WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 17, 13-22. Research Article ISSN 2277– 7105

ENTOMOCIDAL EFFICACY OF ETHANOLIC EXTRACT AND PHYTOCHEMICAL SCREENING OF SOURSOP (*ANNONA MURICATA*) SEEDS AGAINST BRUCHID WEEVILS OF STORED *VIGNA SUBTERRANEAN* L. VERC. IN NIGERIA

[1]*Ubi Godwin Michael, [1]Ogbonna Nneka Constance, [2]Amaefule Comfort Chioma and [3]Anietie Rosemary Bassey

[1]Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria.

[2]Department of Biological Sciences, Abia State University, Uturu, Abia State, Nigeria.

[3]Department of Plant science and ecological studies, Faculty of Biological Sciences, University of Calabar, Nigeria.

Article Received on
24 July 2018,

Revised on 14 August 2018,
Accepted on 04 Sep. 2018,

DOI: 10.20959/wjpr201817-11938

***Corresponding Author**
Ubi Godwin Michael
Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria.

ABSTRACT

Experiment was conducted in 2016/2017 to evaluate the insecticidal properties of Sour-sop fruit seed extracts on the mortality of Bambara groundnut (*Vigna subterranea*) bean weevil, *Callosobruchus maculatus* in the Department of Genetics and Biotechnology, University of Calabar, Nigeria. One hundred bruchid weevils destroying stored Bambara groundnut seeds were counted and introduced into perforated plastic containers containing fifty uninfected and untreated Bambara groundnut seeds. Three (3) g each of cotton wool was soaked differently in 10%, 20%, 30%, 40% and 50% prepared concentrations of ethanol and aqueous extracts of sour-sop seeds and placed inside each of the plastics containing 100 weevils with 50 grains each. The experimental plastics containers were kept in the store under normal room temperature and pressure and observed for mortality and fecundity after 2, 4, 8, 16, 32 and 64 days interval respectively. Five (5) sour-sop seeds were dried, crushed and taken to laboratory for phytochemical analysis using ethanol and aqueous extractants with standard procedures of the Association of Official Analytical Chemist, 2006. The results showed that more of the phytochemical compounds were released when ethanol was used as the extracting solvent suggesting that the active compounds dissolved more in ethanol solvent

than in the aqueous solvent. The results further showed that tannins and phenolic compounds were the most abundant in the sour-sop seeds when ethanol extractant was used. Results of insecticidal properties of ethanol and aqueous seed extract showed 100% mortality of weevils treated with 40% and 50% of the ethanolic seed extract after 2 days of storage. In the same time duration, 50 and 5 insects died respectively when treated with 30% and 20% ethanol seed extract of sour-sop. No mortality was recorded for the control and for weevils treated with 10% ethanolic seed extract of Soursop at this time interval. The results further revealed that after 8 days of treatment application and grain storage, 100% mortality was recorded for the weevils treated with 50% ethanol seed extract of sour-sop. It was revealed from the study that 40% ethanol seed extract of sour-sop was the effective minimum inhibitory concentrated needed to control the bruchid weevils against stored grains. The weevil fecundity was highly inhibited at all the concentrations except in the control experiment were no extract was applied. The study thus recommended that 40% ethanolic extract of soursop seeds can be used as an alternative to synthetic chemicals for controlling Bambara groundnut seeds weevils in storage.

KEYWORDS: Weevil mortality, ethanol and aqueous extracts, *Annona muricata* seeds, phytochemical screening, Bambara groundnut.

1.1 INTRODUCTION

Plants have always found a place in the existence of man as they play a major significant role either as food, medicine or by products for human utilization. Man's life had depended solely on plants not just for food but as key or starting organisms in the food chain of living things. These and many more have made it a fixed characteristic of man to unravel which plants are nutritious, medicinal and perhaps for the production of goods and services.

Legumes have historically been part of inexpensive meals throughout the world as they have a major role in the fight against malnutrition. It is therefore necessary that their levels of consumption, which are already too low in a number of developing countries, be increased (Brink *et al*., 2006). Plant proteins provide nearly 65% of the world supply of proteins for humans; 45-50% legumes and 10-15% cereals.

