

Full Length Research Paper

Preliminary phytochemical analysis and insecticidal activity of ethanolic extracts of four tropical plants (*Vernonia amygdalina*, *Sida acuta*, *Ocimum gratissimum* and *Telfaria occidentalis*) against beans weevil (*Acanthscelides obtectus*)

S. A. Adeniyi^{1*}, C. L. Orjiekwe¹, J. E. Ehiagbonare² and B. D. Arimah³

¹Department of Chemical Sciences, Igbinedion University, Okada, Edo State, Nigeria.

²Department of Biological Sciences, College of Natural and Applied Sciences, Igbinedion University, Okada, Edo State, Nigeria.

³Department of Pharmaceutical Microbiology, College of Pharmacy, Igbinedion University, Okada, Edo State, Nigeria.

Accepted 12 April, 2010

Ethanolic extracts of the leaves of *Ocimum gratissimum*, *Sida acuta*, *Telfaria occidentalis* and *Vernonia amygdalina* were screened for secondary metabolite constituents and insecticidal activity against beans weevil (*Acanthscelides obtectus*). Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, saponins, steroids, tannins, phlobatannins and terpenoids in the plants investigated. Phlobatannins and terpenoids were found to be absent in ethanol extract of *T. occidentalis* (leaf), saponins were also absent in both *O. gratissimum* and *S. acuta* while steroidal compounds were found to be absent in *T. occidentalis* and *V. amygdalina* (leaf). The extracts of *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina* of different concentrations were also investigated for their insecticidal activity against *A. obtectus*. Average mortality indicated that the extracts caused significant mortality on the target insects. The bioassay has indicated that the toxic effect of the extracts was proportional to the concentration and higher concentration has stronger effect. The observed overall mean mortality also increased with increase in time intervals after treatment. The overall mean mortality at 0.25, 0.50, 0.75, 1.00 and 1.50 h after treatment (HAT) indicated that 4% solution of the extracts of *V. amygdalina*, *S. acuta*, *O. gratissimum* and *T. occidentalis* showed the highest mortality of 33.60, 31.47, 28.80 and 15.20, respectively in *A. obtectus* at 1.50 h after treatment. It could be inferred from the study that extract from *V. amygdalina* leaf could cause the highest significant mortality.

Key words: Phytochemical, *Ocimum gratissimum*, *Sida acuta*, *Telfaria occidentalis*, *Vernonia amygdalina*.

INTRODUCTION

Many of the reported tropical plants came under scrutiny, leading to extraction and characterization of their active constituents, which accounted for various uses by man. The most important of these constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins

(Abayomi, 1993). Bisht and Kamal (1994) observed that there is strong need to investigate the chemical composition of many plants to determine their ability to be used as fungicides or insecticides. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities observed by many researchers (Babu and Murugan, 1998; Venketachalam and Jebasan, 2001).

Plants are considered as rich sources of bioactive

*Corresponding author, E-mail: gokeomen@yahoo.com. Tel: +2348058844950.

chemicals and there may be an alternative source of insect control agents (Wink, 1993). Pest control strategies, especially those that are effective, cheap and environmentally non-hazardous are needed. Hence, crude plant extracts have played an important role in this aspect (Mahadevan, 1982). Mankind has used plant parts or extracts to control insects since ancient times. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticide properties (Baladrin, 1985; Rawls, 1986; Sukumar et al., 1991). However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vectors of public health importance (Das et al., 2007).

The protection of stored grains from insect damage is currently dependent on synthetic pesticides (Rahman et al., 2007). The repeated use of synthetic insecticides for insect pests and vectors control has disrupted natural biological control systems. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures (Brown, 1986; Hayes and Laws, 1991; Macêdo et al., 1997; Rahman et al., 2007).

Natural insecticides such as pyrethrum, nicotine and rotenone, among others, have been extensively used until recently for insect control (Baladrin, 1985). It has been reported that many compounds with insecticidal potential have been isolated from the genus *Piper* – Pipericide; isolated from *Piper nigrum* (black piper) has been found to be just as active against adjuki bean weevils as the pyrethroids (Mwangi and Mukiyama, 1988). It has also been reported that essential oils of leaf and bark of some plants demonstrated high larvicidal and insecticidal activity against insect pests (Cheng et al., 2003). Limonoids such as azadirachtin and gedurin present in species from the Meliaceae and Rutaceae are recognized for their toxic effects on insects and are used in several insecticide formulations in many parts of the world (Dua et al., 1995; Nagpal et al., 1996; Harve and Kamath, 2004).

Ocimum gratissimum L; family leguminosae is grown in gardens and used as a tea leaf for fevers. It is widely distributed in tropical and warm temperature regions (Dalziel, 1937). *O. gratissimum* is commonly used in folk medicine to treat different diseases such as upper respiratory tract infection, diarrhoea, skin diseases, pneumonia and also cough and conjunctivitis (Dubey et al., 2000). *O. gratissimum* is grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linlool have been extracted from the oil (Sulistiarini, 1999). Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties (Dubey et al., 2000). The antinociceptive property of the essential oil of the plant has been reported (Rabelo et al., 2003). The whole plant and the essential oil are used in

traditional medicine especially in Africa and India. The essential oil is also an important insect repellent. *O. gratissimum* is germicidal (Nakamura et al., 1999; Pessoa et al., 2002; Holets et al., 2003) and has found wide use in toothpastes and mouth washes as well as topical ointments. It is used as an excellent gargle for some throats and tonsillitis. It is also used as an expectorant and a cough suppressant. The plant extract is used against gastrointestinal helminths of animals and man (Fakae, 2000; Chitwood, 2003). In addition, *O. gratissimum* carminative properties make it a good choice for upset stomach. It is used as an emetic and for hemorrhoids. The plant is also used for the treatment of rheumatism, paralysis, epilepsy, high fever, diarrhoea, sunstroke, influenza, gonorrhoea and mental illness (Dhawan et al., 1977; Sofowora, 1993; Sulistiarini, 1999). In addition, the plant is used as a spice and condiment in the Southern part of Nigeria.

