Journal of Forestry Research and Management. Vol. 18(3).67-74; 2021, ISSN 0189-8418

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Phytochemical and Brine Shrimp Lethality Properties of Methanol Extract of Pumpkin (*Cucurbita pepo* L.) Fruit Shell.

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ABSTRACT

Cucurbita pepo fruit shell which is often regarded as waste and disposed has been reported to be a rich source of nutrients and minerals such as carbohydrates, crude fibre, sodium, potassium and phosphorus. This study was carried out to identify the phytochemical constituents, and determine the bioactivity of *Cucurbita pepo* fruit shell. The methanol extract of the pumpkin fruit shell was subjected to preliminary qualitative phytochemical screening while the bioactivity of the extract was determined using the brine shrimp lethality assay, and mortality data obtained from the brine shrimp assay was subjected to probit analysis to determine the LC_{50} of the extract. The result of the phytochemical analysis showed that the methanol extract of the fruit shell of *Cucurbita pepo* contains saponins, flavonoids, alkaloids, cardiac glycosides and terpenoids while phenols, tannins, phlobatanins and anthraquinones were not detected. The extract was also found to have potent cytotoxic activity with an LC_{50} value of 174 µg/ml in the brine shrimp lethality assay. The fruit shell of *C. pepo* contains important phytochemicals as well as bioactive compounds that may be of therapeutic advantage.

Keywords: Cucurbitaceae, *Cucurbita pepo,* Fruit shell, Phytochemical, Shrimp Lethality Assay.

Introduction

The unused or non-edible parts of fruits and vegetables often classified as wastes have been found to contain nutrients, minerals, phytochemicals, and bioactive compounds that are beneficial to man, and that can also be useful in the food, cosmetics and pharmaceutical industries (Sagar *et al.,* 2018). Plants in the Cucurbitaceae family have been used by humans for thousands of years as food and herbal remedies, and as sources of many other products around the world. In Nigeria, the use of these plants for different purposes vary among cultures and ethnic groups (Ajuru and Okoli, 2013).*Cucurbita pepo*, commonly called pumpkin, marrow, vegetable marrow or gourd, is an annual creeping herb that is cultivated throughout the world mainly for the fruit (Burkill, 1985).

In Nigeria, its local names include “Elegede” in Yoruba, “Kabeewa” in Hausa, “Ukoro” in Igbo, and “Ndise” in Efik languages. The young leaves and immature fruits are widely consumed either as vegetables in soups or used as pot herb (Burkill, 1985; Oloyede, 2012).In traditional herbal medicine, different parts of the plant have been used in the treatment of fever, headache, gastritis, arthritis, burns/wounds, constipation and prostate enlargement (Ratnam *et al.,* 2017).Cold pressed oil from the seeds of *C. pepo* has been shown to be a rich source of bioactive compounds such as phytosterols, phenolic acids and carotenoids that are potent antioxidants; effective in the prevention of prostate cancer, and in the management of diabetes and arthritis (Bharti *et al.*, 2013; Akin *et al.*, 2017).Pumpkin seed oil has also been reported to have wound healing properties (Bardaa*et al.*, 2015). Nutritional investigations into the pulp and seeds of *C. pepo* showed that the pulp contains sodium, potassium and calcium as well as high amounts of carotenoids, and phenolic compounds such as caffeic acid, gallic acid, vanillic acid and rutin (Peiretti*et al.*, 2017; Gulczynski and Gramza-Michalowska, 2019); while the seeds were found to be rich in protein and fat; as well as iron, manganese and potassium (Badu *et al.*, 2019).The fruit shell, which is often regarded as waste and disposed has been reported to be a rich source of nutrients and minerals such as carbohydrates, crude fibre, sodium, potassium and phosphorus among others (Adeniyi and Adam, 2019).

