PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ANALYSIS OF BITTER GARDEN EGG PENICEL EXTRACT

MUHAMMAD, I.[1*] – ISHAQ, Z.[1]

[1] *Faculty of Science, Sokoto State University, Sokoto, Nigeria.*

**Corresponding author*
e-mail: ibrahimmuhammad959[at]gmail.com

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Abstract. Bitter garden eggs are highly valued content of Nigerian food which are mostly consumed in both rural and urban area due to their nutritional and medicinal value, this study seeks to identify the various chemical compounds present in the penicel extract of bitter garden egg found in Sokoto region and also to evaluate its antibacterial active agent (compound) for the possibility of using them in the development of new antibiotics to reduce cost of health care and mortality rate due to resistance of bacteria to already developed drugs. The bitter garden egg penicel was found to a percentage moisture content of 38.80 %. Cool extraction method was adopted to extract the penicel of the bitter garden egg using three different solvent (n-hexane, chloroform and ethanol) and also standard methods were used to assess the antibacterial activity of the extracts. The result obtained from solvent extraction of bitter garden egg penicel revealed that n-hexane, chloroform and ethanol had a mass with percentage yield of 2.19 g, 7.30 %, 0.90 g, 3.19 % and 3.20 g, 11.90 % respectively. The result of the extraction revealed that Bitter garden egg penicel consists of more polar compounds than non-polar ones. The phytochemical screening of the penicel extracts of Bitter garden egg revealed the presence of alkaloids, saponins, steroids, tannins and flavonoids, while antharaquinones and cardiac glycoside were not detected. The results of the antibacterial studies revealed that the fractions has activity on the selected bacteria strain (S. aureus, E. coli and Shigella) with zone of inhibition that ranges between 2-10 mm. these results shows that the penicel of bitter garden egg has a potential for the development of new antibiotic drugs.
Keywords: *phytochemical screening, antibacterial analysis, penicel extract, bitter garden egg, drug resistance*

Introduction

Bitter garden eggs are highly valued content of Nigerian food which is mostly consumed in both rural and urban area due to their nutritional and medicinal value (Dalziel, 1937). In Hausa it is known as Gautandachii, Afufa or Anara in Igbo while Yoruba call it Igbagba, Bura Bura or Targu (Dalziel, 1937). In some parts of the country, the eggplant is domesticated along with leaves to be used as vegetables or traditional medicine (Dalziel, 1937). The uses of the fruit as indigenous medicine ranges from weight loss, treatment of asthma, allergic rhinitis, nasal catarrh, skin infections, rheumatic disease and swollen joint, constipation and dyspepsia (Jaeger and Hepper, 1986). These pharmacological properties of the plant have been attributed to some useful chemical component present in it such as steroid, flavonoids, saponins, tannins, terpenoids and alkaloids (Akintobi et al., 2011; Cushnie and Lamb, 2005; Sharma et al., 2000; El-Olemy et al., 1994; Jaeger and Hepper, 1986; Vohora et al., 1984). Recently, the plant has been reported to be used in ethnomedicinal practices for the treatment of different diseases and also possesses many pharmacological activities that includes antioxidant, anti-inflammatory, antidiabetic, anticancer, antimicrobial, and hepatoprotective properties (Ramamurthy et al., 2016; Peng et al., 2013). In addition to these, the plant is also used to treat fever and cough (Gad et al., 2021). For many

decades, plants have been accepted to be the basis of traditional medicines systems which leads to new drug developments and also, bacteria have always acquired resistance to drugs which are utilized as therapeutic agents. Resistance to antibiotic drugs is a serious health problem that leads to increase in health care cost and mortality rate (Bahl, 2010; Galeotti et al., 2008; Rhen and Cidlowski, 2005). Recently, investigations and oral communications have shown that the penicel of the bitter garden egg fruit is always thrown away as a waste after eaten the fruit due to lack of scientific fact on its nutritional or medicinal values. Testing the antibacterial activity of plants parts that are considered as waste will help a lot in new drug discovery. Therefore, in this study, phytochemical screening and anti-bacterial analysis of penicel extract of bitter garden egg found in Sokoto region, Nigeria were carried out in order to assess its potentials for new drugs development.

Materials and Methods

All solvents and chemicals used were of analytical grade and were obtained from sigma aldrich and BDH Chemicals England respectively. Nutrient Agar and Nutrient Broth were obtained from Titan Biotech L.T.D India.

Collection of bitter garden egg penicel sample

The bitter garden egg fruit was purchased in January, 2022 from Kasuwar daji market in Sokoto state, Nigeria.

Sample treatment

Hand was used to remove the penicel and then washed thoroughly with distilled water to remove dirty and other impurities and was allowed to dry at room temperature for 5 days the dried penicel was ground to fine powder using mortar and pestle. The powdered samples were stored in an air tight water free polyethene bag and used for the analysis.