S. acuta is traditionally used in the treatment of malaria, diarrhoea and many other diseases (Nacoulma/Ouedraogo, 1966). Research focused on malaria led to the identification of alkaloids, principally cryptolepine the major alkaloid of the plant, as its antimalarial agent (Banzouzi et al., 2004; Karou et al., 2003). More recently, Karou and co-workers (2005) found that polyphenol extract of the plant had a weak antioxidant activity through *in vitro* free radicals scavenging assays, on the other hand the extract was very active on pathogenic bacteria and this activity may be influenced by the polymerization size of the phenolic compounds. Phytochemical screening on *S. acuta* resulted in the isolation of several alkaloids and steroidal compounds with the potential to induce quinine reductase and to inhibit 7, 12-dimethylbenz-(a) anthracene-induced preneoplastic lesions in mouse mammary organ (Cao and Qi, 1993; Dinan et al., 2001; Jang et al., 2003).

Among the compounds isolated from *S. acuta*, its alkaloids appeared to be of great interest in pharmacological studies. These alkaloids belong to the family of indoloquinolines. Many investigations have been done on this family of compounds and the results showed that they are new leads in the establishment of drugs against many diseases. For example, cryptolepine 5-methylindolo (2-3b)-quinoline, the main alkaloid of the plant, has been well investigated for its various biological properties. First isolated from *Cryptolepis* species (*C. triangularis* and *Cryptolepis sanguinolenta* (Periplocaceae) from Africa (Clinquant, 1929; Dwuma-Badu et al., 1978), the compound has also been isolated from other plants such as *S. acuta* (Malvaceae) from Sri Lanka (Gunatilaka et al., 1980), *Microphilis guyanensis* (Sapotaceae) and *Genipa Americana* (Rubiaceae) from Surinam (Yang et al., 1999). In Ghana, extracts of roots of *C. sanguinolenta* in which cryptolepine the main alkaloid, have been used clinically to treat malaria, colic and stomach ulcers (Boye and Ampofo, 1983). Cryptolepine itself is found to produce many

pharmacological effects such as antimicrobial (Cimanga et al., 1998), antiprotozoal (Arzel et al., 2001; Wright et al., 2001), antihyperglycemic (Bierer et al., 1998) and cytotoxic effects through GC-rich DNA sequence intercalation that provides basis for design for new anticancer drug (Bonjean et al., 1998; Dassoneville et al., 2000; Guittat et al., 2003; Lisgarten et al., 2002).

Oyolu (1978) reported that the leaves of *Telfaria occidentalis*, together with the edible shoots contain moisture; crude protein, carbohydrates, oils, ash and iron, while Longe et al. (1983) reported that the minerals namely: calcium, potassium, magnesium, iron, sodium and phosphorus are concentrated in the testa, pulp and husk. Oboh (2004) has reported that dietary intake of the leaf could prevent garlic-induced haemolytic anaemia in rats. The aqueous extracts of *T. occidentalis* had been reported to reduce blood glucose level and also have antidiabetic effects in glucose induced hyperglycaemic streptozotocin (STZ) induced diabetic mice (Aderibigbe et al., 1999), while it did not alter the glucose levels in normoglycaemic mice. Recently, Dina et al. (2001) reported that the aqueous extract of *T. occidentalis* leaf could assist in the purging of the gastrointestinal tract as revealed by the purgative effect of the aqueous extracts of *T. occidentalis* leaf on isolated guinea pig ileum and he concluded that there are some pharmacological effects underlying their mode of action.

Iwalokun and co-workers (2004) have also reported that *V. amygdalina* possesses antibacterial, antifungal, antiplasmodial and nematocidal properties. In the present study, the ethanolic extracts of four botanicals namely: *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina* were screened for secondary metabolite constituents and insecticidal activity of the four botanicals against beans weevil (*Acanthscelides obtectus*) were studied and the results were compared.

MATERIALS AND METHODS

Reagents

Ethanol, methanol and HCl (BDH Chemicals, England), chloroform (E. Merck, Germany), acetic anhydride (Fluka, Switzerland), ferric chloride (AnalaR Chemicals, England), sodium hydroxide (Sigma Chemical Co., USA), concentrated sulphuric acid (BDH Chemicals, England), Draggendoff's reagent and Mayer's reagent (E. Merck, Germany). All the chemicals were of analytical grade and used without further purification.

Plant materials

Fresh leaves of four natural plants (*O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina*) were collected from the premises of Crown Estate, Okada in Ovia North-East Local Government Area of Edo State, Nigeria on 15th March, 2009. The plant materials (leaves) were identified and authenticated at the Taxonomy section, Biological Sciences Department, Igbinedion University, Okada, Nigeria.

The leaves were air-dried at room temperature (26°C) for 8

weeks, after which it was milled into a uniform powder with the aid of an electrical grinder. The ethanol extracts were prepared by soaking 100 g each of the dry powdered plant materials in 300 ml of 80% ethanol at room temperature for 48 h. The extracts were filtered after 48 h through a Whatmann filter paper No. 42 (125 mm).

The extracts were concentrated using a rotary evaporator at a maximum temperature of 45°C. The weight of dried crude extracts ranged from 10.6 - 11.8 g from 100 g of the dried powder in 80% ethanol as a solvent. The extracts were then dissolved in distilled water to prepare solutions of different concentrations (1.0, 2.0, 3.0 and 4.0%). The bioassay of the extracts was done for direct toxicity (mortality) test.

METHODS

Phytochemical screening

Chemical tests were carried out on the ethanolic extracts for the qualitative determination of phytochemical constituents as described by Harborne (1973), Trease and Evans (1989) and Sofowora (1993).

Alkaloids

0.5 g of extract was diluted with 10 ml of acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendoff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendoff's reagent) was regarded as positive for the presence of alkaloids.

Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously. An appearance of creamy mass of small bubbles indicated the presence of saponins.

Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration indicating the presence of tannins.

Phlobatannins

About 0.5 g of each plant extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate indicated the presence of phlobatannins.

Flavonoids

About 0.5 g of each plant extract was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicated the presence of flavonoids.