Vigna subterranea (L.) Verdc. [synonym *Voandzeia subterranea* (L.)] is commonly called Bambara ground-nut, Hog-bean, Congo-goober, earth-pea or ground-bean. It is an indigenous African legume, where it is the third most important legume in terms of consumption and

socioeconomic impact in semi-arid Africa, behind peanut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*).

Annona muricata, commonly called soursop (Graviola) due to the sweet and sour flavor of the large fruit, belongs to the family Annonaceae and is indigenous to tropical North and South America. It is also found in parts of Africa. In some parts of Nigeria, the fruit is locally called "Shawa shawa", "Shawa shop" or "chop chop". It is one of the exotic fruits prized for its very pleasant, sub-acidic, aromatic and juicy flesh which consists of edible white pulp and a core of indigestible black seeds (Lutchmedial, 2004). Aside its nutritional importance, soursop has been used indigenously in Nigeria, for centuries (that is bark, leaves, roots, flowers, fruits and seeds) for the treatment of coronary diseases, asthma, liver problems, arthritis etc. Extracts of the leaves, barks and seeds have also shown some astringent, insecticidal, fungicidal, antimicrobial and antibacterial properties. (Asprey,1995).

In recent years, secondary metabolites previously with unknown pharmacological activities have been extensively investigated as source of medicinal and therapeutic agents. There has also been a gradual revival of interest in the use of medicinal plants in developing countries such as Nigeria due to their safety and showing no adverse effect especially when compared to synthetic drugs. The unripe fruits, seeds, leaves and roots have been widely used as biopesticides, bioinsecticides and as insect repellent (Isman and Akthar, 2007).

From time immemorial, man has understood the importance, value, properties and even dangers of the chemicals that are found in plant organelles, be they fresh, dried, fermented or processed in many other ways. Thus, an understanding of the inherent potentials (like nutritive, curative, and poisonous properties) of plants has been of fundamental importance to the survival and evolution of humans and has shaped the way man has lived on utilizing them.

Nigeria in Africa, unarguably, has one of the richest biodiversity hotspot in the world. It forests geographically spans approximately 216,634,000 ha (Farombi, 2003). In today's era, virtually all modern clinical drugs are of plant products in drug development programmes of the pharmaceutical industry. Plants have also been found very useful in the food and nutritional industry. Furthermore, the consumption of plant materials is believed to contribute immensely to the health improvement of man and other animals. Yedjou et al., (2008) had estimated that about 80% of Africans depend on medicinal plants to satisfy their health care

requirements. Thus, the knowledge of the nutritional, medicinal and economic value of plants found in Africa could add value to their cultivation, consumption, conservation and commercialization. Hence, the proper exploitation of this knowledge, could further enhance the fight against hunger, disease and poverty which are daunting challenges in Nigeria. The phytochemical analysis of soursop seed will also enhance our understanding of the chemical constituents and pharmacological value of soursop seeds for further industrial exploitation.

2.1 MATERIALS AND METHODS

One hundred bruchid weevils destroying stored cowpeas were counted and introduced into perforated plastic containers containing fifty uninfected and untreated bamabara groundnut seeds. Three (3) g each of cotton wool was soaked differently in 10%, 20%, 30%, 40% and 50% prepared concentrations of ethanol and aqueous extracts of sour-sop seeds and placed inside each of the plastics containing 100 weevils with 50 grains each. The experimental plastics containing the experiments were in kept in the store, under normal room temperature and pressure and observed for mortality and fecundity after 2, 4, 8, 16, 32 and 64 days interval respectively.

2.2 Methods for phytochemical screening of soursop seed extracts

The seeds of soursop were dried, crushed and taken to laboratory for phytochemical analysis using ethanol and aqeuous extractants following standard procedures of the Association of Official Analytical Chemist, 2006.

Extraction of flavonoids

Two methods were used to extract flavonoids:

a. A portion of the soursop seed extract was heated with 10ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution, a yellow coloration indicated the presence of flavonoids.
b. Dilute ammonia (5ml) was added: a yellow coloration indicated the presence of flavonoids.

Extraction of Alkaloids

Extracts was dissolved individually in dilute Hcl and filtered

a. Filtrate was treated with Mayer's reagents (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids

b. Filtrate was treated with Dragendul's reagent (solution of potassium bismut iodide). Formation of red precipitate indicates the presence of alkaloids.

Filtrate was treated with Hager's reagent (saturated pricic acid solution) presence of alkaloid is confirmed by the formation of yellow colour precipitate.

