Original Research Article

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Antimicrobial activities of *Cajanus cajan* L., *Phaseolus vulgaris* L. and *Vigna unguiculata* L. against some bacterial and fungal isolates

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Abstract

Clinical failures associated with synthetic chemotherapies have turned the search for novel natural antimicrobial agents by pharmaceutical and food industries, in the direction of bioactive phytochemical compounds. The antimicrobial activities of *Cajanus cajan*, *Phaseolus vulgaris* and *Vigna unguiculata* against some selected bacterial and fungal isolates were evaluated. This was done by ethanolic extraction of the plant seeds, which were air-dried at room temperature after extraction and collected in sterile test tubes. Agar well diffusion method was adopted for the antimicrobial susceptibility test against two bacterial isolates (*Listeria ivanovii* and *Escherichia coli*), and two fungal isolates (*Aspergillus fumigatus* and *Candida albicans)*. This study indicated that *P. vulgaris* extract demonstrated the highest antimicrobial activity among the three extracts tested. Comparatively, *Vigna unguiculata* exerted the lowest antimicrobial effect on all the test isolates. MIC results indicated that *C. cajan* inhibited all the strains tested (100%) at the presented concentrations. While *P. vulgaris* inhibited 75% of the isolates, only 50% of the strains was inhibited by *V. unguiculata*. These activities decreased with the extract concentrations. The plants' ability to inhibit both bacteria and fungi suggests that they have broad spectrum antimicrobial activities. Although more studies are needed to further authenticate our findings, our finding is interesting considering that these plants are regularly consumed as foods and could be added to the growing list of useful plants materials/products for the treatment of infectious diseases.

Key words: *Cajanus cajan*, *Phaseolus vulgaris*, *Vigna unguiculata*, antimicrobial, chemotherapies

1 Introduction

Historically, plant-based foods have sustained human existence, serving as natural sources of nutrients. With recent advances in medicine, there is increased awareness on other specific health benefits including antimicrobial potentials of some edible plants. This followed the discovery of numerous bioactive compounds present in those plants. These plant-derived components are utilised in the production of drugs and supplements.

Because of the constant emergence of antibiotic-resistant microbial strains evident in clinical reports [1, 2], the search for novel natural antimicrobial agents is unremitting. Also, the upward trend in the prevalence of some health challenges like diabetes and cancer without yet a specific therapeutic solution, have apparently broadened investigation for alternative treatment approaches. Leguminous plants occupy important place among plants with bioactive components. Studies have shown that in addition to their nutritional values such as sources of macro and micronutrients; *Cajanus cajan*, *Phaseolus vulgaris* and *Vigna unguiculata* have antimicrobial potentials [3, 4]. Consequently, the bioactive component(s) from these plants have found relevance both clinically and in the food industry, where they offer solution to the unnecessary economic loss, and clinical problems resulting from food spoilage and intoxication. Optimal application of these natural compounds offers a reliable technique for preservation and extension of shelf life of processed food products. Ultimately, this could provide a good critical control point against food-borne diseases.

Several other biological activities and health benefits of these plants have been identified. These included antibacterial activity, antidiabetic effects, antioxidant activities, neuroactive properties, hypocholesterolemic effects, hepatoprotective effects [5].

Virtually, extracts from different parts of these bioactive plants are naturally rich in pharmacotherapeutic components with antimicrobial effects. Specifically, they have broad spectrum of antibacterial activities inhibiting both gram positive and gram-negative bacteria [6].

Previous studies have speculated anticancer activities of some phytochemical compounds such as those extracted from legumes [7-9]. However, whether the anticancer claims about leguminous plants are exerted at lower or higher doses remain debatable [10]. Similar to other leguminous plants, health and nutritional values are drivable from various preparations of the plant, *Vigna unguiculata* which antimicrobial effects have also, been described. In a recent finding, it was indicated that *V. unguiculata* seed oil n- hexane extract is effective against microorganisms [11]. The phytochemical constituents of most plants are evenly distributed throughout their organs such that they are

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biochemically and physiologically similar. Thus, the antioxidant activity of *V. unguiculata* leaves for instance, which downregulate cholesterol and blood lipid levels thereby enhancing cardiovascular health, is *ipso facto* present in the seed extracts [12].