Steroids

Two millimeter of acetic anhydride was added to 0.5 g of ethanol extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Terpenoids (Salkowski method)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

Cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Toxicity test

Direct toxicity test with beans weevil was carried out following the method described by Talukdar and Howse (1993) and Rahman et al. (2007). Insects were chilled at 4°C for a period of 10 min. The immobilized insects were individually picked up and 1 ml solutions of different concentrations (0.0, 1.0, 2.0, 3.0 and 4.0% w/v) were applied to the dorsal surface of the thorax of each insect by using a micro capillary tube. Fifty insects per replicate were treated. The insects were then transferred into a 9 cm diameter Petri dishes containing food. Insect mortality rate was recorded after 0.25, 0.50, 0.75, 1.0, 1.5 h after treatment (HAT). All the experiments were conducted in completely randomized design with three replications and turned to statistical analysis. Finally, the mean values were compared using Duncan Multiple Range Test (DMRT), (Duncan, 1955).

Seed viability test

The floatation method described by Ehiagbonare and Enabulele (2007) was used to check the viability of the seeds after treatment with various concentrations of the plant extracts. Fifty seeds per replicate were sprayed with 10 ml solutions of different concentrations (0.0, 1.0, 2.0, 3.0 and 4.0% w/v) of the plant extracts. Percent viability was recorded at 1.5 h after treatment (HAT). All the experiments were conducted in three replications and the mean values were calculated using the following formula:

$$\text{Percent Viability} = (S - S_F / S) \times 100$$

Here, S = Number of treated seeds used per replicate.
S_F = Number of floating seeds per replicate.

RESULTS AND DISCUSSION

Results

Percentage yield of extracts

Table 1 shows the percentage yield of ethanol extract of

O. gratissimum, *S. acuta*, *T. occidentalis* and *V. amygdalina*. The crude extract of the leaf of *V. amygdalina* contains a greater proportion by mass of the component compounds followed by *S. acuta* and *O. gratissimum* while *T. occidentalis* contains the least proportion of the component compounds.

Phytochemical constituents of extracts

The result of the phytochemical screening (Table 2) reveals that alkaloids, tannins and flavonoids were positive in all ethanolic extracts of *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina*. Phlobatannins and terpenoids were detected in the ethanolic extract of *O. gratissimum*, *S. acuta* and *V. amygdalina* while cardiac glycosides were detected in the ethanolic extract of *O. gratissimum*, *S. acuta* and *V. amygdalina*. Also, saponins were detected in both *T. occidentalis* and *V. amygdalina* while steroids were detected only in the ethanolic extract of *S. acuta* and *T. occidentalis*. These phytochemicals may be responsible for their insecticidal properties (Kabar and Gichia, 2001).

The presence of tannins shows that the plants can be used as purgative. They are also used in the treatment of cough, asthma and hay fever (Gills, 1992). The presence of terpenoids revealed that the plants can act mainly as anti-feedant and growth disruptor and possesses considerable toxicity toward insects (Khalid et al., 1989). Terpenoid also plays an important role in wound and scar healing (Hayashi et al., 1993). It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones. The presence of steroidal compounds in the plants is an indication that the plants can be used or expectant mothers or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in synthesis of these hormones (Okwu, 2001).

Results from the present investigation showed that *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina* are very rich in phytochemicals, even though the phytochemical screening of the four plants revealed some differences in their constituents.

Toxicity test

The results of mortality with ethanol extract of *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina* at different concentrations are shown in Tables 3 - 6. The results of average mortality of beans weevil (*A. obtectus*) with ethanol extract of *O. gratissimum* at different concentrations are presented in Table 3. With the crude extract of *O. gratissimum* (leaf), the average mortality indicated that 4.00% concentration resulted in the higher toxicity of 28.80 in *A. obtectus*. It is also observed that 3.00% showed a toxicity of 25.60, 2.00% showed a

Table 1. The percentage yield of ethanol extract of sample (leaves).

Sample	% Yield
<i>Ocimum gratissimum</i>	11.10
<i>Sida acuta</i>	11.40
<i>Telfaria occidentalis</i>	10.60
<i>Vernonia amygdalina</i>	11.80

Table 2. Phytochemical screening of ethanol extracts of samples (leaf).

Phytochemicals	<i>O. gratissimum</i>	<i>S. acuta</i>	<i>T. occidentalis</i>	<i>V. amygdalina</i>
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	-	-	+	+
Steroids	+	+	-	-
Tannins	+	+	+	+
Phlobatannins	+	+	-	+
Terpenoids	+	+	-	+
Cardiac glycosides	+	+	-	+

Key: + = Present, - = Absent.

Table 3. Corrected average mortality with ethanol extract of *O. gratissimum* leaf on *A. obtectus*.

Concentration	Average mortality (HAT)					Overall mean
	0.25	0.50	0.75	1.00	1.5	Mortality
0.00% (control)	0.00	0.00	0.00	0.33	1.00	0.27
1.00	3.00a	10.00a	20.33a	24.33a	27.00a	16.93
2.00	4.33a	13.67a	24.33a	27.67a	35.00b	21.00
3.00	6.67a	16.00b	27.67b	33.33b	44.33c	25.60
4.00	8.33b	17.33c	31.67c	39.33b	47.33c	28.80

HAT, Hours after treatment; different letters in a column are significantly different using Duncan's multiple range test at 5% level.

Table 4. Corrected average mortality with ethanol extract of *S. acuta* leaf on *A. obtectus*.

Concentration	Average mortality (HAT)					Overall mean
	0.25	0.50	0.75	1.00	1.5	Mortality
0.00% (control)	0.00	0.00	0.00	0.33	0.67	0.20
1.00	4.00a	13.67a	20.00a	26.00a	29.33a	18.60
2.00	5.33a	15.67a	27.33b	30.33b	32.67b	22.27
3.00	7.33b	17.33a	30.67c	41.33c	46.33c	28.60
4.00	9.33b	20.33b	34.67c	44.33c	48.67c	31.47

HAT, Hours after treatment; different letters in a column are significantly different using Duncan's Multiple Range test at 5% level.

toxicity of 21.00 while 1.00% showed the average toxicity of 16.93 in *A. obtectus*.

The results of average mortality of beans weevil (*A. obtectus*) with ethanol extract of *S. acuta* at different concentrations are presented in Table 4. With the crude extract of *S. acuta* (leaf), the average mortality indicated that 4.00% concentration resulted in the higher toxicity of

31.47 in *A. obtectus*. It is also observed that 3.00% showed a toxicity of 28.60, 2.00% showed a toxicity of 22.27 whereas 1.00% showed the average toxicity of 18.60 in *A. obtectus*. The results of average mortality of beans weevil (*A. obtectus*) with ethanol extract of *T. occidentalis* at different concentrations are presented in Table 5. With the crude extract of *T. occidentalis* (leaf),

Table 5. Corrected average mortality with ethanol extract of *T. occidentalis* leaf on *A. obtectus*.

