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Antifeedant activity of *Vernonia ocephala* against stored product pest *Tribolium castaneum* (Herbst)

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Abstract

In recent years botanical antifeedants have received increasing attention in developing countries due to continued damaging of grains associated to post harvest insect pest. To develop a sustainable management of post harvest pest using local plant resources in Nigeria, *Vernonia ocephala* ethanol extract, chloroform and petroleum ether fractions were subjected to antifeedant activity against the stored product pest (in maize and maize flour products) *Tribolium castaneum* (Herbst). Wheat wafer disc technique was used in the experiment. The feeding deterrence expressed as Feeding inhibition rate [FI] (%), indicates that ethanol extract was most potent against the insect with a range of 54±1.43 to 86±1.2% and moderate activity of 50±1.11 to 70±3.12% was observed in the chloroform fraction. Phytochemical screening revealed the presence of flavonoids, glycoside, saponins, steroids and triterpenes, cardiac glycoside and tannins in addition; sesquiterpene lactones were identified from chloroform fraction. These findings indicate the potential utility of extracts and fraction of *Vernonia ocephala* for developing active botanical pesticides against the pest *Tribolium castaneum*

Keywords: Antifeedant; *Vernonia ocephala*; Botanical pesticides; *Tribolium castaneum*

Introduction

Plant-derived insecticides have great potential for natural control of post-harvest insect pests, particularly in tropical countries like Nigeria. This stands more important due to increasing incidence of insect resistance against chemical insecticides. Chemical control of stored product-pest has been used for a long time (Isman, 1997). This control measure constitutes problems due to indiscriminate use leading to genetic resistance of pest species (Khanam *et al.* 1990). In addition, synthetic insecticides have in many cases only produced moderate results along with major ecological damages (Franzen, 1993).

Tribolium castaneum (Herbst) is a pest which infests maize crops in the farm and carried into storage where insect population increases (Appert 1987; Adedire and Lajide, 2003). The manifest consequences are the gradual depletion of food quality over storage time resulting to poor products. Hence, the post-harvest loss (PHL) due to the pest attack and other factors in developing countries posed threats to food security (Anomymous, 2011). The need for effective albeit safe control measures of post-harvest pest is imperative. This can be achieved through qualitative research and development (R&D) strategy on botanic based pesticides, which have been found to act concertedly on both behavioural activities and physiological processes of insect

pest (Saxena, 1987). In addition, botanic pesticides have been proven to be specific in action to target pest and relatively harmless to non-target organisms including humans (Golob and Webley, 1980).

Vernonia ocephala Baker (Asteraceae) is an erect under shrub, about 1-2 feet high, with branches from near the base and whitish florets in numerous crowded straw-coloured head of about half inch long. It is perennial and distributed across Northern part of Nigeria (Hutchinson and Dalziel, 1963). Several species belonging to the *Vernonia* genus are known for numerous medicinal uses in traditional medicine across Africa. Some of them including *V. amygdalina*, *V. colorata*, *V. cinerea* and *V. blumeoides* have been reported for antitrypanosomal, antibacterial and insect antifeedant activities (Tandon *et al.*, 1998; Rabe *et al.*, 2002; Aliyu *et al.*, 2011; Yusuf *et al.*, 2012; Ibrahim *et al.*, 2013) among others. Chemical and biological studies of protective allelochemicals especially those affecting insects feeding can make an important contribution to future efforts to control the damage of the crops caused by the insects. To explore the use of plant resources for effective insect post harvest management, this study was designed to evaluate the antifeedant activity of crude extract and fractions of *Vernonia ocephala* against *T. castaneum* which had not been reported hitherto.

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Materials and methods

Collection and preparation of plant material

The plant *V. ocephala* was collected from a local area of Zaria, Kaduna state, on 13th August, 2010. It was authenticated at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen number (1334) was deposited there. The leaves were air-dried and sample pulverized with mortar and pestle to powdered form.

Extraction of plant material

The powdered plant sample (500 g) was percolated using 95% ethanol solution (800 ml) in a closed container on a shaker for 48 hr. It was filtered and the filtrates was then concentrated at reduced pressure to dryness using rotary evaporator at 45°C.

Phytochemical screening

Phytochemical screening of the crude extract and fractions was carried out to detect the presence of secondary metabolites by using standard procedures of Sofowora (Sofowora 1993).

Test for glycoside (FeCl_3 test): To small portion of the extract conc. H_2SO_4 (5 ml) was added to the extract and boiled for 5 minutes. This was then cooled, neutralized with 20% KOH and divided into two (2) portions. Three (3) drops of ferric chloride solution were added to one of the portions giving a green to black precipitate which indicated phenolic aglycone as a result of hydrolysis of glycoside.