The leaves, pulp, seed and seed oil of *C. pepo* have reported to be highly nutritious and as such have been recommended for the fortification of some food that may be lacking in nutrients; the many popular medicinal uses of pumpkin which include anticancer, hypoglycemic, antihypertensive, anti-inflammatory, anti-diabetic, and immunomodulatory among others also contribute to increasing research into the species as medicine (Aamir *et al.*, 2017). There is however insufficient information on the chemical constituents and bioactivity of *Cucurbita pepo* fruit shell (non-edible part) as potential substitute for other parts of the plant as herbal remedy. This study therefore aimed at investigating the phytochemical constituents as well as the bioactivity of the methanol extract of *C. pepo* fruit shell.

Materials and Method

Sample Collection and Preparation

Dry fruits of *Cucurbita pepo* were gathered from the wild at Aba Olu-Ode in Oluyole Local Government Area of Oyo State, Nigeria (Fig. I). The fruits were smashed to break them open after which the dried seeds and pulp were removed and discarded. The dried fruit shell was then washed with clean water, oven-dried at 40^{o}C, pulverized and stored in air-tight containers for analysis.

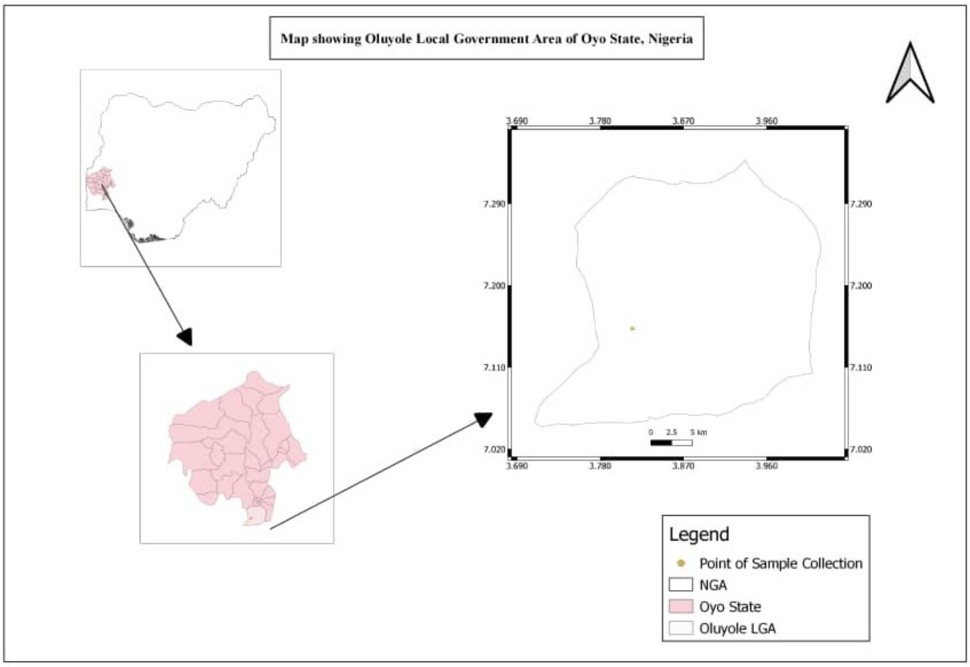

Figure 1. A map showing Aba Olu-Ode in Oluyole LGA, Oyo State, Nigeria.

Extraction procedure

10 g of powdered sample was soaked in 100 ml of absolute methanol in a conical flask for 72 hours, shaken at intervals, filtered through Whatman No 1 filter paper using a Buchner funnel. The filtrate was then evaporated to dryness on steam bath at a maximum temperature of 40 ^{o}C(Pandey and Tripathi, 2014).

Qualitative Phytochemical Screening

The presence of phytochemicals in the samples was determined according to the methods of Trease and Evans (1989) and Sofowora (1993).

Test for Saponins

6 ml of distilled water was added to 2 ml of extract and the mixture was shaken vigorously for 10 minutes. The formation of persistent foam indicated the presence of saponins.

Test for Phenols

2 ml of 5% ferric chloride was added to 2 ml of extract. The appearance of blue colour or deep bluish green solution indicated the presence of phenols.