Concentration	Average mortality (HAT)					Overall mean
	0.25	0.50	0.75	1.00	1.5	Mortality
0.00% (control)	0.00	0.00	0.00	0.33	0.67	0.20
1.00	0.00a	6.00a	8.67a	11.00a	15.33a	8.20
2.00	0.67a	7.33a	12.00b	15.67b	18.33a	10.80
3.00	1.33a	9.67b	14.33b	19.00c	21.33b	13.13
4.00	2.33b	11.00c	18.00c	20.67c	24.00b	15.20

HAT, Hours after treatment; different letters in a column are significantly different using Duncan's multiple range test at 5% level.

Table 6. Corrected average mortality with ethanol extract of *V. amygdalina* leaf on *A. obtectus*.

Concentration	Average mortality (HAT)					Overall mean
	0.25	0.50	0.75	1.00	1.5	Mortality
0.00% (control)	0.00	0.00	0.00	0.33	0.67	0.20
1.00	5.33a	15.00a	23.00a	27.00a	31.00a	20.27
2.00	6.67a	16.67a	27.67a	31.67b	37.00b	23.94
3.00	8.67b	19.00a	33.00b	42.33c	46.67c	29.93
4.00	9.67b	22.33b	40.00c	46.33c	49.67c	33.60

HAT, Hours after treatment; different letters in a column are significantly different using Duncan's Multiple Range test at 5% level.

the average mortality indicated that 4.00% concentration resulted in the higher toxicity of 15.20 in *A. obtectus*. It is also observed that 3.00% showed a toxicity of 13.13, 2.00% showed a toxicity of 10.80 whereas 1.00% showed the average toxicity of 8.20 in *A. obtectus*.

The results of average mortality of beans weevil (*A. obtectus*) with ethanol extract of *V. amygdalina* at different concentrations are presented in Table 6. With the crude extract of *V. amygdalina* (leaf), the average mortality indicated that 4.00% concentration resulted in the higher toxicity of 33.60 in *A. obtectus*. It is also observed that 3.00% showed a toxicity of 29.93, 2.00% showed a toxicity of 23.94 whereas 1.00% showed the average toxicity of 20.27 in *A. obtectus*.

These results also revealed that the extracts from the leaves of *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina* possess good insecticidal potential because of their phytochemical constituents (Nadi, 2001; Kabar and Gichia, 2001). The order of toxicity of four different concentrations were 4.00 > 3.00 > 2.00 > 1.00%. Similar direct toxicity effect of the leaf extracts of Shiyalmutra on rice weevil has been carried out by Roy et al. (2005) and reported the order of toxicity as 3 > 2 > 1%.

The observed overall mean mortality also increased with increase in time intervals after treatment (Figures 1 - 4). Overall mean mortality at 0.25, 0.50, 0.75, 1.00 and most active against *A. obtectus*, followed by *S. acuta*, *O. gratissimum* and the least is the ethanol extract of the leaf of *T. occidentalis*.

Seed viability test

The results of percentage viability of the seeds sprayed with different concentrations of the ethanol extract of *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina* are presented in Table 7. The result of seed viability test reveals that percentage viability of the seeds sprayed with 0.00% concentration of the ethanol extract of *O. gratissimum* is 63.33. The percentage viability of the seeds sprayed with 4.00% concentration of the ethanol extract of *O. gratissimum* resulted in higher 70.00 while 3.00% concentration has percentage viability of 68.67, 2.00% has percentage viability of 66.67 and 1.00% has percentage viability of 64.67.

The percentage viability of the seeds sprayed with 4.00% concentration of the ethanol extract of *Sida acuta* resulted in higher 72.00 while 3.00% concentration has percentage viability of 69.33, 2.00% has percentage viability of 67.33 and 1.00% has percentage viability of 66.00.

The percentage viability of the seeds sprayed with 4.00% concentration of the ethanol extract of *Telfaria occidentalis* resulted in higher 68.67 while 3.00% concentration has percentage viability of 67.33, 2.00% has percentage viability of 66.67 and 1.00% has percentage viability of 63.33.

The percentage viability of the seeds sprayed with 4.00% concentration of the ethanol extract of *V. amygdalina* resulted in higher 72.67 while 3.00%

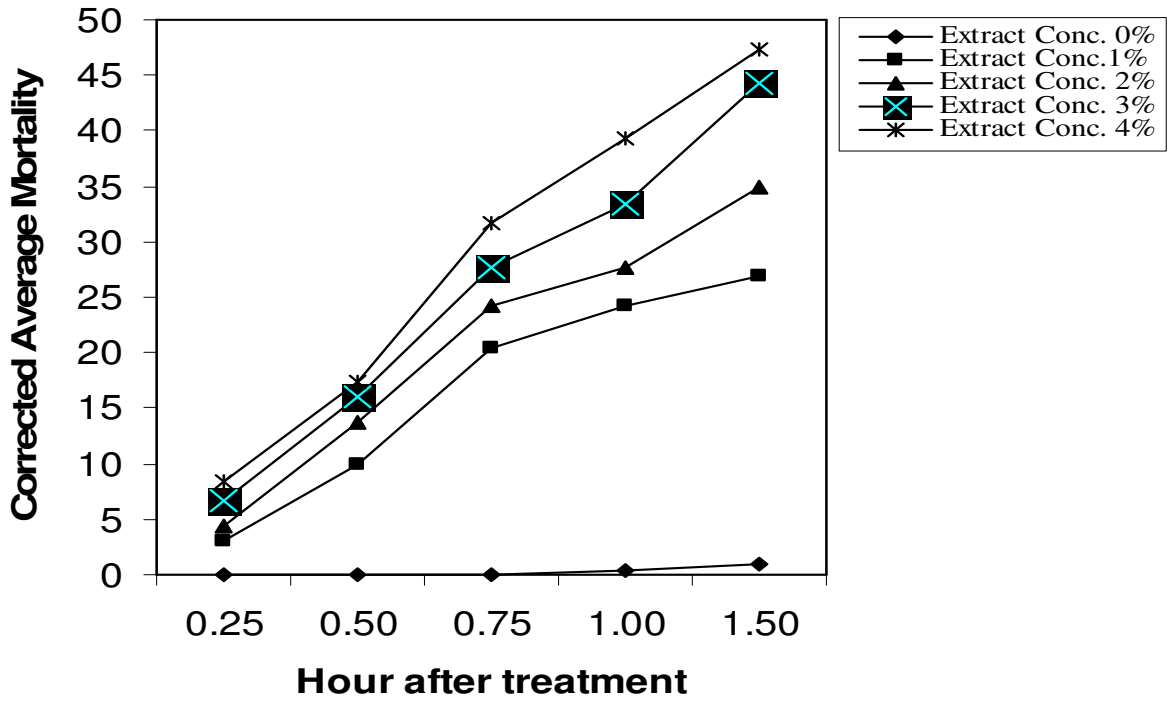


Figure 1. Corrected average mortality with ethanol extract of *Ocimum gratissimum* leaf on *A. obtectus*

