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PRELIMINARY PHYTOCHEMICAL PROFILE AND ANTIMICROBIAL POTENTIALS OF WHITE-GREEN AFRICAN GARDEN EGG (*SOLANUM MACROCARPON*) FRUITS OBTAINED FROM YENAGOA

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ABSTRACT

It is estimated that about 80% of the world's population use medicinal plants either in their crude form or partially in their modified semi-synthetic form for their medical care. The efficacy of these plants in curing diseases is believed to be as a result of active biochemical compounds present, these bioactive compounds vary in compositions and types. Garden egg also known as eggplant is one of such plants. This research was aimed at profiling the preliminary phytochemicals and antimicrobial potentials of white-green African garden egg (*Solanum macrocarpon*) fruits obtained from Yenagoa Nigeria. Qualitative and quantitative phytochemical analysis was done using the standard method of Association of Official Analytical Chemist and agar well diffusion method was employed for antimicrobial assay. Preliminary phytochemical profile shows that Flavonoid content (25.6±0.01%) was highest followed by Saponin (14.3±0.01%), alkaloid (4.4±0.01%), Tannin (2.9±0.01%), Steroid (2.5±0.02%) and Terpenoid (1.6±0.01%). The antimicrobial assay revealed that methanol extract was more sensitive to bacteria [Shigella spp (14.7±0.2 mm), Staphylococcus spp (13.1±0.3 mm) and E. coli (12.3±0.2 mm)] and fungi [Penicillium sp (6.3±0.3 mm)] isolates. While aqueous extracts were more sensitive to bacteria (Vibrio sp with 9.5±0.9 mm) and fungi [Yeast (6.3±0.6 mm) and Mould (9.8±1.5 mm)] isolates. In light of these findings, *S. macrocarpon* fruits obtained from Yenagoa, Bayelsa State, Nigeria, could be considered as a potential source of natural antimicrobial and could be of a great importance for the treatment of infectious diseases caused by the test organisms.

Keywords: Phytochemical profile, Antimicrobial, Extracts, Medicinal plants, Yenagoa.

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1. INTRODUCTION

The emergence and widespread of antimicrobial resistant strains of both bacteria and fungi has compounded the global threat of infectious diseases which is posing severe threat to the general public health. Thus, alternative antimicrobial strategies are needed, which has lead to re-evaluation of the therapeutic use of ancient remedies, such as plants [1]. Medicinal plants are those plants which have a potential to act on disease causing agents, or ameliorate disease agents and thus can be used for treating various ailments [2]. Many natural compounds such as secondary metabolites, have been isolated from plants and which made the basis of many drug inventions today [3, 4]. According to reports more than 80% of the population in Arab and African continents depends on plant based medicines [5]. Consumption of fresh fruits and vegetables gives potential disease resisting capacity to human [6, 7]. Garden egg also known as eggplant is one of such plants. It consists of over 100 species in Africa mainly used as vegetables of which 25 species are found in Nigeria [8, 9].

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The two most widely spread being *Solanum aethiopicum* (the green-striped round shaped garden egg) and *Solanum macrocarpon* (the white-green striped oval garden egg) are eaten raw, boiled or fried to make vegetable sauce [10]. Furthermore, various parts of *S. Melongena* are useful in the treatment of inflammatory conditions, cardiac debility, and neuralgia, ulcers of nose, cholera, bronchitis and asthma. The fruit is a highly valuable vegetable all over the world because of its taste and higher percentage of vitamin B2. The fruit is also used in the treatment of diabetes [11].

Plant derived drugs serve as a prototype to develop effective and less toxic medicines. All parts of *Solanum macrocarpon* are medicinally important, hence this research, Preliminary Phytochemical profile and Antimicrobial Potentials of white-green African garden egg (*Solanum macrocarpon*) Fruits obtained from Yenagoa Nigeria.

2. MATIRIALS & METHODS

2.1. Collection of Samples

White-green striped garden egg fruits were bought from the Swali market in Yenagoa. It was sun-dried for three days and were pulverized and stored in airtight container for laboratory analysis.

2.2. Qualitative Phytochemical Screening

Phytochemical screening of the garden egg fruit samples were carried out by a procedure that was based on those earlier reports [12].

Test for saponins

The plant sample (0.5 g) was added to 5 ml of distilled water in a test tube. The solution was vortexed and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, vortexed and formulation of an emulsion was observed.

Test for terpenoids

Plant sample of 0.5 g was dissolved in 1 ml of chloroform and 1 ml of acetic anhydride added, with 2 ml of concentrated H_2SO_4. Formation of reddish violet colour was observed.