The choice of extraction solvent pH is an important consideration in the investigation of antimicrobial activity of plants [13]. When evaluating the microbiological and toxicological activities of *Phaseolus vulgaris,* it was observed that extracts from acidified extraction solvent had more antibacterial and antifungal activity than extracts from solvents of higher pH [14]. Similarly, ethanolic solvent which was the extraction solvent adopted in this study had proven efficient for phytochemical studies [15, 16].

Certainly, the bioactive potentials of leguminous plants have rekindled interest in their antimicrobial effects and other health benefits in the past decades. However, some of the shortcomings common to those studies included investigation of only one member of the plant family at a given time, and lack of diversity of the test microorganisms. These often make comparison of research outputs very difficult. In the present study, ethanolic extract of three leguminous seeds were tested against diverse groups of microorganisms belonging to bacteria (gram positive and gram negative) and fungi (yeast and mould). This simultaneous investigation of the three plants under similar condition and time could make for better comparison of the outcome.

2 Materials and methods

2.1 Study Area

This work was carried out in the Microbiology Laboratory, University of Nigeria Nsukka. The three months study duration was between June to September 2017.

2.2 Sample collection

The raw plant samples of *Cajanus cajan*, *Phaseolus vulgaris* and *Vigna unguiculata* were purchased from Ogige market, Nsukka Local Government of Enugu state.

2.3 Plant extraction

The collected plant samples were macerated and pulverized into powdered form using a blender. Then, each of the samples were dissolved in the ethanol extraction solvent in the ratio of 1g: 5 mL and left for 48 hr with periodic shaking to mix properly. At the end of the 48 hr extraction period, the solution was sieved and filtered using Whatman filter paper no 1 (GE Healthcare companies, Buckinghamshire, UK) and then exposed to air drying at room temperature. Each of the resulting dried extracts were collected into sterile containers and kept under refrigeration temperature until use.

2.4 Antimicrobial sensitivity test

2.4.1 Preparation of the extracts

Following the method by Nweze and Eze [17] with slight modifications, about 0.5g of each of the ethanolic extracts was dissolved in 5 mL of sterile distilled water containing 10% dimethyl sulphoxide (DMSO). The solution of each extract was homogenised by rigorous shaking. Five serial dilutions of each extract were made by two-fold to achieve concentration gradients of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL.

2.4.2 Test isolates

As previously described [17], overnight bacterial cultures of *Escherichia coli and Listeria ivanovii* on nutrient agar plates, and about 48 hr cultures of *Candida albicans, Aspergillus fumigatus* on SDA plates, (Titan Biotech Sabouraud Dextrose Agar, TM 387 by OF Micro Lab Solution LLP, New Delhi, India) were each adjusted in sterile test tubes of normal saline to turbidity equivalent to 0.5 McFarland standard.

2.4.3 Agar well diffusion test

The antimicrobial susceptibility testing was done as described by Balouirii *et al* [18]. Using sterile swab sticks, each of the standardized cultures was inoculated by spreading the entire surface of Mueller Hinton agar plates (Oxoid, Basingstoke, Hampshire, England). Agar wells of 6 mm in diameter was bored into the inoculated agar plates using sterile cork borer. Then the wells were filled with equal volume (40 µL) of extract concentration gradients. The assay plates prepared in duplicates were incubated at 37 ºC for 18 hr (bacteria) to 48 hr (fungi), and the inhibition zone diameters were recorded and interpreted according to CLSI document [19].

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the plant extracts were determined according to CLSI [19]. Standardized cultures of bacteria were further diluted to achieve a final concentration of 1.0×10^6 CFU/mL. The fungal cultures were adjusted such that the final concentration was 1×10^4 CFU/mL – 5×10^3 CFU/mL. All the preparations were done in Mueller Hinton broth (Oxoid Basingstoke, Hampshire, England) test tubes containing concentration gradients of the extracts. The test tubes were incubated at 37 ºC for 18 hr (bacteria) to 48 hr (fungi). The resulting turbidity were compared to control test tubes (positive controls with conventional antibiotics and inoculum; and negative control test tube broth inoculated with test strains).

2.6 Statistical analysis

Each data was presented as mean ± standard deviation of the values. The number of test strains inhibited by the three plant extracts was compared using Fisher exact test. Statistical significance was considered at $p < 0.05$.