Test for cardiac glycosides (Kella-Killiani test): Small portion of extract was dissolved in glacial acetic acid containing traces of 5% ferric chloride. The test tube was held at an angle of 45° and conc. H_2SO_4 (1 ml) was added down the side. A purple colour at the interfaces indicated cardiac glycoside.

Test for saponins (Frothing test): Small quantity of the extract was dissolved in distilled water (10 ml). This was then shaken vigorously for 30 seconds. and allowed to stand for 15 min. A form for more than 30 min indicated the presence of saponins.

Test for steroids and triterpenes (Lieberman-Burchard's test): To the solution of extract (2 ml) was added acetic anhydride (2 ml) followed by conc. H_2SO_4 (1 ml) was added downside the tube and the color change was recorded immediately and later. Red, pink or purple color indicates the

presence of triterpenes while blue or blue-green indicates steroids.

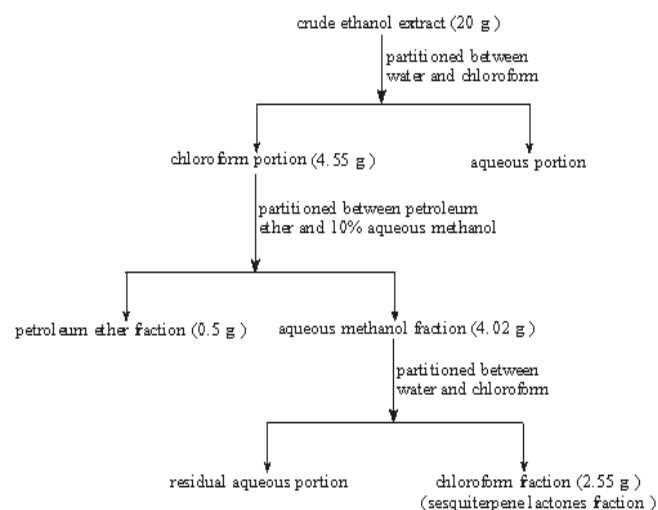
Test for flavonoids (NaOH test): To the extract aqueous solution (5 ml) in a test tube, three drops of aqueous NaOH were added, the formation of and a yellow coloured solution indicated the presence of flavonoids.

Test for tannins (Ferric chloride test): Sample extract (0.5 g) was dissolved in distilled water (10 ml) and filtered. Four drops of ferric chloride solution were added to filtrate. Formation of a blue-black precipitate indicated the presence of hydrolysable tannins while the presence of green precipitate indicated the presence of condensed tannins.

Test for alkaloids (Dragendorff's test): To the extract solution in a test tube, four drops of the Dragendorff's reagent were added; a red precipitate indicated the presence of alkaloids.

Preparation of sesquiterpene lactones fraction

Sesquiterpene lactones fraction was isolated as described by Doskotch and co-workers (Doskotch and El-feraly, 1969). The ethanol extract (20 g) was suspended in water and partitioned with equal amount of chloroform in a separatory funnel. The chloroform portion was further fractionated between 10% methanol and petroleum ether. The aqueous methanol portion was also subjected to fractionation between equal amount of chloroform and water to obtain the sesquiterpene lactone fraction given by the flow chart below:



Test for sesquiterpene lactones (Baljet Reagent)

The chloroform fraction (10 mg) was tested using 2 drops of 1:1 ratio of 1% picric acid in ethanol and 10% sodium

hydroxide. The appearance of orange colour indicated the presence of sesquiterpene lactones (SLs) (Sliva *et al.*, 1998).

Insect culture

The experimental insects were reared under laboratory conditions at $27 \pm 2^\circ\text{C}$ on a diet mixture of wheat meal (190 g) whole meal wheat flour and brewer's yeast (10 g, [19:1]). Test organisms used were starved for 36-48 hr before being introduced into the containers containing the prepared wafer discs (Talukder and Howse, 1995).

Preparation of wheat wafer discs

Wheat wafer discs, made of finely ground flour and water baked at 80°C were used as the test food or substrate. The discs used in the antifeedant bioassay were within range of 0.30-0.35 g.