Extraction of Saponins

0.5g of soursop seed extract was added to 5ml of distilled water in a test tube and 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 after which it was observed for the formation of an emulsion.

Extraction of Triterpenoids

0.5g of soursop seed extract was added in 1ml of chloroform. 1ml of acetic anhydride was also added, followed by the addition of 2ml of concentrated H_2S_{O4}. Formation of reddish violet indicated the presences of triterpenoids.

Extraction of Tannins

Two methods were used to test for the presence of tannins in the soursop seed extracts:

a) To 10ml of freshly prepared 10% KOH in a beaker, 0.5g of raw extract will be added and shaken to dissolve. A dirty precipitate observed indicated the presence of Tannins
b) About 0.5g of soursop seed extract was boiled in 10ml of water in a test tube and then filtered. A drop of 0.1% ferric chloride was added and the solution was observed for brownish green or a blue-black coloration.

Extraction of reducing sugar (Fehling's test)

0.5g of soursop seed extract was dissolved in 5ml distilled water and filtered while hot. The filtrate was hydrolysed with dilute HCI, neutralize with alkali (NaOH) and heated with Fehling's A and B solutions. Formation of red precipitate indicated the presence of reducing sugar.

Extraction of anthraquinones

0.5g of soursop seed extract was boiled with 10ml of H_2SO_4 and filtered. The filtrate was shaken with 5ml of chloroform, the chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes which indicated the presence of anthraquinones.

Extraction of steroids

0.5g of soursop seed extract was dissolved in 10ml of chloroform and equal volume of concentrated H_2SO_4 was added by the sides of the test tubes. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicated the presence of steroids.

Extraction of cardiac glycosides (Keller-Killian test)

To 0.5g of soursop seed extract dissolved in 5ml water was added to 2ml of glacial acetic solution containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated H_2SO_4. A brown ring at the interface l indicated the presence of deoxysugar characteristics of cardenolides. A violet ring appeared below the brown ring while the acetic acid layer a greenish ring was formed above the brown ring and gradually spread through this layer.

3.1 RESULTS

Results of phytochemical screening of the water and ethanol extracts of the seeds of sour-sop revealed that the seeds of sour-sop do possessed to varying degrees some phytochemical compounds that activated their effect against the bruchid weevils. The solvent used for the extraction of the crude extracts influenced the amount (concentration) of some of the extracted components in the crude extracts. This was evident in the differences observed in the results obtained for water and ethanol extracts of the plant seed materials. More of the phytochemical compounds were released when ethanol was used as the extracting solvent suggesting that the active compounds dissolved more in ethanol solvent than in the aqueous solvent (Table 1). The results further showed that tannins and phenolic compounds were more abundant in the seeds with ethanol extraction than other compounds and must have accounted for the greater percent of the insecticidal properties in the seeds of sour-sop.

Table 1: Phytochemical screening and profiling of ethanol and aqueous extract of sour-sop seeds.

Phytochemical	Ethanolic extractant	Aqueous extractant
Alkaloids	++	+
Tannins	+++	++
Saponins	+	+
Phlobotanins	-	-
Anthraquinone	+	-
Flavonoids	+	+
Cardiac glycoside	+	-
Phenols	+++	++

Note: +++ = Very largely present, ++ = Largely present, + = Slight presence - = not present

Results of efficacy of seed extracts of sour-sop on the grain weevils *Callosobruchus maculatus* stored after 64 days are presented in Table 2. It showed 100% mortality of weevils treated with 40% and 50% of the ethanol seed extract after 2 days of storage. In the same time duration, 50 and 5 insects died respectively when treated with 30% and 20% ethanol seed extract of sour-sop. No mortality was recorded for the control experimental unit and for weevils treated with 10% ethanolic seed extract of Soursop at this time interval. The results further revealed that after 8 days of treatment application and grain storage, 100% mortality was recorded for the weevils treated with 30% ethanol seed extract of sour-sop. It was revealed from the study that 40% ethanol seed extract of sour-sop was the effective minimum inhibitory concentrated needed to control the bruchid weevils against stored grains. The weevil fecundity was highly inhibited at all the concentrations except in the control experiment were no extract was applied.

Table 2: Percentage mortality of *Callosobruchus maculatus* bruchid weevils on stored grains treated with different concentrations of Ethanol extract of sour-sop seeds after 2 months of storage.

