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Bioactivity of *Arachis Hypogaea* Shell Extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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ABSTRACT

Groundnut shells (GSs) are abundant renewable by-products which have been underexploited for potential applications. Therefore, this paper reports the bioactive potential of groundnut shell extracts (GSEs) against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The GSs were ground into powder form and subjected to extraction using ethanol, ethyl acetate, and a mixture of ethanol and ethyl acetate using an electrical shaker for 6 h and 12 h; and subsequently centrifuged at 2000 rpm for 20 min. The GSEs were then qualitatively screened for phenol, quinone, saponin tannins, and flavonoids using the standard procedures. More so, antibacterial activities of these GSEs against *P. aeruginosa* (ATCC 29953) and *S. aureus* (ATCC 25923) were tested using Agar well diffusion method on Mueller-Hinton agar (MHA). Therefore, the preliminary phytochemical screening reviewed the presence of saponin, tannin, flavonoid, quinone, and phenol. And the investigation of the antibacterial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* demonstrated that *S. aeureus* was more sensitive to attack by the EtOH derived GSEs; whereas, *P. aeruginosa* was readily affected by the EtOAc GSEs. Generally, *P. aeruginosa* was more inhibited by these GSEs even at the lower concentrations of 25 and 12.5 mg/ mL; especially with the EtOH + EtOAc and EtOAc derived GSEs. EtOH + EtOAc GSE has potential of enhancing these bacterial inhibitions.

GRAPHICAL ABSTRACT

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Introduction

There is increasing search for novel antimicrobial agents because of the multifaceted resistance of microorganism to established drugs [1][2]; so as to prevent and combat new and reemerging infectious diseases [2][3][4]. Therefore, attention has now been highly drawn to folk medicine in order to uncover new leads for formulation of more efficient drugs against microbial infections. Some of these bacteria; *E. coli* and *S. aureus* usually cause food borne diseases and food spoilage [5]. To this direction, natural products (pure or standardized plant extract) have excellently provided opportunities for the search of new drugs because of the unlimited availability of their chemical diversity [1]. The recognition of effective use of some plants in traditional or folk medicine has deepen the search for pharmacological active components from plants in modern scientific medicine as well [6]. In fact, is no longer news that many infectious diseases in the past have been treated with herbal or plant products [7].

Plants are known to possess bioactive chemicals that can provide antioxidant benefits, prevent cancer, reduce blood pressure, accelerate blood clotting, cure infections etc. The intake of phenolic compounds is connected to reduce risk of coronary heart disease.

On the other hand, GSs or peanut shells are abundant agricultural byproducts that contain many nutrients; protein (4-7%), fat (1-2%), carbohydrates (10.6-21.2%), monosaccharide, oligosaccharides, and hemicellulose [8]. They also contain some medicinal chemicals such as; luteolin, *β*-sitosterol, *β*-carotene, saponins, and xylose [9]; as well as antioxidant ingredients (catechol, pyrogallol, and pyrogallic acid) [10]. GS has caught the attention of many for its utilization in food, chemical, medical industries, agriculture, and other fields [11]. Modern techniques (supercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction, ultrasound-assisted extraction, solid-phase extraction etc.) are used also for extraction of bioactive compounds from natural products. With these techniques, we can achieve reduction in solvent consumption and accelerate the extraction process. Agricultural wastes such as the GSs (see Figure 1) are often littered around indiscriminately as they become pollution source in the environment [12]. Fortunately, the utilization of GS for bioactive chemicals will absolutely be in line with Green Chemistry Principles as it concerns efficient resource management and feedstock sustainability [13-15]. Plus, this will reduce stress and pollution on the environment. Hence, this paper reports the bioactive potential of GSs against *S. aureus* and *P. aeruginosa*.

Figure 1: Peanut shell waste

Materials and Reagents

Ethanol, ethyl acetate, lead acetate, NaOH, *staphylococcus aureus and pseudomonas aeruginosa*, Mueller- Hinton Agar (MHA), $BaCl_2$, distilled water, H2SO4, ferric chloride solution, chloroform, swab stick, borer, ammonia solution, mythelated spirit, chloramphenicol (positive control). These chemicals and reagents were of analytical grade. The GSs were gotten from North bank market, Makurdi, Benue state – Nigeria.

Preparation of the GS extract (GSE)

The extracts were obtained as similarly previously reported by Ozor *et al.* [1]; with some slight modifications. About 10 g powder GSs were put into 3 beakers each; A, B, and C. Then 100 mL of ethanol, ethyl acetate, and ethanol + ethyl acetate were added into the different beakers, respectively. These were shaken for 6 h and 12 h in each case. The resulting mixtures were then centrifuged at 2000 rpm for 20 min. After

equilibration for 1 h, the GSEs were filtered using Whatts man no 1 filter paper and were allowed to air dry at room temperature. The dried extracts were collected using sterile container and were stored in the refrigerator at 4 °C for subsequent experiment.