Antifeedant activity test

The antifeedant bioassay of *Vernonia ocephala* extract and fractions was determined using the wheat wafer disc technique (Talukder and Howse, 1995; Morimoto *et al.*, 2006). The feeding deterrence test measures the activity of the test compounds or extracts using the antifeedant index (AFI). The AFI is a measure of the percentage of the weight of the treated wafer discs consumed in comparison with the untreated wafer discs as stated below:

$$\text{AFI} = \frac{\% \text{ weight of treated disc consumed} \times 100}{\% \text{ weight of treated disc consumed} + \% \text{ weight of control disc consumed}}$$

The AFI values obtained are then converted to the feeding inhibition rate FI (%)

$$\text{FI} (\%) = (50 - \text{AFI}) \times 2.$$

A negative control using phostoxin, aluminium phosphide, the primary post harvest chemicals used on maize for stored off-farm product was used as the control in the experimental procedure. Although aluminium phosphide is commonly referred to as a fumigant, it is used to kill adult insects, insect larvae, and mites and is classified as an insecticide by the Environmental Protection Agency (EPA) USDA Report (Anonymous, 2004).

Statistical Analysis

All experiments were carried out in triplicates. The results are given as mean \pm standard deviation (SD). Student's t-test was used for comparison between two means. Analysis of Variance (ANOVA) was used for more than two means. A difference was considered statistically significant when $p \leq 0.05$.

Results and discussion

The results of phytochemical screening indicate the presence of flavonoids, glycosides, saponins, alkaloids and tannins in ethanol crude extract. The chloroform fraction gave a positive test for sesquiterpene lactones, hence referred to as 'lactone rich fraction', whereas the petroleum ether fraction was found to contain only steroids and triterpenes (Table I). Several plant extracts and isolated compounds containing alkaloids, diterpenes, monoterpenes, sesquiterpene lactones and phenolics compounds have been reported for varying degrees of insect deterrents activity (Adeyemi, 2010). This indicates that *V. ocephala* extracts may show some promise due to various secondary metabolites detected (Table I). The results in Table 2 show the antifeedant activity of ethanol extract and lactone rich fraction of *V. ocephala*. The ethanol extract showed maximum activity of 86% at higher concentration. This could be due to glycosides, saponins and alkaloids detected in the ethanol extract (Table 1). The lactone rich fraction however, showed interesting activity with 70% and the least feeding inhibition rate of 54% was observed with the petroleum ether fraction. It was however, observed that increase in concentration of the extract/fraction resulted to increase in the antifeedant activity. The activity between low and high concentrations was also statistically significant ($P \leq 0.05$) suggesting that the antifeedant activity was concentration-dependent (fig. 1).

Chemical characteristics such as functional groups and stereochemistry are important to feeding deterrent ability of a pure compound, such as triterpenoids based on the C-30 structural moiety existing as highly oxygenated derivatives often as glycosides; steroidal saponins or cardenolides are found to be potent antifeedants (Isman, 2002). This implies the functional roles of glycosides moieties in providing the effective toxicity to deter insect feeding. Therefore, the strong deterrent activity of ethanol extract may be due to glycosides containing components (Table I and II). Similarly, the presence of bitter principles such as sesquiterpene lactones in *Vernonia* species has been implicated for their biological activity. This is due to structural characteristics of the α -methylene- γ -lactone moiety (Chaturvedi, 2011). The chloroform fraction of *V. ocephala* contained sesquiterpene lactones whose antifeedant potency agreed with earlier investigation where sesquiterpene lactone, glaucolide A isolated from *V. glauca* had been identified to be effective antifeedant agent (Burnett *et al.*, 1974).

Volatile chemical components from plants such as monoterpenes or essential oils are important bioresources as insect deterrent agents perhaps due to characteristics lipophilic chemistry. Asawalam and co-workers (Asawalam