Percentage Mortality of Weevils (%)						
Days After Ethanol Seed Extract Treatment Application						
Ethanol Seed Extract	**2days**	**4days**	**8days**	**16days**	**32days**	**64days**
10%	0	2	4	5	10	40
20%	5	20	25	25	25	50
30%	50	70	100	100	100	100
40%	100	100	100	100	100	100
50%	100	100	100	100	100	100
0% (control)	0	0	0	0	0	0

Results of efficacy of aqueous seed extracts of sour-sop on the grain weevils *Callosobruchus maculatus* stored after 64 days are also presented in Table 3. It shows that compared to ethanolic seed extract of soursop, aqueous seed extract had very low effect on the weevils. As shown in table 3, a maximum of 14% mortality of weevils was recorded when stored grains were treated with 50% of aqeuous seed extract of soursop after 64 days of storage. In the same time duration, 11% and 10% insects died respectively when treated with 40% and 30% aqueous seed extract of soursop. Results further showed that no mortality was recorded for the all the treatments and control for weevils after 4 days of treatment application. The results further revealed the potency of aqueous seed extract of soursop began to be effective after 8 days of treatment application recording only 2% mortality of weevils in stored grains treated with 30%, 40% and 50% concentrations of aqueous seed extract respectively. The percentage

mortality of weevils increased slightly and marginally with increasing concentrations of the aqueous seed extract after 16 and 32 days of grain storage respectively. The weevil fecundity was highly inhibited at all the concentrations except in the control experiment were no extract was applied.

Table 3: Percentage mortality of *Callosobruchus maculatus* bruchid weevils on stored grains treated with different concentrations of Aqueous extract of sour-sop seeds after 2 months of storage.

Percentage Mortality of Weevils (%)						
Days after Aqueous seed extract treatment application						
Aqueous Seed Extract	**2days**	**4days**	**8days**	**16days**	**32days**	**64days**
10%	0	0	0	2	2	5
20%	0	0	0	4	6	6
30%	0	0	2	4	8	10
40%	0	0	2	4	9	11
50%	0	0	2	4	10	14
0%(Control)	0	0	0	0	0	0

3.2 DISCUSSION

The findings from this study has shown that, the bruchid weevil, Callosobruchus maculatus, which is very destructive against stored grain legumes like Bamabara groundnut (*Vigna subterranea*); Cowpea (*Vigna unguiculata*), Pigeon pea (*Cajanus cajan*), Common bean (*Phaseolus vulgaris*) etc. can be completely controlled in store using ethanolic extract of soursop (*Annona muricata*) seeds. The findings has also shown that, to control the bruchid weevils attacking the grain legumes, there was no direct contact between the stored grains and the soursop seed extracted liquid, thus the extract can be used as a fumigant. The results of the phytochemical profiles of the soursop seed extract had revealed that most of the phytochemical dissolved in ethanol compared to water, with tannins and phenolic compounds dissolving in higher concentrations. These compounds were thus responsible for the aromatic odour which was entomocidal to the bruchid weevils. The study had also shown that with a minimal lethal concentration of 40% of sour-sop seed extract, all the weevils died within 48 hrs of application. This by implication further suggest that application of any dose greater than 50% or simply doubling of the concentration can kill all the weevils within or in less than 24 hrs after application. This result can be harnessed in the pharmaceutical industry for the production of fumigants and insecticides for the control of insect borne diseases and insect vectors of human diseases such mosquitoes. These findings are in conformity with the reports of ken and Robert, 2014; Tai, *et al.*, 2011, Ming *et al.*, 1998 and Roblot *et al.*, 1993).

4.1 CONCLUSION

The study has revealed that ehanolic extract of phytochemicals contained in the seeds of Soursop (*Anona muricata*) possess insecticidal properties and are entomocidal which can be effectively used against bruchid weevils ravaging stored grain legumes in this region, which have plunged the teeming growing populace into hunger and malnutrition. The study findings was very significant because it also revealed that the bruchid weevils can be controlled with the ethanolic seed extract by mere aroma and not by direct contact between the edible seeds of the legume and the liquid extract. Therefore, the use of the seed extract of soursop as a fumigating agent could best help to achieve the desired goal. Much more, the phytochemicals therein soursop seeds can be harnessed by the pharmaceutical industry for the development of therapeutic drugs for the control of other insect vectors of human diseases as well as insect borne diseases.

