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Proximate, Phytochemical Profiling and Antibacterial Activity of Aqueous *Solanum Melongena* Linn Leaf Extract

Dunkwu-Okafor, A[a], Atuanya, E. I.[a] and Obayagbona, O. N.[b*]

[a]Microbiology Department, Faculty of Life Sciences, University of Benin, Benin City, 300001, Nigeria

[b]Environmental Management and Toxicology Department, Faculty of Life Sciences, University of Benin, Benin City, 300001, Nigeria

[*]Corresponding author, email; Omoregbe.Obayagbona@uniben.edu, GSM: +2347065279704, 300001, Nigeria

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Abstract

The antibacterial, nutritional and phytochemical properties of Solanum melongena Linn. aqueous leaf extracts were evaluated using routine procedures which included; Salkowski test, agar well diffusion and broth dilution respectively. Test clinical isolates utilized were; Staphylococcus aureus, Escherichia coli, and Salmonella sp. Varying quantities of the phytochemical compounds; Phenols, flavonoids, saponins, alkaloids and terpenoids were detected in the examined powdered leaves. The leaves had comparatively high mean concentration of saponins; 14.20% while the mean terpenoids value; 2.34% was the least amongst the detected phytochemicals. The ash content of the eggplant leaves had the least mean concentration; 2.19 %, whilst the moisture value was the highest; 31.56 %. S. aureus was the sensitive isolate as it elicited the highest mean growth inhibitory zone; 21.00 mm, while the minimal mean growth inhibitory zone; 4.80 mg/ml, was elaborated by E. coli. The Minimum Inhibitory Concentration (MIC) value of the aqueous leaf extract was 800 mg/ml. More research aimed at profiling and identifying bioactive compounds potentially present in extracts prepared from parts of the eggplant is needed.

1. Introduction

Globally, several groups of scientists; Pharmacologists, microbiologists, botanists, and natural-product chemists have and are continually in a search for phytochemicals that could be utilized for the treatment of various diseases [1]. Natural products such as plants have been documented as a very successful source of medicines and health supplements [2]. Each plant has been described as a natural factory with the propensity to elaborate a plethora of complex and unusual chemical moieties whose structures and functions are very diverse [3]. Worldwide, there are about one hundred and twenty (120) unique phytochemicals identified from plants which have been regarded as vital drugs and are currently in usage against a multitude of aliments [2].

Lester and Hasan, [4] reported that globally, *Solanum melongena* L. (Eggplant) was/is commonly cultivated and consumed. It has been observed that, generally in the south Asian subcontinent countries such as India, the plant is prepared and cooked in a variety of forms and relished as an accompaniment to the main food delicacies such as rice or roasted bread (made from wheat or sorghum flour) [5]. *S. melongena* is known to bear different colours, shapes, sizes of fruits [6]. The fruit has described as been pendent, fleshly berry and the colour is known to vary from shinning purple to white, green, yellow and black often with strips and patches on the skin [7]. The fruit shape can vary from long cylindrical to round, oblong and oval shape and the plant is also known to undergo self-pollination [7]. Nutritionally, the eggplant is known to be a very good source of

nutrients such as; Dietary fiber, potassium, manganese, copper, vitamin B1, vitamin B6, magnesium and niacin [8]. The plant is known to possess several nutritional and pharmacological properties that make them a relevant addition to diets [9]. Sanchez-Mata *et al.* [10] attributed this trend to the appreciable reserve of nutrients and phytochemical compounds, such as anthocyanin, fiber, phenols, ascorbic acid, glycoalkaloids and α-chaconine that ensure the dietary addition of the eggplant very valuable to the end consumer. Ossamulu *et al.* [11] and Horbowicz *et al.* [12] reported that anthocyanin, the primary phenolic constituent present in eggplant peel is known to be responsible for the pigmentation of the plant fruit. Mazza *et al.* [13] opined that the bitter taste of eggplant fruit has been attributed to its polyphenol content.