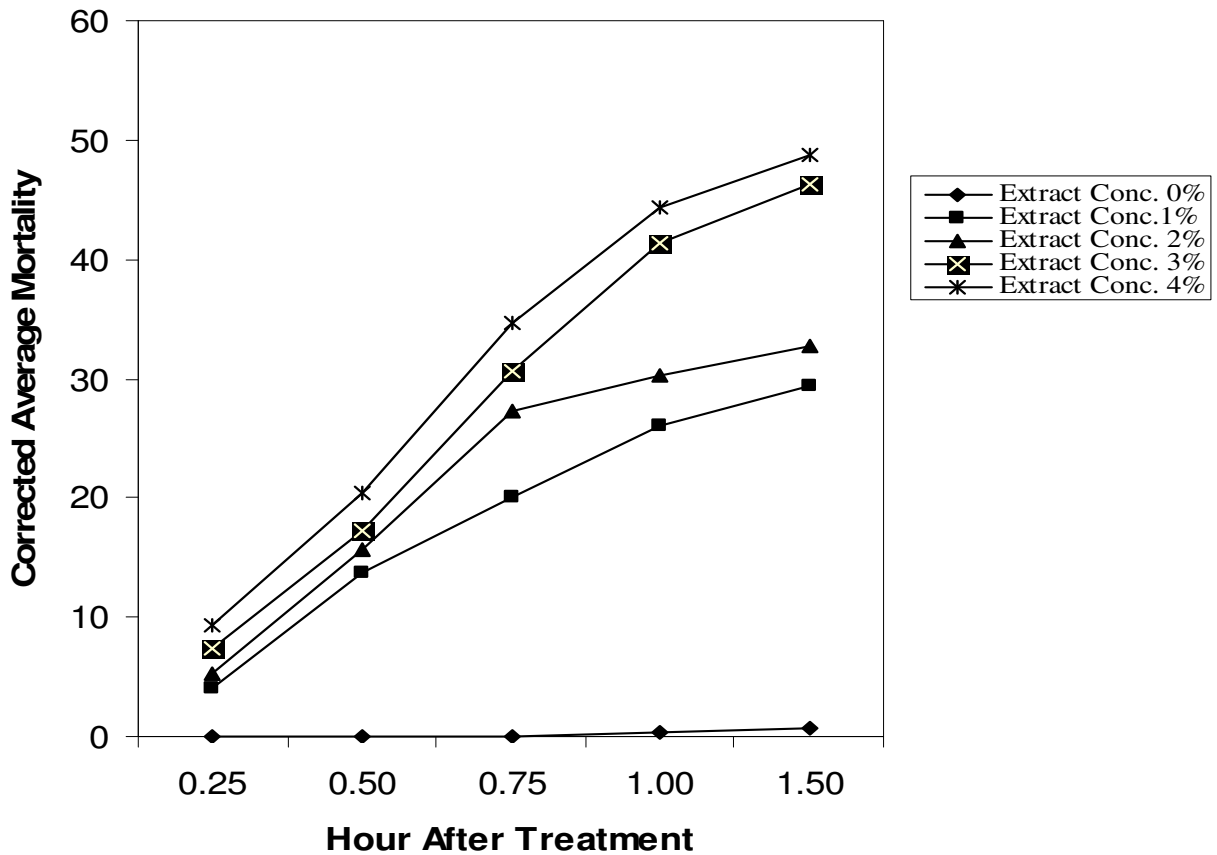


Figure 2. Corrected average mortality with ethanol extract of *Sida acuta* leaf on *A. obtectus*.

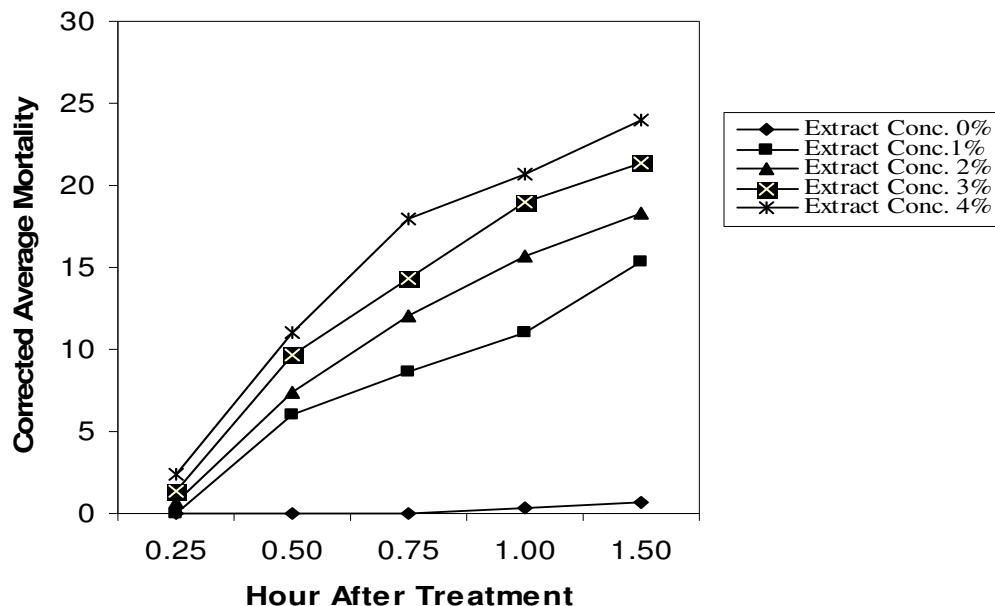


Figure 3. Corrected average mortality with ethanol extract of *T. occidentalis* leaf on *A. obtectus*.

