Bayero Journal of Pure and Applied Sciences, 4(1): 145 - 147
Received: August, 2010
Accepted: June, 2011
ISSN 2006 - 6996

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *CUCURBITA PEPO* (PUMPKIN) AGAINST *STAPHYLOCOCCUS AUREUS* AND *SALMONELLA TYPHI*

***Chonoko, U. G. and Rufai, A. B.**
Department of Applied Science College of Science and Technology Kaduna Polytechnic, Kaduna.
*Correspondence author

ABSTRACT
Phytochemical screening and antibacterial activity of the extracts of Cucurbita pepo (backpeel and seeds) against Staphylococcus aureus and Salmonella typhi were carried out using standard procedures. The extraction was achieved using percolation method with ethanol and methanol as solvents. Higher yield of the extract was obtained from the backpeel. Phytochemical screening revealed differences in the presence of phytochemicals among the extracts. The result showed the presence of saponins in both methanol and ethanol extracts but tannins were recorded only in back peel methanol extract. The results of antibacterial activity showed that ethanol extract of Cucurbita pepo (backpeel) was active against Staphylococcus aureus and Salmonella typhi (07mm). The methanol extract was active against S. aureus but inactive against S. typhi at 500 µg/disc. Similarly, both ethanol and methanol extracts were active against S. aureus but inactive against S. typhi (06mm) at 500 µg/disc. The implication of this study in relation to ethnomedicinal uses of this plant has been discussed.

Key words: Phytochemical Screening, Antibacterial activity, Cucurbita pepo, Staphylococcus aureus and Salmonella typhi.

INTRODUCTION

Cucurbita pepo (pumpkin) belongs to the family Cucurbitaceae which includes cucumbers, melons, squash and gourds. The word 'pumpkin' comes from a Greek word "pepon" meaning large melon. While the English name is pumpkin or pompion, a term which dated as far back as 1547BC. The cucurbitaceae include the genera of Citrullus eg *C. lanatus* (water melon), Cucumis eg *C. melo* (Cucumber) (Bradley, 1992).

Cultivated pumpkins are believed to have originated for central Africa as far back as 5500BC and are now grown all over the world (Satyavati *et al*., 1976). They vary considerably in shape, size and flavour. *Cucurbita pepo* (Pumpkin) has long been used as food and a source of lamp oil, but now, it serves as a raw material of paramedicinal product (Dermarderosian and Butler, 2002).

Pumpkin is used as an emollient to soften the dryness of the skin and pimples spots (Duke and Ayensu 1985). They also reported that the seed oil of pumpkins has been widely used in culinary and nutiaceutical
arena due to its many nutritional benefits. For medicinal purposes, pumpkin has been used as a gentle and safe remedy for a number of complaint especially as an effective tapeworm expeller for children and pregnant women for whom stronger acting and toxic remedies are unsuitable (Cucurbitaceae 1995).

Pumpkin seed can be eaten fresh or roasted for the relief of abdominal cramps and distension due to intestinal worms. About 800 peeled seeds is said to make a safe and effective treatment for tape worm. They are ground into fine flour, then made into an emulsion with water and eaten. It is then necessary to take a purge in order to expel the tapeworms or other parasites from the body (Michael *et al.,* 2008) .This study seeks to screen *Cucurbita pepo* for the presence of phytochemicals and to determine the antibacterial activity of the plant extracts (back peel & seed) against *Staphylococcus aureus* and *Salmonella typhi*.

MATERIALS AND METHODS

Sample Collection

Cucurbita pepo (pumpkin) fruit was bought from Kaduna central market in February, 2009. It was packaged in a polythene bag and transported to the Department of Applied Science, Kaduna polytechnic. Authentication of the plant specie was done by comparing it with the voucher specimens available at the Departmental herbarium. The pumpkin was properly washed and sliced using knife. The backpeel and seeds were air dried by spreading on sacks in an aerated room for 6 weeks. They were then ground in to powder using pestle and mortar in the laboratory as described by Mukhtar and Tukur (1999).

Extraction

Fifty grams (50g) each of the powdered plant materials was soaked in 500ml of ethanol and methanol in separate conical flasks and kept for two weeks in a shaker after which the mixture was filtered. The filtrate was evaporated at room temperature (Fatope *et al*., 1993).

Phytochemical screening

Test for Saponins

The plant extract (1ml) was transferred into a test tube. Distilled water (1ml) was added to the test tube and shaken vigorously. Persistent froth that last for about 15 minutes would indicate the presence of saponins (Sofowora, 1993).