Phytochemical Screening

The GSEs were then qualitatively screened for phenol, quinone, saponin tannins, and flavonoids using the standard procedures as also reported in [1, 16-18].

Test for tannins (Wohler's Test)

A few drops of basic lead acetate solution were added to 1.6 mL of the GS extract. Formation of a white precipitate indicated the presence of tannin [18].

Test for saponins

About 2.5 mL of the extract was mixed with a few drops of distilled water and the mixture was shaken vigorously. The appearance of copious lather formation implied the presence of saponin [18].

Test for flavonoid (Shindo's Test)

About 1.3 mL of the GS extract was mixed with 0.5 g of Mg turnings and the mixture boiled for 5 min. Positive test for flavonoid was found by the appearance of orange - red colour [18].

Screening for Quinone

About 1 mL of the extract was mixed with conc. H_2SO_4. The presence of the light -green colour indicated that quinone was present [18].

Screening for Phenol

A few drops of ferric chloride solution were added to 2 mL of the extract in a watch glass. Bluish-green colouration was observed because of the presence of phenol [18].

Antibacterial Analysis

Bacteria strains used

The test bacteria strains used were *Staphylococcus aureus* ATCC 25923 and Pseudomonas *aeruginosa* ATCC 29953.

Evaluation of antibacterial activity

The antibacterial activities were carried out as previously reported in [1, 17, 19-20]. Antibacterial activities of these GSEs were evaluated using Agar well diffusion method on Mueller-Hinton agar (MHA). The inhibition zones were reported in millimeter (mm). *Pseudomonas aeruginosa* (ATCC 29953) *and Staphylococcus aureus* (ATCC 25923) were used as references for the antibacterial assay. In a nutshell, MHA agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter, 6 mm) were filled with 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg of the GSEs and the plates were incubated at 37 °C for 18 -24 h. After the incubation period, the diameters of the growth inhibition zones were measured. Chloramphenicol was used as positive control. All tests were performed in triplicate.

Result and Dissection

Phytochemical screening

Phytochemical screening reviewed the presence of saponin, tannin, flavonoid, quinone, and phenol; as previously been found [17][21]. Similarly, the extraction of polyphenols from peanut shells by ultrahigh pressure (PPSUP) has also been reported (with 71.3 mg of gallic acid equivalents (GAE) /g) using 75% ethanol, 300 MPa, at 4 min, and 1:25 ratio of material : liquid [5].

Bioactivity potentials GS extracts

The various inhibition zones of the bacterial activity of all the GSEs at different concentration against the test organisms are as presented in Table 1 and also in Figure 2.

Table 1: Inhibition zones (mm) for the test organisms at different concentrations of the GSEs

Sample	Test Organism	Growth inhibition (mm) of the test organisms at different concentration of the GSEs			
		100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL
Control (Positive)	*S. aeureus*	15.33 ± 0.58	11.33 ± 0.58	9.66 ± 0.58	7.33 ± 0.58
	P. aeruginosa	16.33 ± 0.58	12.33 ± 0.58	10.66 ± 0.58	7.33 ± 0.58
GSEA-6 h	*S. aeureus*	6.33 ± 0.58	4.66 ± 0.58	-	-
	P. aeruginosa	3.66 ± 0.58	2.66 ± 0.58	-	-
GSEB-12 h	*S. aeureus*	6.33 ± 0.58	4.66 ± 0.58	-	-
	P. aeruginosa	3.66 ± 0.58	2.66 ± 0.58	-	-
GSEC -6 h	*S. aeureus*	6.66 ± 0.58	6.00 ± 0.00	-	-
	P. aeruginosa	7.00 ± 1.00	5.66 ± 0.58	4.33 ± 0.58	3.66 ± 0.58
GSED-12 h	*S. aeureus*	10.66 ± 0.58	7.33 ± 0.58	-	-
	P. aeruginosa	3.33 ± 0.58	1.66 ± 0.58	-	-
GSEE -6 h	*S. aeureus*	6.00 ± 0.00	-	-	-
	P. aeruginosa	7.33 ± 0.58	6.33 ± 0.58	5.00 ± 0.00	4.33 ± 0.58
GSEF-12 h	*S. aeureus*	-	-	-	-
	P. aeruginosa	7.66 ± 0.58	5.66 ± 0.58	4.33 ± 0.58	-

Note: *Ground shell extract (GSE) A is EtOH- 6 h; GSEB is EtOH-12 h; GSEC is EtOH + EtOAc-6 h; GSED is EtOH + EtOAc-12 h; GSEE is EtOAc -6 h; GSEF is EtOAc -12 h; and - = No antimicrobial activity*

