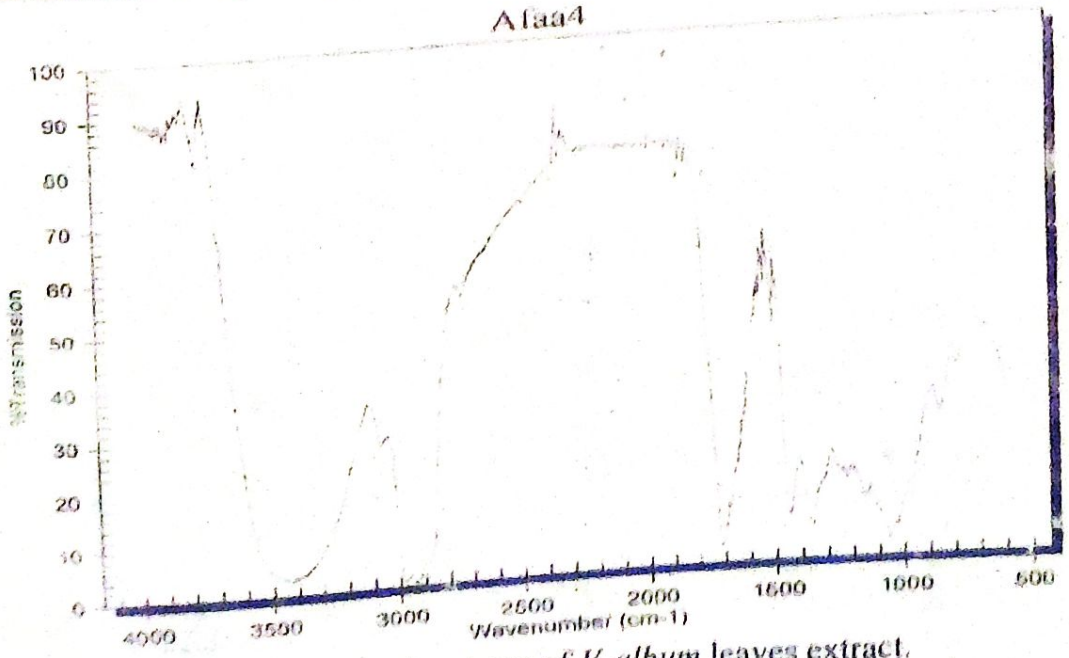

Figure 2: Infrared Spectrum of *V. album* leaves extract.

Discussion

Alkanloids contain nitrogen, which frequently forms part of a heterocyclic ring and makes them basic in natures. These compounds offer protection against predators, acts as a nitrogen reserve and growth regulators, maintain ionic balance, and possibly serve as nitrogen excretion product. They are toxic to insects (Morrison and Boyd, 1992) Ivbijaro and Agbaje 1986 reported that alkaloids detected in *Piper guineense* extract is responsible for the insecticidal activity of the fruit on *Callosobruchus maculatus*.

Flavonoids represent a widespread group of water soluble phenol derivatives, with numerous biological activities including anti-inflammatory, anti-allergic, antithrombotic, vasoprotective and antimicrobial effects (Alverz et al., 2008). Flavonoids also function in defense against herbivores and in regulation of auxin transport, several flavonoids, such as apigenin, kaempferol, myricetin, quircetin and quercitin, have been found to act in the gastrointestinal tracts as antiulcer, antiplasmodic and antidiarrhoeal agents (Prabhuji et al., 2009). Finar, (1986) reported that flavonoids are generally toxic to insects.

Saponins found in plant parts are characterized by their bitter taste and ability to haemolyse red blood cell. Saponins are used in India Ayurveda medicine for the treatment of skin disorders and in human diets for controlling cholesterol. There are reports that saponin – based antibiotics, fungicides and insecticides have been produced (Fabricant and Farnsworth, 2001). Adesina, *et al* 1980 reported that saponin detected from root and stem-back of *Tetrapleura tetraptera* have mollusicidal effect on *Bulinus globosus*.

Tannins are astringent, bitter plant polyphenols. Their astringency is responsible for dry and pucker feeling in the mouth following the consumption of red wine, strong tea of unripe fruit. Tannins have been known to possess antiviral, antibacterial, antifungal, pesticidal and antitumor properties (Sofowora, 2006).

Amines are compounds that readily soluble in water. They also dissolve in less polar solvent like ether, alcohol and benzene. They have 'fishy' odours. They are generally toxic and are readily absorbed through the skin often with fatal results (Morrison and Boyd, 1992). Okwute, 1992 reported that piperine-type amide detected in *Piper guineense* was implicated as the insecticidal agent. The characteristics of amides are similar to those of amines. Ketones are polar compounds with higher boiling points than non-polar compounds of comparable molecular weight. They are appreciably soluble in water and most organic solvents. They are generally toxic to insects (Morrison and Boyd, 1992).

CONCLUSION

The efficacy of mistletoes leaves extract as pesticidal agent on *Z variegatus* may be due to the active ingredient detected in the plant as shown in table 2 and figure 2. This view is strongly supported by the fact that the active ingredients detected in the plant have been found to be toxic to insects or small mammals. Therefore, *V. album*, apart from its antimicrobial activity and antioxidant property that had been reported by earlier workers, is also a potential source of biological active chemical compounds for use in agriculture and allied field.

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