Test for Tannins

Two mls of each of the extract was diluted with distilled water in separate test tubes and 2-3 drops of 5% ferric chloride ($Fecl_3$) was added. A green –black or blue colouration would indicate the presence of tannin (Ciulci, 1994)

Test for Flavonoids

To each of the extracts (2ml) was transferred into different test-tube. Ten percent sodiumhydroxide (1ml) was added to each of the test tubes with the extracts. Three drops of dilute hydrochloric acid (HCL) were added to each of the extract. A change in colour from yellow to colorless indicates positive result. (Trease and Evans, 2002).

Test for Alkaloids

Each of the extracts (1ml) was stirred with 1% aqueous HCL (3ml) on a hot water bath and then filtered. The filtrates were treated with Meyer's reagent. A buff precipitate indicates presence alkaloids. (Trease and Evans, 2002).

Test for Steroids

Salkowski test was adopted in which the extract (2ml) was each transferred in to a test tube containing chloroform (2ml), concentrated sulphuric acid (H_2SO_4) was subsequently added to form a lower layer. A reddish brown ring at the interface of the two liquids and a violet colour in the supernatant layer indicated the presence of steroids (Sofowora, 1993).

Antimicrobial disc preparation

Discs of about 6mm diameter were made from What man's No.1 filter paper using a paper puncher. The discs were transferred in to Bijou bottles and sterilized in the oven at 121^{o}C for 15 minutes. Sensitivity discs were prepared by serial doubling dilution of the extract in Dimethyl Sulfoxide (DMSO). The paper discs were placed in the solution such that each disc take up 0.01m to make the disc potencies of 500, 1000 and 2000µg/disc.

Test Isolates

The test isolates used for this research were confirmed *Staphylococcus aureus* and *Salmonella typhi* isolates obtained from Barau Dikko Specialist Hospital, Kaduna in 2009.

Standardization of Inoculum

Few colonies of the confirmed isolates were dispensed in sterile normal saline to match the 0.5 McFarland Standard for sensitivity test as described by NCCLS (1999).

Antimicrobial Assay

Antimicrobial assay was achieved by disc diffusion method (NCCLS, 1999). Standardized inoculum of *Staphylococcus aureus* and *Salmonella typhi* were swabbed on to the surface of prepared and solidified Mueller Hinton Agar in separate Petri – dishes. This was followed by placing the prepared discs of the extracts and standard antibiotic discs on to the surface of inoculated media at intervals. The plates were incubated at 37^{0}C for 24 hrs. After incubation, zone of inhibition (diameter) formed by the organisms were measured to determine antibacterial effectiveness of the different concentrations of the extracts used.

RESULTS AND DISCUSSION

The results of the percentage yield of *Cucurbita pepo* (pumpkin) backpeel and seed is presented in Table 1. Higher yield was observed for backpeel (73.8%) as compared to the seed which yielded (51.7%). The result of this study will help in providing extracts that may be useful in ethno botanical studies. It also help in providing information for the treatment of bacterial infections using *C. pepo* plant part extractives.

Phytochemical screening of the plant extract showed that saponins were present in all the extracts. However, only flavonoids were not present in backpeel methanol extract but were recorded in the ethanol extract. Tannins were absent in the seed ethanol extract while flavonoids, alkaloids, and steroids were present. Saponins and tannins were also present in seed ethanol extract but flavonoids, alkaloids, and steroids were absent (Table 2). The presence of phenolic compounds predominantly in the seed and pulp of *Cucurbita. pepo* such as alkaloids, sapponins and steroids which have been found to be used as anti inflammatory and anti- oxidant agents (David 1989). Waterman (1992) also reported that the main class of phenolics predominant in both the fruit and leaf extract were alkaloids and flavonoids which are found useful in medicine as antimicrobial, anti inflammatory and anti-oxidant agents.

The result of sensitivity test showed that both back peel and seed extract of *Cucurbita pepo* were active against the test isolates as compared to the sensitivity of the isolates to standard Streptomycin disc with ethanol extract being active on both *Staphylococcus aureus* and *Salmonella typhi* while methanol extract against *Staphylococcus aureus* at 500 µg/disc concentration (Table 3) The methanol seed extract was observed to be more active than ethanol extract with *Salmonella typhi* being less sensitive (06mm) to both extracts at 500 µg/disc concentration (Table 4). A significant antibacterial activity exhibited by the plant materials may be linked to the presence of steroids flavonoids, tannins, alkaloids and saponins which were reported to posses antimicrobial activity (Bourgard *et al*., 1994).