Test for tannins

The pulverized garden egg fruits (0.5 g) were boiled in 10 ml of water in a test tube and filtered. A few drops of 0.1 % ferric chloride were added and the solution observed for brownish green or a blue-black colouration.

Test for cardiac glycosides (keller-killiani test)

Garden egg fruits sample of 0.5 g, dissolved in water (5 ml) were added 2 ml of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of deoxysugar characteristics 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.

Test for flavonoids

Five (5) ml of dilute ammonia was added to a portion of an aqueous filtrate of the sample. Then, 1 ml concentrated sulphuric acid was added. A yellow colouration indicated the presence of flavonoids.

Test for alkaloids

The plant sample was dissolved in dilute HCl and filtered. Filtrates were treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Test for steroids

Acetic anhydride (2 ml) was added to 0.5 g of the sample and filtered. Sulphuric acid (2 ml) was added to the filtrate and observed for color change from violet to blue or green, which indicates the presence of steroid.

2.3. Quantitative Phytochemical Analysis

Total Tannin Content Determination

Total tannins were determined by slightly modified Folin and Ciocalteu method. 0.5 ml of the sample was added with 3.8 ml of distilled water and 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution was added. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5 mg/ml) were used as standard solutions. The result of tannins is expressed in terms of tannic acid in mg/ml of the sample.

Total Alkaloid Content Determination

Forty (40) ml of 10% acetic acid in ethanol was added to 1 g of the powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the sample until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Total Flavonoid Content Determination

Total flavonoid content of the sample was determined by following the Aluminum chloride method. The plant concentrate was mixed with distilled H_2O and $NaNO_2$ solution. After 6 min, $AlCl_3$ solution was added and enabled to stand for 6 min, NaOH solution was added to the mixture. Immediately distilled H_2O was added to bring to the final volume and then the mixture was extensively mixed and enabled to stand for another 15 min. Optical density of the mixture was recorded at 510 nm. Rutin was used as a standard compound for the evaluation of total flavonoid. The total flavonoids were calculated using the standard curve, and expressed as rutin equivalent in mg/g of the sample.

Total Saponin Content Determination

The pulverized sample were dissolved in 80 % methanol, 2 ml of Vanilin in ethanol was added, mixed well and the 2 ml of 72 % sulphuric acid solution was added, mixed well and heated on a water bath at 60°C for 10 min, absorbance was measured at 544 nm against reagent blank. Diosgenin is used as a standard material and the assay compared with Diosgenin equivalents.

Total Terpenoid Content Determination

Garden egg fruits (1 g) were marcarated with 50 ml of ethanol and filtered. To the filtrate (2.5 ml), 2.5 ml of 5 % aqueous phosphomolybdic acid solution was added and

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2.5 ml of concentrated H_2SO_4 was gradually added and mixed. The mixture was left to stand for 30 min and then made up to 12.5 ml with ethanol. The absorbance was taken at 700 nm.

Total Steroid Content Determination

The steroid content of the plant sample was determined using the method described by reference [13]. A portion of 2 ml was taken from a solution of 2.5 g of powdered plant material prepared in 50 ml of distilled water after vigorous shaking for 1 hour. The extract solution was washed with 3 ml of 0.1M NaOH (pH 9) and later mixed with 2 ml of chloroform and 3 ml of ice cold acetic anhydride followed by the cautious addition of two drops of concentrated H_2SO_4. The absorbance of both sample and blank were measured using a spectrophotometer at 420 nm.

2.4. Plant Sample Extraction

The extraction reagents were methanol and aqueous. About 10 g of the garden egg fruit sample was placed in a beaker and 25ml of methanol added and mixed by vortexing. It was centrifuged at 3000 rmp for 10 minutes. The supernatant was collected and transferred to a stoppered test tube by filtration. The resulting supernatant was evaporated to dryness with a gentle stream of nitrogen and reconstituted in 10 ml dimethyl sulphoxide and was mixed by vortexing. The same procedure was repeated for that of aqueous.

2.5. Preparation of Dried Filter Paper Discs

Whatman filter paper no. 102 was used to prepare discs. Approximately 5mm in diameter was perforated using a perforator. These were placed in a petri dish after sterilization in autoclave.

2.6. Plant Extract Disc Placement

Plant disc containing 3 ml (3 µl) concentration, as well as garden egg fruit were made using filter paper and then placed on the plates using sterile forcep. One sterile antibiotic disc was placed on the surface of an agar plate using a forcep. The forcep was sterilized by immersing in alcohol each time before placing another antibiotic disc. The disc was then gently pressed with the forcep to ensure complete contact with the agar surface and placed away from the edge of the plates so that it is easily measured. Once all discs were in place, the plates were inverted, and placed in a 37°C incubator for 24 hours.