Test for Flavonoids

2 ml of dilute sodium hydroxide (20% v/v) was added to 2 ml of extract. Few drops of 70% hydrochloric acid were then added to the mixture. The appearance and disappearance of yellow colour indicated the presence of flavonoids.

Test for Alkaloids

1 ml of extract was mixed with 5 ml of 1% hydrochloric acid, the mixture stirred on steam bath and filtered while hot. The residue was washed with few drops of distilled water and the filtrate was allowed to cool. To1 ml of the filtrate was added few drops of Wagner's, Meyer's or Dragendorff's reagents. The

formation of reddish brown precipitate indicated the presence of alkaloids.

Test for Tannins

2 ml of extract was mixed with 2 ml of distilled water and filtered. Few drops of 10% ferric chloride were then added to 1 ml of extract. The appearance of brownish blue or bluish black colour indicated the presence of tannins.

Test for Cardiac Glycosides

1 ml of extract was mixed with 0.5 ml of glacial acetic acid containing 3 drops of 1% ferric chloride. 2 ml of concentrated sulphuric acid was then gently poured down the side of the tube. The formation of a brown ring at the interphase indicated the presence of cardiac glycosides.

Test for Phlobatanins

1 ml of extract was boiled with 0.5 ml of 1% hydrochloric acid. The appearance of red precipitate indicated the presence of phlobatanins.

Test for Terpenoids

0.5 ml of chloroform was added to 1 ml of extract. Few drops of concentratedsulphuric acid were then added to the mixture. The formation of reddish brown precipitate indicated the presence of terpenoids.

Test for Anthraquinones

2 ml of extract was shaken with 5 ml of chloroform for 10 minutes and filtered. 5 ml of 10% ammonia solution was added to filtrate. The formation of pink, red or violet colour in the ammonia phase indicated the presence of free anthraquinones.

Brine Shrimp Lethality Assay (BSLA)

This was carried out at the Biomedicinal Research Centre Laboratory, FRIN and was done according to the method of Otang *et al.* (2013).*Artemiasalina*(brine shrimp) eggs were procured from Brine Shrimp Direct, USA, while the sea water was collected from the beach in Lagos, Nigeria. The eggs were introduced into filtered sea water in a beaker and incubated for 48hours under constant illumination at a temperature of 28 ^{o}C to facilitate hatching and maturation of the nauplii. After 48 hours, two-fold serial dilution of extract with filtered sea water was done to yield concentrations of 2,1, 0.5, 0.25 and 0.125 mg/ml. Ten hatched nauplii/larvae were then introduced into each test tube containing 9 ml of varying extract concentrations after which the volume was made up to 10 ml with sea water. Each concentration was replicated three times while sea water only served as negative control. The number of live nauplii was counted after 24 hours of introducing them into the extract. Larvae were considered dead when no external movement was observed during several seconds of observation. Percentage mortality was calculated as shown below:

%Mortality = (number of dead nauplii / total number of nauplii) × 100

Statistical Analysis

Data obtained from the brine shrimp lethality assay were subjected to Probit analysis for the determination of LC_{50} at 95% confidence intervals. LC_{50}value less than 1000 µg/ml is considered to be toxic while LC_{50} value greater than 1000 µg/ml is considered to be non-toxic (Supraja *et al.*, 2018).

Results and Discussion

Phytochemical screening

The result of the phytochemical screening of the fruit shell of *C. pepo* as depicted in Table 1 showed the presence of saponins, flavonoids, alkaloids, cardiac glycosides and

Journal of Forestry Research and Management. Vol. 18(3).67-74; 2021, ISSN 0189-8418

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terpenoids while phenols, tannins, phlobatanins and anthraquinones were not detected.