3 Results

3.1 Antimicrobial sensitivity

The agar well diffusion results as presented in Table 1 showed the inhibition zone diameter (± standard deviation) recorded for each extract against the four microbial strains. *P. vulgaris* extract demonstrated the highest antimicrobial activity among the three extracts tested, except for *Listeria ivanovii* where *Cajanus cajan* produced the highest zone of inhibition at 100 mg/mL and 50 mg/mL concentrations. On the other hand, *Vigna unguiculata* exerted the lowest antimicrobial effect on all the test isolates except for *Escherichia coli*, whose susceptibility to *Cajanus cajan* was comparable with other extracts.

Table 1. Inhibition Zone Diameter (mm) of the extracts (mg/ml) against the test organisms

	Inhibition zone diameter of extracts at different concentrations (mg/mL)				
Organisms/Extracts	**100**	**50**	**25**	**12.5**	**6.25**
Aspergillus fumigatus					
Cajanus cajan	9.50 ± 0.7	8.70 ± 0.1	8.10 ± 0.03	7.20 ± 0.3	6.20 ± 0.1
Vigna unguiculata	9.20 ± 0.2	8.10 ± 0.1	7.20 ± 0.03	7.20 ± 0.1	6.90 ± 0.1
Phaseolus vulgaris	11.20 ± 0.2	9.30 ± 0.1	9.10 ± 0.1	8.40 ± 0.1	7.50 ± 0.1
Candida albicans					
Cajanus cajan	7.15 ± 0.2	6.35 ± 0.1	6.25 ± 0.1	6.15 ± 0.1	6.05 ± 0.1
Vigna unguiculata	6.75 ± 0.1	6.50 ± 0.1	6.35 ± 0.1	6.20 ± 0.1	6.05 ± 0.1
Phaseolus vulgaris	9.10 ± 0.6	8.30 ± 0.2	7.10 ± 0.1	6.35 ± 0.2	6.10 ± 0.1
Listeria ivanovii					
Cajanus cajan	9.60 ± 0.6	9.05 ± 0.1	8.20 ± 0.1	7.90 ± 0.1	7.50 ± 0.04
Vigna unguiculata	8.90 ± 0.1	8.70 ± 0.3	8.30 ± 0.3	8.15 ± 0.2	7.90 ± 0.1
Phaseolus vulgaris	9.20 ± 0.1	8.90 ± 0.1	8.40 ± 0.4	8.45 ± 0.1	7.90 ± 0.2
Escherichia coli					
Cajanus cajan	8.10 ± 0.1	7.70 ± 0.1	7.10 ± 0.1	6.20 ± 0.1	6.10 ± 0.1
Vigna unguiculata	10.10 ± 0.1	7.90 ± 0.1	6.30 ± 0.1	6.60 ± 0.1	6.30 ± 0.5
Phaseolus vulgaris	10.90 ± 0.1	9.50 ± 0.7	8.50 ± 0.7	7.10 ± 0.1	6.20 ± 0.1

3.2 Minimum Inhibitory Concentration (MIC)

MIC results indicated that *C. cajan* inhibited all the strains tested (100%) at the presented concentrations (Table 2). While *P. Vulgaris* inhibited 75% of the isolates, only 50% of the strains was inhibited by *V. unguiculata.* However, their difference in inhibitory activities are not significant (Fisher exact test at $p < 0.05$).

Table 2. Minimum Inhibitory Concentration (MIC) of the extracts (mg/mL) against clinical isolates

Organism/source	***C. cajan***	***P. vulgaris***	***V. unguiculata***
E. coli	25.00	12.50	25.00
L. ivanovii	25.00	12.50	>100
A. fumigatus	12.50	>100	>100
C. albicans	6.25	25.00	6.25
MIC range	6.25-25.00	12.50-25.00	6.25-25.00
Geometric mean	14.90	7.90	3.50

Key: >100 = absence of inhibition at the highest concentration of the extract tested (100 mg/mL).