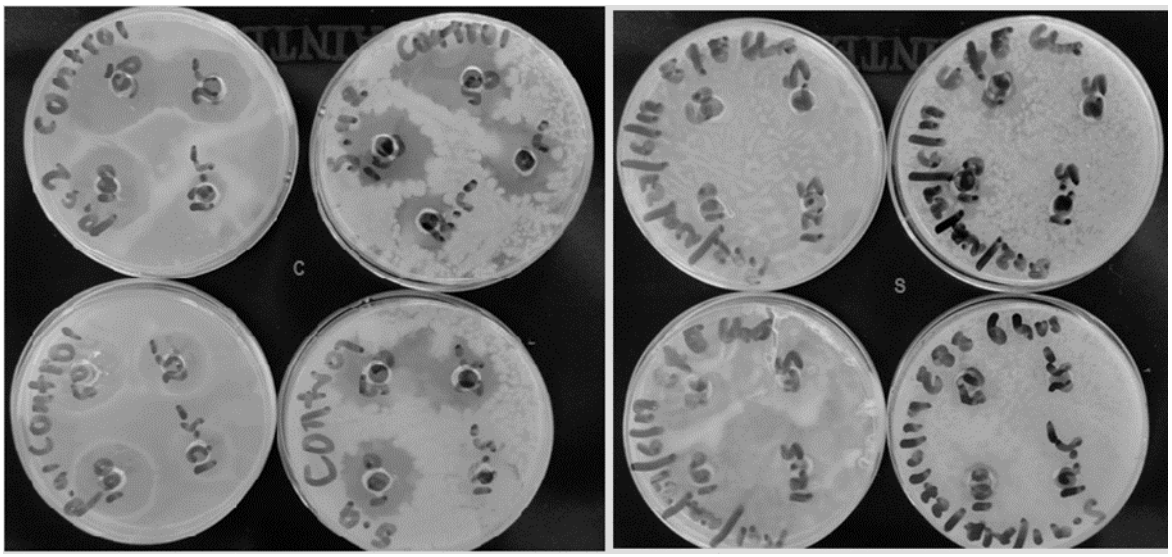

Figure 2: Plates showing antibacterial activities for the control (C) and for some of the extract samples (S)

The bacterial inhibitions were directly proportional to the concentration of the GSEs; 100 mg/mL and 50 mg/mL produced more bacterial inhibitions than 25 and 12.5 mg/mL. Therefore, GSEA-6 h, GSEB-12 h, and GSED-12 h at 25 and 12.5 mg/ mL were neither active against *S. aureus nor P. aeruginosa*. GSEC -6 h, GSEE -6 h were also ineffective for *S. aureus* at 25 and 12.5 mg/ mL. More so, it was observed that GSEF-12 h was not active in this experiment against *S. aureus* at all the tested concentrations of the GSEs. It was also found that *S. aeureus* was more sensitive to attack by the EtOH - derived GSEs; whereas, *P. aeruginosa* was readily affected

by the EtOAc GSEs. In general, *P. aeruginosa* was more inhibited by these GSEs even at the lower concentrations of 25 and 12.5 mg/ mL; especially with the EtOH + EtOAc - and EtOAc - derived GSEs. EtOH + EtOAc GSE has potential of enhancing these bacterial inhibitions. It is important to add that effect of duration of extraction was not observed on the bacterial inhibitions. That is to say that the bacterial inhibitions zones of the extracts at 6 h and 12 h were similar.

In a nutshell, the results have shown potential in the use of GSE as antibacterial agent; hence validating the previous claims and usage of the GSs or GSEs for medicinal purposes [5]. This is so because GS contains many nutrients [8], medicinal ingredients like luteolin, β-sitosterol, β-carotene, saponins, and xylose [9], as well as antioxidant ingredients (catechol, pyrogallol, and pyrogallic acid [10][22][23]. Similarly, growth inhibition zones of extracts of PGSUP with 5000 µg/mL resulted into 9.00 and 9.50 mm for *E. coli* and *S. aureus*, respectively. On the other hand, for infuse method sample with 5000 µg/mL, the growth inhibition zones were 8.12 and 8.45 mm for *E. coli* and *S. aureus*, respectively [5]. The results showed that GSE can be used as an antiseptic aid in food and other related applications [5].

Conclusion

The study revealed that GSs contain some secondary metabolites such as quinone, saponin, tannin, phenols and flavonoids. The investigation of the antibacterial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed that *S. aeureus* was more sensitive to attack by the EtOH - derived GSEs; whereas, *P. aeruginosa* was readily affected by the EtOAc GSEs. In general, *P. aeruginosa* was more inhibited by these GSEs even at the lower concentrations of the GSEs (25 and 12.5 mg/ mL); especially with the EtOH + EtOAc - and EtOAc - derived GSEs. EtOH + EtOAc GSEs have potential of enhancing these bacterial inhibitions. There should be more detail researches about the potentials of GSE with a view of developing antibacterial agents from it.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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