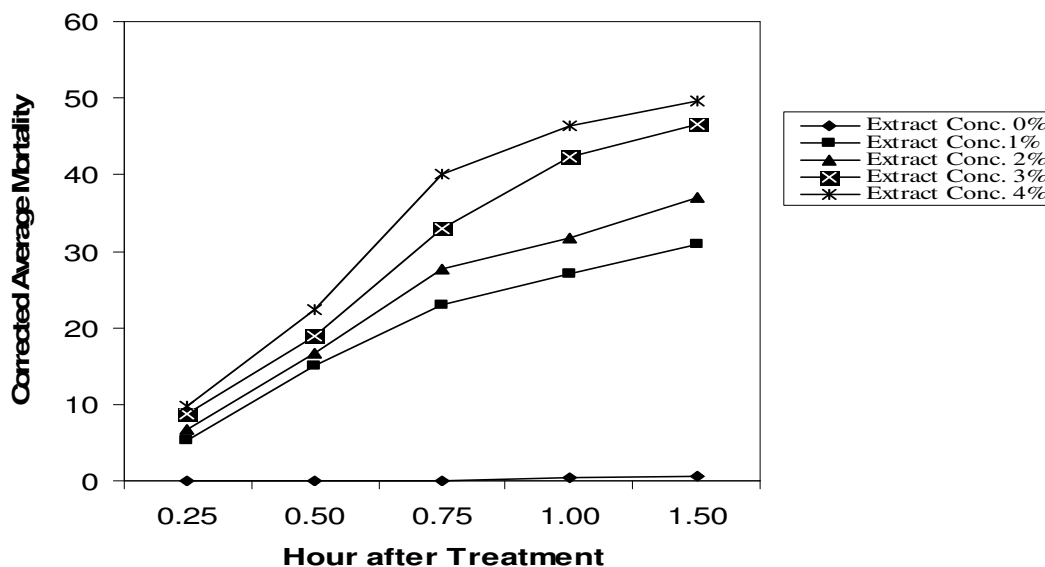


Figure 4. Corrected average mortality with ethanol extract of *V. amygdalina* leaf on *A. obtectus*.

1.50 h after treatment indicated that 4.00% solution showed the highest mortality of 28.80, 31.47, 15.20 and 33.60 in *A. obtectus* at 1.50 HAT with *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina* respectively. As a result, overall mean mortality of all the plants showed parallel response to the level of concentration at different time intervals after treatment. Moreover, the ethanol extract of the leaf of *V. amygdalina* was found to be the concentration has percentage viability of 70.00, 2.00% has percentage viability of 68.67 and 1.00% has

percentage viability of 67.33. As a result, percentage viability of the seeds showed parallel response to the level of concentration after treatment.

Conclusion

This study suggested that *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina* possess insecticidal properties and they can be used to control variety of

Table 7. Percentage viability of the seeds sprayed with the ethanol extract of *O. gratissimum*, *S. acuta*, *T. occidentalis* and *V. amygdalina*.

Concentration	<i>O. gratissimum</i>	<i>S. acuta</i>	<i>T. occidentalis</i>	<i>V. amygdalina</i>
0.00% (control)	63.33	63.33	63.33	63.33
1.00	64.67	66.00	63.33	67.33
2.00	66.67	67.33	66.67	68.67
3.00	68.67	69.33	67.33	70.00
4.00	70.00	72.00	68.67	72.67

insect pests and vectors. However, further work is necessary to elucidate the structures of the biologically active components that are responsible for the insecticidal activity of these plants.