Table 1: Results of the phytochemical screening

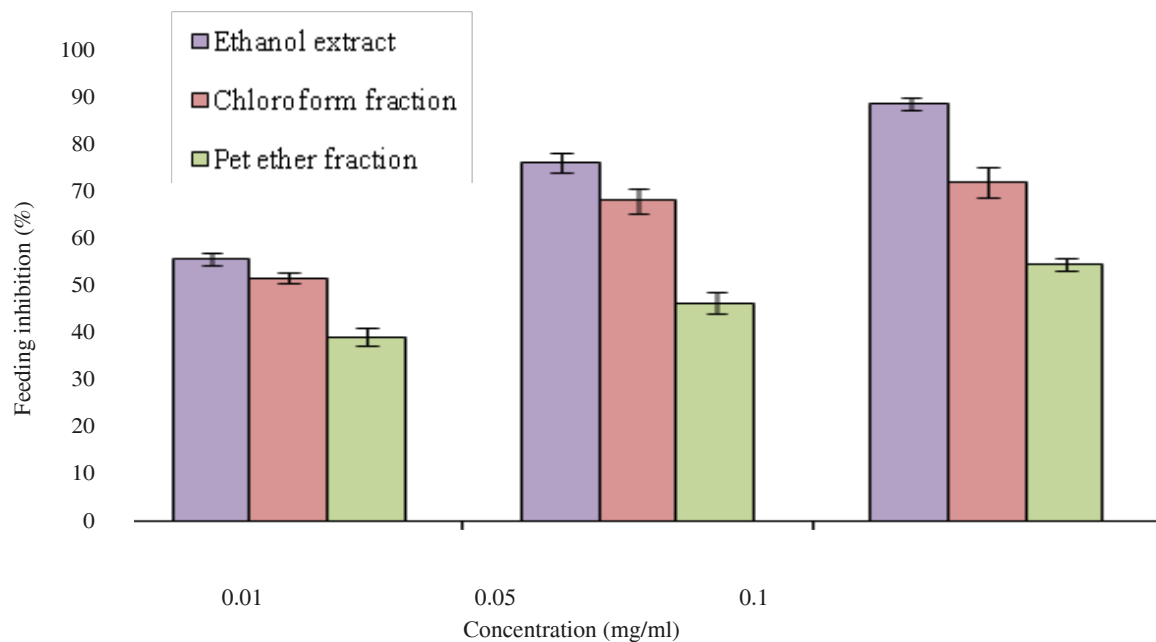
| Constituents | Test | Ethanol extract | Pet ether fraction | Chloroform fraction |
|--------------------------|---------------------------|-----------------|--------------------|---------------------|
| Flavonoids | NaOH | | + | - |
| Glycoside | FeCl ₃ | | + | - |
| Saponins | Frothing test | | + | - |
| Steroids and Triterpenes | Lieberman Burchard's test | | + | + |
| Cardiac glycoside | Kella-killiani test | | + | - |
| Alkaloids | Dragendoff's test | | + | - |
| Tannins | FeCl ₃ test | | + | - |

Key: + = positive, - = negative

Table 2: Results of Feeding inhibition rate (%)

| Concentrations (mg/ml) | Ethanol extract | Pet ether fraction | Chloroform fraction | Phostoxin (standard) pesticide |
|------------------------|-----------------|--------------------|---------------------|--------------------------------|
| 0.01 | 86±1.20 | 70±3.12 | 53±1.21 | ND |
| 0.05 | 74±2.00 | 66±2.43 | 45±2.17 | ND |
| 0.1 | 54±1.43 | 50±1.11 | 38±1.90 | 60±1.94 |

ND: Not determined, FI (%) were presented ±Standard deviation of triplicate determinations

**Fig. 1. Antifeedant activity of *Vernonia oocephala***

and Hassanali, 2006) have demonstrated the repellent ability of *V. amygdalina* essential oil containing 1, 8-cineole, β -pinene, and myrtenal against maize weevil. This indicates that *V. ocephala* might contain some of these terpenoid chemo-types with repellent ability that presumably enhanced the antifeedant activity. It is also suggestive that the activity of both the lactone rich fraction and ethanol extracts may have been mediated by synergistic interactions of the various chemical components. The potency of crude ethanol extracts > lactone rich fraction > petroleum ether fraction. Our results have re-affirmed the potential utility of *Vernonia species* as plant based alternatives to insect post-harvest management. This is because the extract/lactone rich fraction of *V. ocephala* (0.01 mg/ml) was comparable ($P \leq 0.05$) to standard pesticide-phostoxin (aluminum phosphide) at low concentration, which indicates the significance of the extracts or chemical constituents in formulating effective bio pesticides against *T. castaneum*.

Conclusion

In this study *Vernonia ocephala* extracts and fraction have shown considerably moderate to good antifeedant activity against post-harvest insects *T. castaneum* using the wheat wafers disc technique, and the chemical components such as triterpenoids, phenolic compounds, glycosides and sesquiterpene lactones have been tested to be present. These compounds may also be analytically quantified from the extracts/fraction to provide the basis for formulation of optimally potent bio pesticides. However, isolation and characterization of pure compounds could be interesting trend of this research.

Aknowledgements

To Ahmadu Bello University, Zaria-Nigeria for financial support and the National Research Institute for Chemical Technology (NARICT)- Basawa, Zaria-Nigeria for providing some of the facilities.

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Received: 1 January 2014; Revised: 6 July 2014;

Accepted: 25 August 2014.