Extraction of penicel

The penicel powder (30g) was extracted by maceration in a 1000cm^3 glass stoppered conical flask. N-hexane (150cm^3) was first used to defat (extract) the penicel to remove non polar compound. The marc was allowed to dry and then extracted with chloroform to remove moderately polar compound and then extract with ethanol to remove polar compound. The extraction was done according to the order of increasing polarity of the solvents. In each case the penicel was left in contact with the solvent for 24 hours with intermittent stirring to ensure maximum extraction. After 24 hours, the extracts were filtered using Whitman filter No.1 and the filtrates were transferred to evaporation dishes and concentrated using air circulated oven at temperature between 80-100 °C.

Qualitative phytochemical screening of extract

The tests for flavonoids, tannins, saponnins, steroids/triterpenoids, alkaloids, cardiac glycosides and anthraquinones were carried according to the methods described by Harborne (1998), Sofowora (1996) as well as Trease and Evans (1989).

Antibacterial studies

Test organisms

The test organisms were obtained from the Department of microbiology, Faculty of Sciences, Sokoto State University, Sokoto, Nigeria. The microorganisms are standard laboratory strains of Staphylococcus aureus (gram +ve), Shigella (gram -ve), Escherichia coli (gram -ve).

Susceptibility test

The antibacterial test was conducted using the petri dish plate method described by Salunkhe and Chavan (1989). The Nutrient Agar plates prepared according to manufacturer's instruction were allowed to solidify for 15 minutes at room temperature and incubated without inoculum for 24 hours at 37 °C to ensure the sterility of the medium. The Nutrient Agar plates were flooded with 1 ml of the inoculum and the excess was removed using Pasteur pipette. Five wells (cups) of about 6 mm in diameter were cut on each Nutrient Agar plate using a sterile cork borer and the agar plugs were removed using sterile ampoule file. The extract solution (0.1mL) was placed in each of the wells and were allowed to settle for two hours at room temperature and then incubated for 24 hours at 37 °C. The inhibition zone was observed and then recorded in millimeters using a transparent ruler. Standard antibiotic was used.

Minimum inhibitory concentration (MIC)

This was carried out as described by Salunkhe and Chavan (1989). Minimum inhibitory concentration (MIC) was defined as the lowest concentration where no visible turbidity would be observed in the test tubes. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test extracts. In this test, the micro-organisms were prepared using the broth dilution technique. The stock solution (20mg/mL) of the peniecl extract was prepared by dissolving 0.2g of the peniecl extract in 10 mL Dmso. Four concentration were prepared (0.25mg/mL, 0.5mg/mL, 1.0mg/mL and 2.0mg/mL), from the stock solution using serial dilution technique and later inoculated with 0.2 mL suspension of the test organisms. After 24 hours incubation at 37 °C, the tubes were observed for turbidity. The lowest concentration where no turbidity was observed was determine and noted as the Minimum Inhibitory Concentration (MIC).

Minimum bactericidal concentration (MBC)

The minimal bactericidal concentration was determined from broth dilution test resulting from the MIC tubes as described previously by inoculating the content of each test tube on a nutrient agar plate. The plates were then incubated at 37 °C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration (Salunkhe and Chavan, 1989).

Results and Discussion

Extraction and qualitative phytochemical screening

The percentage yield and mass of the fractions obtained from n-hexane chloroform and ethanol fractions of bitter garden egg penicel are 2.19g, 0.9g, 3.2g, with percentage yield of 7.3%, 3.19% and 11.9%, respectively. This result indicates that ethanolic fraction had the highest percentage yield. The result of the extraction revealed that the bitter garden egg penicel consist of non-polar and polar compounds. The results of the qualitative phytochemical screening of the bitter garden egg penicel fractions revealed the presence of alkaloids, saponins, tannins, flavonoids and steroids in all the three fractions, while antharaquinones and cardiac glycoside were not detected in all the fractions (*Table 1*).

***Table 1**. Qualitative phytochemical screening of bitter garden egg penicel extracts.*

Phytochemicals	n-Hexane	Chloroform	Ethanol
Alkaloids			
(a) Mayers test	+	+	+
(b) Wagners test	+	+	+
(c) Dragendorffs test	+	+	+
Flavoniods			
(a) Sodium hydroxide	+	+	+
(b) Ethyl acetate test	+	+	+
(c) Shinodas test	+	+	+
Steroids			
(a) Salkowskis test	+	+	+
(b) Lieberman-Buchards test	+	+	+
Saponius			
(a) Foam test	+	+	+
Tannins			
(a) Iron (III) chloride test	+	+	+
(b) Lead acetate	+	+	+
Antharaquinones			
(a) Borntragers test	-	-	-
Cardiac glycoside			
(a) Keller-Killiani test	-	-	-

Notes: + means detected; - means not detected.