REFERENCES

- Abayomi S (1993). Historical review of Traditional Medicine in Africa. Spectrum Book Ltd, Ibadan pp. 9-25.
- Aderibigbe AO, Lawal BA, Oluwagbemi JO (1999). The antihyperglycemic effect of *Telfairia occidentalis* in mice. *Afri. J. Med. Sci.* 28: 171-175.
- Arzel E, ROcca P, Grellier P, Labaeid M, Frappier F, Guritte F, Gaspard C, Marais F, Godard A, Queguiner G (2001). New synthesis of benzo- δ -carbolines, cryptolepines and their salts: *in vitro* cytotoxic, antiplasmodial and antitrypanosomal activities of δ -carbolines, benzo- δ -carbolines and cryptolephines. *J. Med. Chem.* 44: 949-960.
- Babu R, Murugan K (1998). Interactive effect of neem seed kerna and neem gum extract on the control of *Culex quinquefasciatus* Say, *Neem Newsletter* 15(2): 9-11.
- Baladrin MF (1985). Natural Plant Chemicals: Sources of Industrial and Medicinal Materials. *Science* 228: 1154-1160.
- Banzouzi JT, Prado R, Menan H, Valentin A, Roumestan C, Mallie M, Pelissier Y, Blache Y (2004). Studies on medicinal plants of Ivory Coast: Investigation of *Sida acuta* for *in vitro* antiplasmodial activities and identification of an active constituent. *Phytomed.* 11: 338-341.
- Bierer DE, Fort DM, Mendez CD, Luo J, IMbach PA, Dubenko LG, Jolad SD, Gerber RE, Litvak J, Lu Q, Zhang P, Reed JM, Waldeck N, Bruening RC, Noamesi BK, Hector RF, Carlson TJ, King SR (1998). Ethnobotanical-directed discovery of the antihyperglycemic properties of cryptolepine: its isolation from *Cryptolepis sanguinolenta*, synthesis and *in vitro* and *in vivo* activities. *J. Med. Chem.* 41: 894-901.
- Bisht SS, Kamal R (1994). Garlic extract: An antifungal treatment for the control of storage of apple. *Proc. Nat. Acad. India* 64: 233-234.
- Bonjean K, De Pauw-Gillet MC, Defresne MP, Colson P, Houssier C, Dassonneville L, Bailly C, Greimers R, Wright C, Quentin-Leclercq J, Tits M, Angenot L (1998). The DNA Intercalatin alkaloid cryptolepine interferes with topoisomerase II and inhibits the primarily DNA synthesis in B16 melanoma cells. *Biochemistry* 37: 5136-5146.
- Boye GL, Ampofo O (1983). Proceedings on the first international seminar on cryptolepine (eds Boakye – Yiadom K, Bamgbose S.O.A.) 37 (University of Kumasi, Ghana).
- Brown AWA (1986). Insecticide resistance in mosquitoes: pragmatic review. *J. Am. Mosq. Control Ass.* 2: 123-140.
- Cao JH, Qi YP (1993). Studies on the chemical constituents of the herb huanghuaren (*Sida acuta* Burm.f.). *Zhongguo Zhong Yao Za Zhi* 18: 681-703.
- Cheng SS, Chang HT, Chang ST, Tsai KH, Chen WJ (2003). Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae, *Biores Technol.* 89(1): 99-102.
- Chitwood DJ (2003). Phytochemical based strategies for nematode control. *Annual Rev. Phytopathol.* 40: 221-249.
- Cimanga K, De Bruyne T, Pieters L, Totte J, Tona L, Kambu K, Vanden Berghe D, Vlietinck AJ (1998). Antibacterial and antifungal activities of neocryptolepine, biscryptolepine and cryptoquinoline alkaloids isolated from *Cryptolepis sanguinolenta*. *Phytomedicine* 5: 209-214.
- Clinquart E (1929). Sur la composition de *Cryptolepis triangularis*, plante congolaise. *Bull. Acad. Roy. Med. Belg.* 9: 627-635.
- Daiziel IM (1937). Pipercease. Useful plants of west Tropical Africa handbook. African Press, Ibadan, Nigeria pp. 16-17.
- Das NG, Goswami D, Rabha B (2007). Preliminary evaluation of mosquito larvicidal efficacy of plant extracts, *J. Vect. Borne Dis.* 44: 145-148.
- Dassonneville L, Lansiaux A, Wattelet A, Wattez N, Mathieu C, Van Miert S, Pieters L, Bailly C (2000). Cytotoxicity and cell cycle effect of the plant alkaloids cryptolepine and neocryptolepine: relation to drug-induced apoptosis. *Eur. J. Pharmacol.* 409: 9-18.
- Dhawan BN, Patnik GR, Rastogy RAT, Singh KK, Tandol TS (1997). Screening of Indian plants for biological activity. *YL India Exp. B.* 15: 108.
- Dina OA, Saba AB, Akhiromen IO, Adedapo AA, Davies OOE (2001). The effect of aqueous leaf extract of *Telfaria occidentalis* on isolated guinea pig ileum. *Afr. J. Biomed. Res.* 4: 53-54.
- Dinan L, Bourne P, Whittin P (2001). Phytoecdysteroid profiles in seeds of *Sida* spp. (Malvaceae). *Phytochem. Anal.* 12: 110-119.
- Dua VK, Nagpal BN, Sharma VP (1995). Repellent action of neem cream against mosquitoes. *Indian J. Malariol.* 32: 47-53.
- Dubey NK, Tiwari TN, Mandin D, Andriamboavonijy H, Chaumont JP (2000). Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). *Fitoterapia* 71(15): 567-569.
- Duncan DE (1955). Multiple range and multiple tests, *Biometrics* 11: 1-42.
- Dwuma-Badu D, Ayin JKS, Fiagbe NIY, Knapp JE, Shiff PL, Slatkin Djj (1978). Quindoline from *Cryptolepis sanguinolenta* J. *Pharm. Sci.* 67: 433-635.
- Ehiagbonare JE, Enabulele SA (2007). Effect of storage regime, presorting treatments, light and dark on seed germination of *Chrysophyllum deleuyoi* (De Wild). *Nig. J. Appl. Sci.* 25: 151-156.
- Fakae BB, Campbell AM, Barrett J, scott IM, Teesdale-Spittle PH, Liebau E, Brophy PM (2000). Inhibition of glutathione-S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigeria medicinal plants. *Phytother. Res.* 14(8): 630-634.
- Gills LS (1992). Ethnomedical uses of Plants in Nigeria. University of Benin Press, Nigeria p. 276.
- Guittat L, Alberti P, Rosu F, Van Miert S, Thetiot E, Pieters L, Gabelica V, De Pauw E, Ottaviani A, Roiu JF, Mergny JL (2003). Interaction of cryptolepine and neocryptolepine with unusual DNA structures. *Bioch.* 85: 535-541.
- Gunatilaka AAL, Sotheeswaran S, Balasubramaiam B, Chandraseka AI, Sriyani HTB (1980). Studies on medicinal plants of Sri Lanka III: Pharmacologically important alkaloids of some *Sida* species. *Planta Medica* 39: 66-72.
- Harborne JB (1973). *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis.* Chapman and Hall Ltd. London pp. 49-188.
- Harve G, Kamath V (2004). Larvicidal activity of plant extract used alone and in combination with known synthetic larvicidal agents against *Aedes aegypti*, *Indian J. Exptl. Biol.*, 42: 1216-1219.
- Hayashi T, Okamuka K, Kawasaki M, Morita N (1993). Production of diterpenoids by cultured cells from two Chemotypes *Scoparia Dulcis*. *Phytochemistry* 35(2): 353-356.
- Hayes JB Jr, Laws ER Jr (1991). *Handbook of pesticide toxicology, v.1.* Academic Press, San Diego pp. 24-28.