4 Discussion

Antimicrobial activities of the ethanolic seed extracts of *Cajanus cajan*, *Phaseolus vulgaris* and *Vigna unguiculata* were presented in this study. Earlier findings demonstrated their extra nutritional values, where they are associated with enhanced health benefits including prevention of infectious and non-infectious diseases [4]. These plants as investigated showed activity against the test isolates, although at varying degrees. However, among the plants, no significant difference in their inhibitory activities was recorded (Fisher exact test, $p < 0.05$). Findings from the susceptibility test (by agar well diffusion) indicated that *P. vulgaris* produced the highest inhibition zone diameter when used against the fungal isolates, and against one of the two bacterial strains tested. On the other hand, the MIC test showed that *C. cajan* has excellent inhibitory activity against all the test isolates. This has corroborated a previous observation that in addition to their antibacterial effects, these plants including *C. cajan* are popular for their good antifungal activity [20].

Evidently, *C. cajan's* therapeutic effects against cutaneous diseases especially in the traditional medicine was reported [21]. The antimicrobial activities of *C. cajan* may be related to the presence of the phytochemical compounds, flavonoids, which are common bioactive components of medicinal plants [22]. Additionally, some studies have provided their antiviral evidence. In one of the studies using ethanolic and aqueous *C. cajan* extracts *in vivo*, their antiviral activities against measles virus was shown [23].

Anticancer effect of some leguminous plants remains ambiguous due to conflicting findings. Their phytoestrogenic potential was implicated as an inducer of human breast cancer by enhancing the proliferation of estrogen receptor proteins and those that regulate apoptosis during the initial neoplasma formation [24]. Corroborating this, another observation in an animal study also, indicated that some phytocomponents of *Cajanus cajan* viz: isoflavones, resveratrol, and flavones have phytoestrogenic activity, which at high exposure pose risk of a reversible histotoxicity [25]. However, a contrasting evidence of cancer preventive activity was suggested in an *in vivo* study, in which mice groups administered with flavonoids sub unit of *Cajanus cajan* countered lethal effects of mutagenic cyclophosphamide in mice [26]. Moreover, high circulating serum concentration of the genistein sub-component of isoflavones had resulted in reduced risk of cancer. It was reported that strategic supplementation, when there is minimal endogenous oestrogen level, could promote phytoestrogens' anticancer effect [27]. A related observation by Bilal *et al* [28], traced the anticancer effect of phytoestrogens to their ability to influence multiple targets, and their functions in epigenetic modulation such as micro RNAs, DNA methylation and histone acetylation. These are accomplished especially at effective concentration and exposure from early stage of life.

P. vulgaris seed is a natural reservoir of important plant antifungal fraction, lectin. Probably, this compound mediates the plant's antifungal activity, whose mechanism of action was related to interference with the fungal chitin wall synthesis, given the analogous structure of chitinase and lectin [29]. Furthermore, Ekowati *et al* [30], identified lectin as the most active phytohemaglutinin component responsible for the *P. vulgaris* antimicrobial activity, particularly for its immunomodulatory effects in defense against viral infections.

V. unguiculata's bioactive component, globulins have demonstrated comparative antibacterial activity with conventional antibiotics, which was associated with its high cationic concentration enabling intercellular interactions [31]. Similar to present findings, evidence of bactericidal activity of *V. unguiculata* was revealed by Franco *et al* [32], in addition to its generally known antimicrobial potentials.

Although, the *V. unguiculata* alpha and beta sub-units have equipotent antibacterial effects, Ye *et al* [33] reported that the alpha sub-unit has comparatively higher antifungal activity than its beta fraction. In the same findings, they reported that gamma thionin II, belonging to the plant defensins family could exert anti- bactericidal activity on the test bacterial strains, with specific higher activity against gram negative bacteria. *V. unguiculata* antioxidant property is well studied, however, its antiparasitic protozoan activity is seemingly the opposite effect of its antioxidant activity, which was suggested as a consequence of free radicals from reactive oxygen induction by the plant's defensin peptides [34]. However, induction of free radicals including nitrogen (II) oxide, could result only at high concentration of peptides Cp -Thionin II following a similar activity observed from its synthetic analogue, KT43C [35].

5 Conclusion

These results help to improve our initial understanding that the utility of these leguminous plants extends beyond their basic nutritional values, and includes therapeutic effects against microbial infections. Their ability to inhibit both bacteria and fungi suggested that they are broad spectrum antimicrobial agents and can be applied in the management of wider range of health problems including non-infectious conditions like cancer.

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