In spite of the widespread dietary consumption of the eggplant fruits in different parts of Nigeria and the impressive list of phytochemical and nutrachemical properties associated with the plant, there is scant available literature on the potential phytochemical composition and antimicrobial attributes of the eggplant leaves. This study is aimed at investigating the antibacterial activity of aqueous *S. melongena* leaf extract and also, selected phytochemical and proximate properties of the plant leaves.

2. Methodology

2.1. Collection of *S. melongena* L. leaves

Fresh *S. melongena* L. leaves were purchased from Uselu market, Uselu quarters, Benin City during the month of April, 2019 and the leaves were identified by Mr. O. Eguagie of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. Sorting of the leaves was done and the leaves were subjected to air drying at room temperature for a two-week period. The dried leaves were then grounded into fine powder respectively with the aid of a sterile pestle and the powdered leaves were kept in air-tight plastic containers pending further analysis.

2.2. Preparation of aqueous leaf extract for phytochemical screening and antibacterial susceptibility testing

Aqueous extract of the sample was prepared by soaking 50 g of the powdered leaf samples in 250 ml of distilled water for about 12 hr. The extract was separated from the supernatant with the aid of a sterile muslin cloth and the extract was further concentrated using a water bath which further removed residual traces of the solvent supernatant.

2.3. Phytochemical screening of the aqueous leaf extract

The aqueous extract was screened for the presence of alkaloids using procedure as reported by Islam *et al.* [14]. The qualitative presence of terpenoids in the leaf extract was ascertained using the Salkowski test as detailed by Islam *et al.* [14]. The extract was screened for flavonoids using method detailed by Sofowora [15]. The qualitative saponin and phenol content of the aqueous extract was evaluated using procedures detailed by Ajiboye *et al.* [16].

2.4. Quantitative analysis of the selected phytochemicals in the raw powdered leaves

The alkaloid and phenolic profiles of the powdered leaf was evaluated with the aid of procedure as detailed by Harborne [17] and Thangi *et al.* [18] respectively. The total flavonoid and saponin values of the powdered leaves were evaluated using methods stated by Ladi *et al.* [19]. The terpenoid value of the leaves was ascertained using procedures described by Malik *et al.* [20]. All the quantitative analyses were conducted in triplicates and values expressed as means ± standard deviation.

2.5. Proximate (nutritional) evaluation of the powdered leaves

The total ash and moisture value of the powdered leaves was evaluated using methods stated by Ladi *et al.* [19]. The crude protein value of the leaves was elucidated using the micro-kjeldahl method as detailed by A.O.A.C [21] and Ladi *et al.* [19]. The crude fiber and lipid profiles of the powdered leaves were determined by procedures described by Chatepa and Mbewe, [22]. As with the phytochemical analyses, the nutritional experiments were conducted in triplicates and values expressed as mean ± standard deviation.

2.6. Source of clinical bacterial isolates

The bacterial isolates utilized in this research were; *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* sp. They were obtained from the Medical Microbiology laboratory of the University of Benin Teaching Hospital (UBTH), Ugbowo, Benin City. The isolates were sub-cultured onto a basal enriched solid medium; Nutrient agar and re-identified with the aid of biochemical tests such as Gram staining, catalase and coagulase production [23], [24], [25].

2.7. Standardization of test bacterial Inoculum

The test bacterial isolates were sub-cultured on freshly prepared Mueller Hinton agar plates and incubated for 24h respectively. Portions of the streaked colonies were transferred into test tubes which contained five (5) ml of sterile nutrient broth and incubated overnight at 35^{o}C. The density of the resultant bacterial culture suspension was compared to that of freshly prepared Barium sulphate solution (opacity standard) {0.5ml of 1% Barium in Chloride to 99.5ml of 1% H_2SO_4 (0.36 Normal) [26]. The turbidity of the bacterial suspension was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standard (10^6 cfu/ml).