- Holets FB, Ueda-Nakamura T, Filho BPD, Cortez DAG, Morgado-Diaz JA, Nakamura CV (2003). Effect of essential oil of *Ocimum gratissimum* on the trypanosomatid *Herpetomonas samuelpeessoai*. Act. Protonzool 42: 269-276.
- Iwalokun AU, Bamiro BA, Durojaiye SB (2004). An antimicrobial evaluation of *V. amygdalina* (compositae) against gram positive and gram negative bacteria from Lagos, Nigeria. W. Afr. J. Pharmacol. Drug Res. 19: 9-15.
- Jang DS, Park EJ, Kang YH, Su BN, Hawthorne ME, Vigo JS, Graham JG, Cabieses F, Fong HH, Mehta RG, Pezzuto JM, Kinghorn AD (2003). Compounds obtained from *Sida acuta* with the potential to induce quinone reductase and to inhibit 7, 12 -dimethylbenz[a]anthracene-induced preneoplastic lesions in a mouse mammary organ culture model. Arch. Pharm. Res. 26(8): 585-590.
- Kabaru JM, Gichia L (2001). Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (rhizophoraceae) Lam. against three anthropoids: Afr. J. Sci. Tech (AJST). Sci. & Eng. Series 2(2): 44-49.
- Karou D, Dicko MH, sanon S, Simpore J, Traore AS (2003). Antimalarial activity of *Sida acuta* Burm f. (Malvaceae) and *Pterocarpus erinaceus* Poir (Fabaceae). J. Ethnopharmacol. 89: 291-294.
- Karou D, Dico MH, Simpore J, Traore AS (2005). Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. Afr. J. Biotechnol. 4: 823-828.
- Khalid SA, Duddeck H, Gonzalez-Sierra M (1989). Isolation and characterization of an antimalarial agent of the neem tree *Azadirachta indica*. J. Nat. Prod. 52: 922-926.
- Lisgarten JN, Coll M, Portugal J, Wright CW, Aymami J. (2002). The antimalarial and cytotoxic drug cryptolepine intercalates into DNA at cytosine-Cytosine sites. Nat. Struct. Biol. 9: 57-60.
- Longe OG, Farimu GO, Fetuga BL (1983). Nutritional value of fluted pumpkin. J. Agric. Food Chem. 31: 989-992.
- Macêdo ME, Consoli RAGB, Grandi TSM, Antônio MG dos Anjos, Alaide B de Oliveira, Mendes NM, Queiróz RO, Zani CL (1997). Screening of *Asteraceae* (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae), Mem. Inst. Oswaldo Cruz., Riode Janeiro 92(4): 565-570.
- Mahadevan A (1982). Biochemical aspects of plant disease resistance. Parti performed inhibitory substances-prohibitions. Today and Tomorrow Printers and Publishers. New Delhi, India pp. 125-129.
- Mwangi RW, Mukiyama TK (1988). Evaluation of *Melia volkensii* extract fractions as mosquito larvicides, J. Am. Mosq. Control Assoc. 4: 442-447.
- Nacoulma Ouedraogo OG (1996). Plantes medicinales et pratiques medicales traditionnelles au Burkina Faso: cas du plateau central MOssi. These D'Etat, Universite de Ouagadougou.
- Nadi KJ (2001). Toxicity of plant extracts to *T. granarium*. Pak. J. Biol. Sci. 4(12): 1503-1505.
- Nagpal BN, Srivastava A, Sharma VP (1996). Control of mosquito breeding using scrapings treated with neem oil. Indian J. Malariol. 32: 64-69.
- Nakamura CV, Nakamura TU, Bando E, Melo AJN, Cortez DAG, Dias Filho BP (1999). Antibacterial activity of *Ocimum gratissimum* essential oil. Mem. Inst. Oswaldo Cruz 94: 675-678.
- Oboh G (2004). Prevention of garlic-induced haemolytic anaemia by some tropical green leafy vegetables. Biomed. Res. 15: 134-137.
- Okwu DE (2001). Evaluation of chemical composition of Indigenous spices and flavouring agents. Global J. Pure Appl. Sci. 7(3): 455-459.
- Oyolu C (1978). Relatively unknown vegetables: Fluted pumpkin (*Telfairia occidentalis*). Proc. 1st Annual Conference of Horticultural Society Nigeria Nihort, Ibadan Nigeria.
- Pessoa LM, Morais SM, Bevilaqua CML, Luciano JHS (2002). Antihelmintic activity of essential oil of *Ocimum gratissimum* Linn and eugenol against *Haemaphysalis contortus*. Vet. Parasitol. 109: 59-63.
- Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA, Criddle DN (2003). Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in ice. Bra. J. Med. Biol. Res. 36: 521-524.
- Rahman SS, Rahman MM, Khan MMR, Begum SA, Roy B, Fakruddin Shahed SM (2007). Ethanolic extract of melgota (*Macaranga postulate*) for repellency, insecticidal activity against rice weevil (*Sitophilus oryzae*). Afr. J. Biotechnol. 6(4): 379-383.
- Rawls RL (1986). Experts probe issues, chemistry of light-activated pesticides. Chem. Eng. News, Sep. 22: 21-24.
- Roy B, Amin R, Uddin MN (2005). Leaf extracts *Shiyalmutra* (*Blumea lacera*) as botanical insecticides against lesser grain borer and rice weevil, J. Biol. Sci. 5(2): 201-204.
- Sofowora A (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books, Ibadan p. 150.
- Sofowora LA (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan pp. 55-71.
- Sukamar K, Perich MJ, Boobar LR (1991). Botanical derivatives in mosquito control: a review. J. Am. Mosq. Control Ass. 7: 210-237.
- Sulistiarini D, Oye LPA, Nguyen XD (1999). *Ocimum gratissimum* L. In: Plant Resources of South-East Asia. No. 19: Essential oils Plants. Prosea Foundation, Bogor, Indonesia pp. 140-142.
- Talukdar FA, Howse PE (1993). Deterrent and insecticidal effect of extract of pithraj, *Aphanamixis polystacha* against *Tribolium Castaneum*. J. Chem. Ecol. 19: 2463-2471.
- Trease GE, Evans WC (1989). Pharmacognosy. 13th edn. Bailliere Tindall, London pp. 176-180.
- Venketachalam MR, Jebasan A (2001). Repellent activity of *Ferronia elephantum* Corr, (Rutaceae) leaf extract against *Aedes aegypti*, Biores Technol. 76(3): 287-288.
- Wink M (1993). Production and application of phytochemicals from agricultural perspective. In: Van Beck TA, Breteler H, Editors, Phytochemistry and agriculture, Clarendon Press, Oxford, UK pp. 171-213.
- Wright CW, Addae-Kyereme J, Breen AG, Brown JE, Cox MF, Croft SL, Gokcek Y, Kendrick H, Philips RM, Pollet PL (2001). Synthesis and evaluation of cryptolepine analogues for their potential as new antimalarial agents. J. Med. Chem. 44: 3187-3194.
- Yang SW, Abdel-Dader M, Malone S, werkhoven MCM, Wisse JH, Bursuker I, Neddermann K, Fairchild C, Raventos-Suarez C, Menendez AT, Lane K, Kingston DGI (1999). Synthesis and biological evaluation analogues of cryptolepine, an alkaloid isolated from the Surinm rainforest. J. Nat. Prod. 62: 976-983.