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Antimicrobial and Phytochemical Evaluation of *Vigna subterranean* (L.) Verdc. (Bambara groundnut)

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Abstract

Vigna subterranean, an underutilized legume commonly utilized in local therapy to resolve health conditions mediated by bacterial and fungal infections, was the primary purpose of this research work. The main objectives were to carry out a comprehensive examination of specific chemical components of the plant and explore the antibacterial and antifungal efficiency of the ethanolic extract from the root of V. subterranean. After the samples were collected, they were dried in the shade, pulverized, and then macerated (with 100% ethanol) to extract the components. The resulting liquid extract was subjected to evaporation to attain dryness and then underwent both phytochemical screening and antimicrobial assessment employing varying concentrations (0.5, 1.0, and 1.5 mg/ml) to evaluate the susceptibility of clinical bacterial strains (*Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus aureus*) and also a fungus (Candida albicans) utilizing established protocols, with Ciprofloxacin and Fluconazole utilized as positive controls for antibacterial and antifungal investigations, respectively. The phytocompounds present are steroid, alkaloids, tannins, saponins and flavonoids while anthraquinone was conspicuously absent. The results revealed that the effectiveness of the extract from this plant is dose-dependent, with the most significant inhibition zone observed at 1.5 mm/ml for both antibacterial and antifungal evaluations. The antimicrobial properties demonstrated were enhanced when plant extracts were augmented with Ciprofloxacin against the test isolates. The findings derived from this study emphasize the potential of the *V. subterranean root* as a promising reservoir for antimicrobial agents, thereby corroborating the historical usage of the plant in traditional medicine to combat microbial infections.

Keywords: Antimicrobial activity, Bambara groundnut, Phytochemical analysis, Opportunity Crop

Introduction

Vigna subterranean, also identified as Bambara groundnut (BG), is a legume characterized by subterranean fruit-set and is farmed by smallholders across a significant portion of semi-arid Africa. This crop, originating from Africa, belongs to the legume species and is prevalent in regions located to the south of the Sahara [1]. The significance of food legumes in combating infectious diseases is noteworthy. Hence, it is imperative to augment their consumption levels, which are currently inadequate in numerous developing nations [2]. Legumes function as a vital protein source for a considerable segment of the populace in impoverished countries across the globe, given their status as the most cost-effective, readily stored, and transportable unprocessed protein source for both rural and urban residents. The Bambara groundnut, with its elevated carbohydrate content of 65% and relatively substantial protein content of 18%, represents a comprehensive and vital dietary component essential for addressing various ailments [3]. Nevertheless, limited research endeavours have delved into the possibilities arising from the examination of *V. subterranean* root extract for the identification and quantification of diverse bioactive compounds. This examination helps determine the biological activities and possible medicinal properties of the extract. To determine the potential of the root extract as a natural antibacterial agent, it is necessary to measure its antimicrobial efficacy. These studies provide insightful information about how well it works against bacterial and fungal infections, information that may be useful in the creation of new antimicrobial drugs or alternative methods of treatment. The significant increase in mortality rates due to infectious diseases has been attributed to pathogenic microbes developing resistance to antimicrobial agents and the indiscriminate use of synthetic antibiotics. This has prompted a continuous quest for antimicrobials, particularly those of natural origin. Medicinal plants harbour a diverse array of bioactive compounds with healing properties, some of which have been traditionally utilized as antimicrobials. Therefore, natural plant-based solutions offer exciting potential as alternatives, especially in light of the growing bacterial resistance to antibiotics. Because of their known antibacterial qualities, some plant extracts and phytochemicals can be very significant in therapeutic interventions [4].

Medicinal herbs have been used historically all over the world to cure a lot of illnesses, like asthma, skin problems, intestinal issues, urinary, respiratory, hepatic, and heart diseases. For plants to live and thrive in their natural habitat, they must manufacture a vast variety of biologically active chemicals [5]. These molecules serve to protect plants from abiotic stress, insect infestations, and disease outbreaks. The renewed interest in medicinal plants in both developed and developing nations stems from their proven efficacy as natural antimicrobial agents in combating infectious diseases, unlike synthetic drugs [6]. Pharmaceutical formulations often utilize the natural components of medicinal plants either in the form of extracts or as pure substances. Moreover, medicinal plants are acknowledged as a plentiful source of traditional treatments, and many modern pharmaceuticals are developed from them. For thousands of years, people have used medicinal plants to treat illnesses, preserve food, improve flavour, and control outbreaks of disease. The secondary metabolites produced by plant species that are widely used are responsible for most of their biological traits. Plant-derived compounds are involved in mitigating the growth of microorganisms in different settings [7].