ACKNOWLEDGEMENT

The authors which to acknowledge the Training and Technical Assistance Department of the Cross River State Fadama Development project and the research coordinating unit of Farm focus international.org.ng.

COMPETING INTEREST

The authors declare that there are no competing interests in this manuscript.

AUTHORS CONTRIBUTION

All authors contributed immensely in the development of this manuscript from the design, writing of manuscript sections, statistical analysis, proof reading / editing and financial contribution.

REFERENCES

1. Abbo, E.S, Olurin, T.O and odeyemi, G (2006) Studies on the storage stability of soursop (Annona muricata L.) juice. *African Journal of Biotechnology*, 19: 1808-1812.
2. Asprey, G.F and Thompson, P (1995) Medicinal plants of Jamaica. *West Indian medicinal journal*, 4: 69–72.
3. Brown, J, Laurentin, H and Davila, M (2003) Genetic relationships between nine *Annona muricata* accessions using RAPD markers. *Fruits*, 58: 255-259.
4. Erlindha, G and Lia Kartika, S (2014) Antimicrobial and biological activity of extracts of herbs parasite soursop (Dendropthoe pentandre L). *Ecobotany*, 1: 33-37.

5. Farombi, E.O (20030 African indigenous plants with chemotherapeutic potentials and biotech nological approach to the production of bioactive prophylactic agents. *African journal of Biotechnology*, 2: 662 -667.
6. Gleye, C. Laurens, A. Hocquemiller, R., Laprevote, O., Serani, L and cave, A (1997) Cohibins A and B, acetogenins from roots of *Annona muricata* L. *Ecobotany*, 3: 51-56.
7. Hodgkin, T., Brown, A., Hintum, T and Morales, E (1995) Morphological Characterization of soursop (*Annona muricata* L.) *Journal of Agricultural Science*, 1(2): 141-145.
8. Idayat, S.I., Samira, A and Muslim, A (2014) Extraction and Phytochemical Screening of The root and leaf of *Annona muricata* L. *Journal of Agricultural Sciences*, 2(2): 445-450.
9. Ken, L and Robert E. (2014) Phytochemical screening, antioxidant activity and in vitro Anticancer potential of ethanolic and water leaves extracts of *Annona muricata. Journal of Microbiology*, 2(3): 128-133.
10. Kim, G., Zeng, L Agali, F., Rogers, L. Wu, F., Sastrodihardo, S and Mclaughin, J (1998) muricoreancin and murihexocin C, monotet rahydrofuran acetogenins, from leaves of *Annona muricata*. L. *Phytochemistry*, 3: 565-571.
11. Lutchmedial, M., ramlal, R., Badrie, N., and Chang-Yen, I(2004). Nutritional and sensory ouality of stirred soursop (*Annona muricata* L.) Yorghurt. *International Journal of food Science Nutrition*, 55: 407 - 414.
12. Ming, L., Hua, T., Lan, Z.H., Qing, M., Jiang, H., Neng, H. and Jun, Z (1998). Cyclopeptide from the seeds of *Annona muricata*. *Phytochemistry*, 3: 555 – 556.
13. Prashat, T, Bimlesh, K, Mandeep, K. Gurpreet K and Harleen K.(2011). Phytochemical screening and Extraction: A review: *Internationale pharmaceutica sciencia*, 2: 203 -208.
14. Roblot, F. Laugel, T., Lebeuf, M., Cave, A and Laprevote, O (1993) Two acetogenins from *Annona muricata* seeds. *Phytochemistry*, 3: 281 – 285.
15. Sarah, Z. Ratna, D. and Siti, N (2005). Identification of Soursop seeds (*Annona muricata*) seeds extracts as a candidate against the Aedes aegypti L in Mosquito vector control. *Phyto-chemistry*, 2: 123 – 127.
16. Tai, H., Kedari, F and Ayesha A.K (2011) *Annona muricata*: A review of its traditional uses. *Phytochemistry and Pharmacology*, 2: 34 – 39.
17. Usunobun, U., Okolie, N., Anyanwu, O and Adegbegi, A.J (2014) phytochemical screening and proximate composition of *Annona muricata* leaves. *Journal of ecobotany*, 3: 112 – 116.