Table 1. Phytochemical composition of *Curcubita pepo* fruit shell

Phytochemical	Methanol extract
Saponins	+
Phenols	-
Flavonoids	+
Alkaloids	+
Tannins	-
Cardiac glycosides	+
Phlobatanins	-
Terpenoids	+
Anthraquinones	-

\+ indicates present; - indicates absent

The presence of phytochemicals otherwise referred to as secondary metabolites in plants, confers therapeutic potentials on them, and has made such plants to be useful in biotechnology, agriculture, pharmacy and medicine (Bribi, 2018). Phytochemicals have been shown to be effective in the treatment and management of chronic and severe disorders like arthritis, diabetes, cardiovascular and neurodegenerative diseases (Zhang *et al.,* 2015). Saponins have found wide use and application in the food, cosmetics and pharmaceutical industries (Anwar and Hussain, 2017).

Apart from the high commercial value of saponins, they are also biologically active, having laxative, antitussive, hypoglycemic, anti-inflammatory, antimicrobial, cardio-protective, cytotoxic as well as antitumor properties (Guclu-Ustundag and Mazza, 2007; Anwar and Hussain, 2017). Saponins have also been found to help in liver function, and reduction of blood cholesterol (Egbunna and Ifemeje, 2016). The presence of saponins in *C. pepo* fruit shell may render it useful as a potential source of laxative, antitussive, antimicrobial or hypoglycemic agent. Flavonoids are among the group of phytochemicals often referred to as antioxidant phytochemicals. They are known to possess potent antioxidant, anti-inflammatory, and free radicals scavenging activities; they also play preventive roles in chronic diseases such as coronary heart diseases, organ damage, cancer and viral infections (Kumar and Pandey, 2013; Zhang *et al.*, 2015). The presence of flavonoids in the fruit shell of *C. pepo* may therefore be an indicator of its therapeutic potential for the management of arthritis, inflammation and other oxidative stress-related diseases(Aamir *et al.,* 2017). Alkaloids are nitrogen-containing natural compounds with diverse pharmacological activities ranging from antimicrobial, antimalarial, analgesic, sedative, muscle-relaxing, hypotensive, and anti-carcinogenic activities (Bribi, 2018).

The fruit shell of *C. pepo*could be used in the treatment of microbial infections, malaria, hypertension and cancer. Cardiac glycosides such as digoxin and digitoxin are used in the treatment of congestive heart failure and cancer (Pongrakhananon, 2013). The detection of cardiac glycosides in the

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methanol extract of *C. pepo* fruit shell may be an indicator of its potential in the management of heart failure and cancer. Terpenoids of plant origin play important role in traditional medicine basically because of their aromatic properties, and have been known to be effective anti-inflammatory agents against respiratory inflammation, arthritis, atopic dermatitis among others (Kim *et al.*, 2020). The presence of terpenoids in the fruit shell of *Curcubita pepo* makes it useful for the treatment of inflammatory disorders.

Brine Shrimp Lethality Assay

The brine shrimp lethality assay is a simple, rapid, convenient, reliable and inexpensive bioassay used for the screening of extracts, pesticides, and other natural products for the presence of bioactive compounds (Meyer *et al.*, 1982; Ogbole *et al.*,2016).The result of the brine shrimp lethality assay showed that the methanol extract of *Cucurbita pepo* fruit shell possessed a dose dependent cytotoxic activity as depicted in Table 2. The extract was also found to have an LC_{50} value of 174 µg/ml which is considered to be in the toxic range (less than 1000 µg/ml), implying that the fruit shell of C. pepo can be further investigated for its potential role medicinal value and possible role in disease management.

Table 2. Brine shrimp lethality assay of methanol extract of *Cucurbita pepo* fruit shell.

Concentration (µg/ml)	%Mortality
125	47
250	57
500	87
1000	90
2000	100
Control (sea water only)	0

This therefore implies that the fruit shell of *C. pepo* most likely contains important bioactive compounds (LC_{50} 174 µg/ml) with therapeutic potentials.

Conclusion

Although the fruit shell of *C. pepo* is non-edible, this study has shown that it may have potential therapeutic applications as it contains important phytochemicalsas well asand bioactive compounds. This part of the C. pepo fruit that should therefore be further investigated for theirin order to determine itsconcentrations of phytochemicals,and various potential applications especially in phytomedicine, as well as its anticancer, anti-inflammatory and antioxidant properties.

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