The primary objective of this study is to determine the chemical composition of root extract from the plant and determine the impact of ethanolic root extract of *V. subterranean* on harmful microorganisms. Hence, these investigations strive to confirm the traditional medicinal uses of roots of *Vigna subterranean* and investigate its potential applications in contemporary healthcare and pharmaceuticals. The objectives include the extraction of the dried, powdered root sample using ethanol, performing initial phytochemical analysis, and assessing the antimicrobial efficacy of the extract.

Materials and Methods

The collection, Authentication, and Preparation of Plants

Seeds of *Vigna subterranean* was obtained from the Genetic Resources Centre, International Institute of Tropical Agriculture (IITA), in Ibadan, Nigeria. The Field experiments were established at the Institute for Agricultural Research (IAR), which is located in Ahmadu Bello University (ABU), Zaria. Verification of the dried root was conducted at the herbarium unit within the Department of Biological Sciences at the Nigerian Defence Academy (NDA) in Kaduna, Nigeria, and was assigned specific voucher number (NDA/….13). Subsequently, the root underwent air drying in a shaded area, followed by grinding into a fine powder using a vibrating cup mill machine, and finally, it was stored in a sealed container until needed.

Sample Extraction Process

The methodology outlined by Maiyama [15] was used for sample processing with slight adjustments. A total of 90g of the sample was subjected to extraction using absolute ethanol (1:5 w/v) within a sealed container through maceration, accompanied by regular agitation over a period of three days under warm environmental conditions. After this period, the mixture underwent decantation, and the remaining residue underwent subsequent extraction cycles using fresh solvent (absolute ethanol) until reaching a colourless state. The resulting extract was then sieved through muslin cloth and Whatmann no. 1 filter paper, followed by concentration on a water bath set at 45°C. Subsequently, the concentrated extract was dried and stored in a designated container for future analytical investigations.

Phytochemical Analysis

As reported by Evans et al. [8] and Sofowora [18], the investigation was conducted to detect the presence of steroids/triterpenes, alkaloids, tannins, flavonoids, saponins, and anthraquinone.

Antimicrobial Investigations

Cultures for Testing

Bacterial and fungi strains sourced from the microbiology laboratory at the National Agency for Food and Drug Administration and Control (NAFDAC) in Kaduna state, Nigeria were cultured on Tryptone soy agar (TSA) and Saboraud Dextrose Agar (SDA) agar respectively.

Preparation of Microbial Growth Media

The procedure used to produce tryptone soy agar media (TSA) was to suspend 65 g of solid medium agar in one litre of distilled water, then mix the mixture well. The mixture was then heated to boiling point while being stirred often to ensure that all of the ingredients were completely dissolved. The medium was also put in an autoclave at 121 °C for about fifteen minutes, and then cooled to 45 °C overnight. After incubation, standard microbiological procedures were used for the identification.

Evaluation of Antibacterial Efficacy (Agar Well Diffusion Method)

A standardized inoculum (0.5 McFarland turbidity standard = 1.0x10^8 cfu/cm^3) of each test bacterium (0.1cm^3) was evenly distributed on aseptic tryptone soy agar plates. Subsequently, wells measuring 8.0 mm in diameter were aseptically generated in an agar. The extract was thinned with 10% dimethylsulfoxide (DMSO) to yield concentrations of 0.5, 1.0, and 1.5 cm^3, which were then introduced into the wells. The susceptibility of the microorganisms to the extract was determined by measuring the diameter of inhibition zones surrounding the wells using a transparent ruler. Ciprofloxacin was utilized as a reference substance, and its interaction with the extract was also examined.

Evaluation of Antifungal Efficacy (Agar Well Diffusion Method)
A Saboraud Dextrose agar plate devoid of contaminants was utilized for the experiment, and it was inoculated with the standard inoculum of the test fungus to promote consistent growth. Post ensuring the plate's dryness, aseptic cavities were created through it utilizing a sterile corkborer with an 8.0 mm diameter. The extract was prepared using 10% dimethylsulphoxide (DMSO) and then serially diluted to achieve concentrations of 0.5, 1.0, and 1.5 cm^3. Subsequently, 200 µL of each extract concentration was dispensed into the bored wells, and incubation in a controlled environment at 37 °C was carried out for 48 hours to allow for diffusion into the medium. A positive control containing 0.5% was incorporated into a single well at the centre of a petri dish. Assessment was checked by the pattern of zones of inhibition surrounding the wells. The susceptibility level of the test fungus was assessed based on the diameter of these zones [8]. Furthermore, the efficacy of Fluconazole as a control drug, both alone and in combination with the extract, was also evaluated.

Data Analyses
Results were presented as mean of 3 determinations.

Results

The ethanolic root extract of Vigna subterranean underwent phytochemical screening, revealing the presence of various bioactive compounds, including steroids, tannins, flavonoids, saponins, and alkaloids. Notably, anthraquinones were absent in the extract, as indicated in the results presented below.