2.7. Bacteria/ fungi suspension preparation

Media used: Nutrient agar, buffered peptone water, *shigella* agar, macconky agar and cetrimide agar. These media were prepared according to manufacturer's instruction. Using a sterile inoculating loop and needle for bacteria and fungi respectively, through aseptic techniques the test organisms of each colony was taken from the subculture plate. The organism was suspended in 4 ml of normal saline and vortexed for overall suspension. Mcfarland standard solution was used as a reference to adjust the turbidity of individual bacterium isolate in the suspension (1×10^8). And 10 fold serial dilutions was made and plated for the antimicrobial sensitivity test.

2.8. Inoculation of Isolates on the Nutrient Agar Plate Proper

A sterile swab stick was dipped into the bacterial/ fungi suspension and the test organisms were suspended in 4 ml of buffered peptone water. The swab was rotated against the side of the tube using firm pressure to remove excess fluid, but the swab was not dipped wet. The dried surface of the nutrient agar plate was inoculated by streaking the swab over the entire agar surface by rotating the plate at 60 degrees each time to ensure an even distribution of the inoculum.

3. RESULTS & DISCUSSION

3.1. Preliminary Phytochemical Profile

Morbidity and mortality of bacterial infections are on the increase partly due to inadequacy and high cost of new generation antibiotics as well as widespread resistance to old generation antibiotics [14]. Therefore, there is need to look for new substances from other sources with proven antimicrobial potentials. Consequently, this has led to the search for effective antimicrobial agents of plant origin. The present study profiled the phytochemical components and antimicrobial potentials of aqueous and methanol fruit extracts of white-green garden egg.

The phytochemical screening conducted on the white-green garden eggplant sample revealed the presence of chemical constituents which has been shown to possess some pharmacological activities they are; alkaloids, saponins, tannins, terpenoids, steroids, glycosides and flavonoids **(Table 1)**. Alkaloids content were 4.4±0.01% in the plant sample and can be efficient therapeutically. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bacterial effect [15]. Pharmacological activities have been reported about saponins such as antibiotics, antifungal, antiviral, hepatoprotective anti-inflammatory and anti-ulcer [16]. Saponin content was 14.3±0.01% on the garden egg sample. Tannin content was 2.9±0.01% on the plant sample. Tannins are basically used for the treatment of inflammation, leucorrhoea, gonorrhoea, burn, piles and diarrhea [17].

Flavonoids (25.6±0.01%) were the most active compound in the plant samples. Flavonoids are plant nutrients that when consumed in the form of fruits and vegetables are non-toxic as well as potentially beneficial to the human body; up till now, more than 200 different flavonoids have been isolated from vegetables [18]. Terpenoid content was 1.6±0.01% suggests the usefulness of the plant as a potential fertility agent and has demonstrated antimicrobial, anticarcinogenic (perilla alcohol), antimalarial (artemisinin), anti-ulcer and hepaticidal effects [19]. Steroid content was 2.5±0.02% as shown in **Table 2**. The findings of this work are synonymous with the works of references [12, 13, 19].

3.2. Antimicrobial Potentials

The sensitivity of the test microorganisms is related to the inhibition zone size in millimeters via agar well diffusion assay. Table 3 shows the Biochemical characteristics of the bacterial isolates and identification of fungi isolates with cultural morphology are shown in **Table 4**.

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The antimicrobial assay revealed that methanol extract was more sensitive to bacterial [*Shigella spp* (14.7±0.2 mm), *Staphylococcus spp* (13.1±0.3 mm) and *E. coli* (12.3±0.2 mm)] and fungi [*Penicillium sp* (6.3±0.3 mm)] isolates. While aqueous extracts were more sensitive to *Vibrio sp* with 9.5±0.9 mm, Yeast (6.3±0.6 mm) and Mould (9.8±1.5 mm) bacteria and fungi isolates respectively as shown in **Table 5 and 6**. Our results are in agreement with previous reports of references [20, 21].

These findings support the traditional knowledge of local users and it is a preliminary, scientific and validation for the use of garden egg fruits for antimicrobial activity to promote proper conservation and sustainable use of the plant resources. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings.