Table 1: percentage yield of Extracts

Sample	Initial weight (g)	weight of Extract (g)	Yield (%)
Backpeel	30	24.6	73.8
Seed	30	16.1	51.7

Table 2: Phytochemical Properties of the extracts

Phytochemicals	BME	BEE	SME	SEE
Sapponins	+	+	+	+
Tannins	+	-	+	-
Flavonoids	-	+	-	+
Alkaloids	+	+	-	+
Steroids	+	-	-	+

KEY: **BME=** Backpeel methanol extract, **BEE=** Backpeel ethanol extract**, SME=** Seed methanol extract**, SEE=** Seed ethanol extract, + = presence, - = Absence

Table 3: Sensitivity of *Staphylococcus aureus* and *Salmonella typhi* to backpeel extract

	EE			ME			
	Concentration (µg/disc)						
	500	1000	2000	500	1000	2000	SXT (30µg)
	Zones of Inhibition (mm)						
Staphylococcus aureus	07	08	09	07	09	10	10
Salmonella typhi	07	07	08	06	08	08	12

KEY: EE = Ethanol extracts, **ME =** Methanol extracts, **SXT =** Streptomycin disc

Table 4: Sensitivity of *Staphylococcus aureus* and *Salmonella typhi* to seed extract

	EE			ME			
	Concentration (µg/disc)						
	500	1000	2000	500	1000	2000	SXT (30µg)
	Zones of Inhibition (mm)						
Staphylococcus aureus	07	07	09	07	08	09	11
Salmonella typhi	06	07	08	06	08	09	12

KEY: EE = Ethanol extracts, **ME =** Methanol extracts, **SXT =** Streptomycin disc

CONCLUSION

High yield of extract was obtained from the backpeel of the fruit. *C pepo* has the potentials for production of antibacterial agents. The backpeel and seeds contains steroids and alkaloids. *Staphylococcus aureus* and *Salmonella typhi* were both observed susceptible to the extracts at 500 µg/disc.

Recommendations

Based on the findings from this study, it is recommended that:

Further research should be carried out especially in the area of toxicity of the plant. The extracts should also be tested on other pathogenic microorganisms. The backpeel of this plant should be used during extraction for higher yield of extract.

REFERENCES

Akinyemi, K. O., Oladapo, O., Okwara, C. E., Ibe, C. C. and Fasure, K. A. (2005). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicilin resistant *S. aureus* activity. *BMC Complimentary Alternative Medicine*. 5:6

Bourgard, F., Putaraud, A. and Guckert, A. (1994). *Extraction of Coumanins from plant material* (Leguminocea). Phytochemical Analysis Pp 127-132.

Bradley, P. R. (1992). *British Herbal Compendium.* British Herbal Medicine Association, Bournemouth, Dorset, UK. Vol **1**, pp: 112–4.

Ciulci, I. (1994). Methodology for the analysis of vegetable drugs. Chemical industries branch, Division of industrial operations. UNIDO, Romania: 24, 26 and 67.

Cucurbitaceae. (1995). In *Van Nostrand's Scientific Encyclopedia* (8th ed.). New York: Van Nostrand Reinhold.

David, G.W. (1989). Antimicrobial chemotherapy oxford mechanical Publication machine Pp. 91 – 99

Duke, J. A and Ayensu E. S. (1985). Medicinal plant of china Reference Publication. ISBN. 0-972-56-20-4

Dermarderosian, A. and Butler, J. (2002). The review of natural products St Louis, Moo facts and coupasim Pp.23-20

Fatope, A. O., Ibrahim, H. and Takeda, Y. (1993). Screening of higher plants reputed as pesticides using brine shrimp lethality bioassay. International Journal of Pharmacognosy **31:** 250-256.

Michael, O. D., George, L. G. and Jayson K. H. (2008). "Pumpkin Production." *Agricultural Alternatives. Penn State College of Agricultural Sciences*. .

Mukhtar, M. D. and Tukur A. (1999). In-vitro screening for activity of *Pistia stratiotes* extracts. *NISEB Journal* **1 (1)**: 51-60.

NCCLS (1999). Performance standard ofr the antimicrobial susceptibility testing. National Committee for Clinical Laboratory approved Standard M100-59.

Satyavati, G.V., Raina, M. K. and Sharma, M. (1976). *Medicinal Plants of India.* Indian Council of Medical Research, New Delhi. Vol. **I**, pp: 201–06.

Sofowora, E. A. (1993). *Medical Plant and Traditional Medicine in Africa* (2nd editiotin), Spectrum Books Limited Pp 44-58, 210-212.

Trease and Evans (2002) *Early Readers in phytochemisry* University of Nettingham, U. K. (4th edition), 121-134

Waterman, P. H.(1992). Searching for Bioactive compounds various Strategies *Journal of National products* **53**(1) 13-22.