Table 1: Findings from the analysis of phytochemical constituents in the extract

Test	+ Present / - absent
Steroids	+
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Anthraquinone	-

The antibacterial activity of the extract was evaluated against *E. coli* and *S. aureus*. Against E. coli, the 0.5% extract exhibited a zone of inhibition of 10.3 mm, which increased to 22.1 mm when combined with Ciprofloxacin. For comparison, Ciprofloxacin alone showed a zone of inhibition of 30.2 mm (Table 2). Against *S. aureus*, the zones of inhibition increased with extract concentration, ranging from 9.2 mm to 16.3 mm. Notably, when combined with Ciprofloxacin, the zones of inhibition increased in a concentration-dependent manner, ranging from 19.3 mm to 25.7 mm. These findings suggest that the extract has antibacterial properties that can be enhanced by combination with Ciprofloxacin. Against the clinical isolate *C. albicans*, Ciprofloxacin alone exhibited a zone of inhibition of 31.5mm, while the combination of Ciprofloxacin and the plant extract showed a slightly reduced zone of inhibition of 26.5mm.

Discussion

The screening revealed the presence of alkaloids, tannins, flavonoids, saponins and steroids with anthraquinone conspicuously absent. The findings from the qualitative screening corresponds with the findings of [15]. The outcome underscores the concentration-dependent nature of the efficacy of the extract, mirroring the pattern observed in the antibacterial and antifungal tests respectively. Several researchers have documented congruent findings. The conventional drugs (ciprofloxacin) exhibited antifungal/antibacterial properties, showcasing higher efficacy compared to the extract

due to its pure form [23]. If the observed diameter of the inhibition zone (DIZ) is equal to or greater than 9 mm surrounding the perforations, potential antimicrobial activity may be present [18]. All concentrations exhibited susceptibility in bacterial test organisms, with *P. aeruginosa* demonstrating slightly heightened sensitivity in comparison to *E. coli*.

Table 2: Zones of inhibition exhibited by extracts against the test microorganisms (clinical isolates)

Treatments	Concentration of extracts			Drug
	O.5%	1.00%	1.50%	(0.5%)
	Zones of inhibition (mm)			
Escherichia coli				
Extract	10.3	14.2	18.2	-
Ciprofloxacin	-	-	-	30.2
Extract + Ciprofloxacin	22.1	24.5	27.2	-
Staphylococcus aureus				
Extract	9.2	14.2	16.3	-
Ciprofloxacin	-	-	-	29.6
Extract + Ciprofloxacin	19.3	23.1	25.7	-
Pseudomonas aeruginosa				
Extract	13.2	17.4	20.1	-
Ciprofloxacin	-	-	-	33.5
Extract + Ciprofloxacin	23.3	26.2	29.5	-
Candida albicans				
Extract	11.3	14.5	18.2	-
Ciprofloxacin	-	-	-	31.5
Extract + Ciprofloxacin	22.1	25.4	26.4	-

The results indicated a direct correlation between the extract's efficacy and its concentration level, a finding consistent with a previous study [19]. As per the earlier discussed antibacterial attributes, the extracts contain a secondary metabolite capable of halting the growth of specific microorganisms. The possession of components such as tannins and saponins, acknowledged for their antimicrobial qualities [20], confers the extracts with their antibacterial characteristics. These components are presumed to disrupt the bacterial cell membrane [21]. The substantial inhibition zone of the extract against *P. aeruginosa* implies its potential application in treating wounds and sores, while its efficacy against *E. coli* suggests utility in addressing diarrhoea and dysentery [22]. The growth of *C. albicans* was impeded by the crude ethanol extract of *V. subterranean* root across all concentrations. The presence of phytochemicals like steroids may underlie the observed antifungal and antibacterial [24] activities. The antimicrobial efficacy could also be related to the tannins present in the root extract of the plant [25]. A synergistic effect was observed in all antimicrobial outcomes when the extract was combined with Ciprofloxacin, resulting in enhanced activity. However, it is worth noting that Ciprofloxacin alone consistently demonstrated larger zones of inhibition compared to the extract alone or in combination.

Conclusion

The root crude ethanol extract of *V. subterranea*, obtained through maceration, was found to contain steroids, alkaloids, tannins, and flavonoids. Antimicrobial investigations, conducted using the agar well diffusion method, demonstrated the effectiveness of the extract against clinical isolates of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The extract exhibited the highest sensitivity to the microorganisms tested when used at the highest

concentration. These findings provide further credence to the historical use of this plant in traditional medicine for treating microbial infections.

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Conflict of Interest

No conflicts of interest were disclosed by the contributors.

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