Antibacterial activity of bitter garden egg penicel extract

Table 2 to *Table 7* show the details of the results obtained from the antibacterial activity test of the n-hexane, chloroform and ethanol fractions of bitter garden egg penicel extract. The results deduced that all the three fractions, has activity on the selected bacterial strain. The chloroform extract exhibited good antibacterial activity on all the test organisms with zones of inhibition ranging between 2-10 mm (*Table 2*). The n-hexane fraction showed a high level of activity on gram positive bacteria than gram negative bacteria, but all the organisms were found to be susceptible (*Table 3*).

***Table 2**. Antibacterial activity of chloroform fraction of bitter garden egg pecicel.*

Concentration (mg/ml)	S-aureus	E-coli	Shigella
0.25	2.00	6.00	2.00
0.50	5.00	5.00	2.00
1.00	8.00	4.00	2.00
2.00	10.00	3.00	6.00

***Table 3**. Antibacterial activity of n-Hexane fraction of bitter garden egg penicel.*

Concentration (mg/ml)	S-aureus	E-coli	Shigella
0.25	0.00	2.00	8.00
0.50	4.00	2.00	7.00
1.00	5.00	3.00	6.00
2.00	6.00	4.00	5.00

The demonstrated activity shown by the fractions may be due to the presence of tannins, saponins and alkaloids (*Table 1*) that are known to have some antibacterial activity as reported by Harborne and Harborne (1973). This results when compared with Amoxicillin (Standard Antibiotic for *Table 4*) the zone of inhibition produced by the antibiotic against the test organisms was found to be appreciable in relation to those activities produced by the extracts for S-aureus, E-coli and Shigella under study. However, according to Harborne and Harborne (1973), diameter of zones of inhibition ≥10mm is considered active.

***Table 4**. Antibacterial activity of standard antibiotic (Amoxicillin).*

Antibiotic concentration	S-aureus	E-coli	Shigella
Amoxicillin (125mg/ml)	12.00	10.00	10.00

Also from these results, it can be deduced that the n-hexane fraction is bacteriocidal on S-aureus, E-coli, and Shigella at concentration of 2.0mg/mL, 0.5mg/mL, and 0.5mg/mL respectively while chloroform fractions shows concentration of 0.5mg/mL, 0.25mg/mL and 0.25mg/mL for S-aureus, E-coli, and Shigella, respectively. Also ethanol fractions are bactericidal on all the organisms at concentration of 1.0 mg/mL, 0.25mg/mL and 0.25mg/mL for S-aureus, E-coli, and Shigella (*Table 5* to *Table 7*). In view of the above results, all the extracts showed considerable activity against S. aureus, a gram positive bacterium known to play a significant role in invasive skin diseases and also on E-coli (gram -ve) and Shigella (gram -ve) (Garrod and O'grady, 1971). The antibacterial activities of the extracts are related to the plant secondary metabolites detected. In line with these findings, Bhumi and Savithramma (2014) reported that tannins had been widely used as an application to sprains, bruises and superficial wounds. According to Usman and Osuji (2007), cytotoxicity is the common biological property of alkaloids and it has been associated with medicinal uses for centuries. The analgesic, antispasmodic and antibacterial properties of alkaloids have also been reported by several workers. Penicel extracts contain steroids which are very important compounds because it has a relationship to body compounds such as sex hormones (Halilu et al., 2013).

***Table 5**. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of n-Hexane fraction of bitter garden egg penicel.*

Test organism	MIC (mg/ml)	MBC (mg/ml)
S-aurens	2.00	2.00
E-coli	0.50	0.50
Shigella	0.50	0.50

***Table 6**. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of chloroform fraction of bitter garden egg penicel.*

Test organism	MIC (mg/ml)	MBC (mg/ml)

S-aurens	0.50	0.25
E-coli	0.25	0.25
Shigella	0.25	1.00

Table 7*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol fraction of bitter garden egg penicel.*

Test organism	MIC (mg/ml)	MBC (mg/ml)
S-aurens	1.00	1.00
E-coli	0.25	0.25
Shigella	0.25	1.00

Conclusion

Based on the percentage yield of the extract obtained from the penicel of bitter garden egg, it could be concluded that n-hexane and ethanol extracts produced more of the phytochemicals than chloroform extracts. Similarly, the penicel contain alkaloids, saponins, steroid, tannins and flavonoids, but no cardiac glycosides and anthraquinones. The penicel shows strong antibacterial activity against Staphylococcus aureus (gram +ve) Echerichia coli (gram -ve) and Shigella (gram -ve). This confirms the assertion that the penicel has good potentials for the development of new drugs. Thus, if properly harnessed, it can reduce the cost of health care and mortality rate due to resistance of bacteria to the already manufactured drugs.

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Conflict of interest

The authors declare that there is no conflict of interest involve in this research study.

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