2.8. Determination of antibacterial activity of the aqueous leaf extract

The agar-well diffusion method as detailed by Vollekova *et al.* [27], was utilized to ascertain the inhibitory effects of the aqueous leaf extract concentrates on test bacterial cultures. The test was conducted by preparing several aqueous leaf extract concentrates; 500mg/ml, 400 mg/ml, 300 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml respectively. Prepared and labeled Mueller Hinton plates were then inoculated with two (2) ml of the respective standardized bacterial broth suspensions. The bacterial lawn was done with the aid of a sterile glass rod. The seeded plates were allowed to dry and upon drying, a 5 mm sterile cork borer was utilized to create two (2) equidistant holes in the center of the labeled culture plates. The holes were filled with 0.2 ml of the respective aqueous leaf extract concentrates. The agar plates were left at room temperature for ten (10) minutes, so as to permit the diffusion of the extract concentrate into the agar. The plates were then incubated at 35 ^{0}C for 24 h and at the end of this period, the agar plates were assessed for the presence of visible zones of growth inhibition surrounding the well. The mean inhibitory zones were measured using a meter rule and expressed in millimeters.

2.9. Minimum Inhibitory Concentration (MIC) Determination

The Minimum Inhibitory Concentration (MIC) of the *S. melongena* L. aqueous leaf extracts was evaluated using the broth dilution method [28]. A maximal concentration of the aqueous leaf extract;800 mg/ml was constituted by the dissolution of a specific amount of the leaf extract; 0.08 g, into 10 ml of sterile DMSO. This concentrate was further diluted to give concentrates which varied from 400 mg /ml to 200 mg/ml respectively. Zero point one (0.1) ml of the standardized bacterial broth cultures were inoculated into the labeled tubes which contained the diluted extract concentrates. The tubes were incubated at 37 ^{O}C for 24h and the least concentration of the extract which inhibited inoculum growth was regarded as the minimum inhibitory concentration.

2.10. Statistical analysis

One way Analysis of variance (ANOVA) analysis of the respective mean inhibitory zones was conducted ($\alpha = 0.05$) using SPSS version 21. Duncan Multiple Range (DMR) test was utilized to locate the cause of any significant differences in the examined mean zones.

3. Results and Discussion

Table 1: Phytochemical profile of *S. melongena* L. powdered leaves

Parameters	Qualitative analysis	Quantitative analysis (%)
Phenol	+	2.39 ± 0.12
Flavonoids	+	16.08 ± 0.07
Saponins	+	14.20 ± 0.21
Alkaloids	+	9.53 ± 0.22
Terpenoids	+	2.34 ± 0.11

+ ; present - ; absent

Table 2: Nutritive values (%) of *S. melongena L.* powdered leaves

Parameters	Values (%)
Crude fiber	[a]6.38 ± 0.52
Lipid	8.22 ± 0.11
Ash	2.19 ± 0.04
Moisture	31.56 ±1.4
Crude protein	9.35 ± 0.11

a: Values are in mean ± Std. deviation

Table 3: Antibacterial activity of the leaf extract concentrates

Leaf extract concentrate (mg/ml)	*S. aureus* inhibitory zone (mm)	*E.coli* inhibitory zone (mm)	*Salmonella* sp. inhibitory zone (mm)
500	[a]21.00[b] ± 0.58	20.67[b] ± 0.58	20.31[b] ± 0.01
400	17.33[b] ± 0.33	19.67[b] ± 0.33	19.33[b] ± 0.33
300	15.67[b] ± 0.33	18.00[b] ± 0.58	17.67[b] ± 0.58
200	11.33[b] ± 0.88	15.67[b] ± 0.33	16.63[b] ± 0.33
100	6.67[b] ± 0.33	5.67[b] ± 0.33	11.67[b] ± 0.33
50	5.00[b] ± 0.00	4.80[b] ± 0.00	4.87[b] ± 0.00

a: Values are in mean ± Std. deviation, Means succeeded by alphabet "b "are not significantly different (*P>0.05*) from each other