Table 1: Qualitative phytochemical profile of garden egg

Plant Sample	Phytochemicals						
	Alkaloid	Tannin	Flavonoid	Saponin	Glycosides	Terpenoid	Steroid
Garden egg fruits	+	+	+	+	+	+	+

Note: + (presence)

Table 2: Quantitative phytochemical profile of garden egg

Plant Sample	Phytochemicals %					
	Alkaloid	Tannin	Flavonoid	Saponin	Terpenoid	Steroid
Garden egg fruits	4.4±0.01	2.9±0.01	25.6±0.01	14.3±0.01	1.6±0.01	2.5±0.02

Note: % - Percentage, (Means ± SD)

Table 3: Biochemical characteristics of some bacteria

Bacterial Isolates		*Shigella sp*	*E. coli*	*Staphylococcus sp.*	*Vibrio sp*
Cell morphology (cell shape)		Rod	Rod	Coccus	Comma
Colony (cell shape)		Round	Spindle	Circular	Curved
Gram reaction		Negative	Negative	Positive	Negative
Biochemical Test	Nitrate reductive	Positive	Positive	Positive	Positive
	Oxidase	Negative	Negative	Negative	Positive
	Catalase	Positive	Positive	Positive	Negative
	Methyl red	Positive	Positive	Positive	Negative
	Voges Proskauer	Negative	Negative	Positive	Positive
	Indole	Negative	Positive	Negative	Positive
	Citrate	Negative	Negative	Positive	Positive
	Hydrogen sulfide reduction	Negative	Negative	Negative	Negative
	Ureas activity	Negative	Negative	Positive	Negative

Table 4: Identification of fungi with cultural morphology

Fungi Isolates	Microscopic observation (Medium)	Microscopic observation (gram reaction)
Yeast	White colour, creamy growth on the media surface	Pink colour large cells obtained by gram's staining, oval, budding cells obtained by LPCB staining.
Penicillium sp	Greyish-green colour colonies, smooth colonies.	Brush like conidiophores and branched mycelium spores arranged on conidiophores
Mould	Black huge colonial growth	Heavy mycelial growth arranged in filamentous form.

Table 5: Antibacterial activity of white-green garden egg fruits extracts

Bacterial Isolates	Solvent extracts	
	Methanol	Aqueous
Shigella sp	14.7±0.2	11.0±1.0
E. coli	12.3±0.2	5.7±0.1
Staphylococcus sp	13.1±0.3	6.0±1.0
Vibrio sp	8.3±0.1	9.5±0.9

(Diameter of inhibition zone in mm) (Means ± SD)

Table 6: Antifungal activity of white-green garden egg fruits extracts

Fungi Isolates	Solvent extracts	
	Methanol	Aqueous
Penicillium sp	6.3±0.3	5.3±1.5
Yeast	5.4±0.2	6.3±0.6
Mould	6.6±0.1	9.8±1.5

(Diameter of inhibition zone in mm) (Means ± SD)

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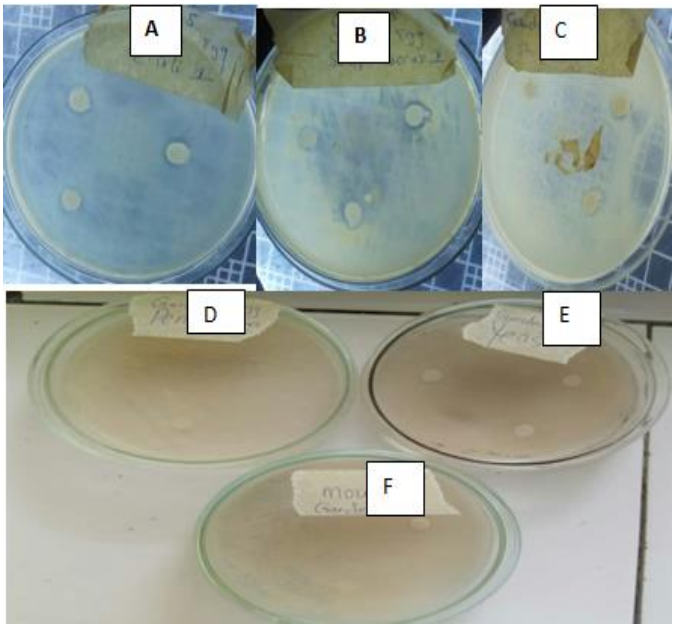

Figure 1: Antimicrobial Activity of Garden egg sample on test organisms; (A) *E. coli* (B) *Staphylococcus sp* (C) *Shigella sp* (D) *Penicillium sp* (E) Yeast (F) Mould

4. CONCLUSION

The level of zones of inhibition of the microbial isolates in this study shows the potentials of the white green garden egg fruit extracts. However, the antimicrobial effect of the plant could be attributed to the bioactive compounds such as the phytochemical constituent present in the plants. Generally, the activity of plant extracts against disease causing microorganisms and their use in traditional remedies is considered to be a function of the phytochemicals in the plants, for they are known to act by different mechanism and exert antimicrobial action. The isolate analysis has been shown to be a good source of antimicrobial agent and phyto-constituents therefore could be potential source of bioactive compounds with beneficial biological activities.

DECLARATIONS

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Conflict of interest: The authors declare that they have no competing interests.

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