Table 4: Minimum Inhibitory Concentration (MIC) values of the aqueous *S. melongena* L. leaf concentrates

Test isolates	800 mg/ml	400 mg/ml	200 mg/ml	MIC
S. aureus	-	-	+	800
E. coli	-	-	+	800
Salmonella sp.	-	-	+	800

+: turbidity/growth, -: No growth or turbidity

The phytochemical screening of the *S. melongena* L. powdered leaves revealed the presence of several phytochemicals which included; Phenols, flavonoids, saponins, alkaloids and terpenoids (Table 1). Quantitative phytochemical analysis revealed varying mean concentrations of these compounds in the examined leaves (Table 1). The leaves had comparatively high quantity of saponins; 14.20% while the mean terpenoids concentration; 2.34% was the least amongst the phytochemicals present in the powdered leaves (Table 1). Umamageswari and Yasmeen, [29] also reported the presence of these phytochemical compounds in eggplants. The presence of these bioactive phytochemicals in the powdered leaves is indicative of the medicinal value of the *S. melongena* leaves, Al-Janabi, and Al-Rubeey, [8] stated that was a revived interest in the study of the medicinal properties of *S. melongena* based on its phenolic and alkaloid contents. The authors also reported that antioxidant activities are a primary function of several phenolic moieties present in the eggplant; Caffeic and chlorogenic acids and flavonoids which include nasunin. Naturally, eggplant is known to produce these compounds to protect them against oxidative stress emanating from continuous exposure to harmful elements, as well as from infection by plant pathogenic microorganisms [8].

Amongst the macro-nutrients detected in the *S. melongena L.* leaves, ash content had the least concentration; 2.19 %, while moisture had the maximal amount; 31.56 % (Table 2). The nutritional profile of the examined eggplants leaves would indicate the availability of macro nutrients in the leaves of the plants, aside of the fruit which is consumed globally as a culinary delicacy. Umamageswari and Yasmeen, [29] stated that *S. melongena* Linn (Garden egg) was a culinary vegetable, which since antiquity, has been utilized in the Indian medicinal system.

The antibacterial activity of the aqueous leaf extract is shown in Tables 3 and 4. Amongst the test cultures, *S. aureus* was the sensitive isolate as it elicited the highest mean growth inhibitory zone; 21.00 mm when exposed to the maximal concentrate of the extract; 500 mg/ml (Table 3). The minimal mean growth inhibitory zone; 4.80 mg/ml, was elaborated by *E. coli* exposed to the least extract concentrate; 50 mg/ml (Table 3). The differences between the respective mean inhibitory zones was statistically insignificant different ($P>0.05$) (Table 3). The MIC value of the aqueous leaf extract for all the exposed bacterial cultures was 800 mg/ml respectively (Table 4). The level of suspectibility of the exposed bacterial cultures was directly proportional to the amount of leaf extract concentrate utilized. This trend would suggest the the antibacterial activity of the aquoeus leaf extract was dose/ concentration dependent extract. This observation is similar to a trend documented by Idu *et al*. [30] with respect to the expressed antibacterial activity of *Khaya senegalensis* seed oil. Al-Janabi, and Al-Rubeey, [8] observed a similar trend with regards to the antimicrobial activity of extracts prepared from several parts; roots, stems, leaves, flowers and fruits of eggplants cultivated in Iraq. They also reported that the plant derived extract antimicrobial activity was dependent on the concentrations and the higher the plant extract concentration, the greater the inhibitory action of the extracts to the exposed test microbial culture.

4. Conclusion

The crude aqueous leaf extract of *S. melongena* L. had varying concentrations of different phytochemicals which included; phenols, flavonoids, saponins, alkaloids and terpenoids. The moisture content of the powdered leaf was high and the aqueous extract displayed antibacterial activity which was dose dependent. The potentials of elucidating novel bioactive phytochemicals from eggplant is promising and more research aimed at profiling and identifying bioactive compounds potentially present in extracts prepared from parts of the eggplants is recommended.

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