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Antimicrobial Properties and Phytochemical Composition of *Garcinia kola*, *Bryophyllum pinnatum*, and *Allium sativum* Juices on Some Clinical Pathogens

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Abstract

Background: Medicinal plants have been in use since the origin of man. Many important chemical substances with biological functions that could be used for treatment and prevention of attack from bacteria, fungi, herbivorous mammals and insects are produced by different plants. Such compounds with useful properties have been recorded in their numbers, about 12,000 accounting for about 10% of total plant species. **Aim:** The aim of the study was to determine the antimicrobial efficacies of herbal extracts on some clinical pathogens. **Methods:** The antimicrobial activities of pressed juices of *Allium sativum* (garlic), *Bryophyllum pinnatum* and *Garcinia kola* neats and their dilutions were tested on pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus* and *Candida albicans* to determine their susceptibility to the juices and their combinations. Agar well diffusion method was employed on Muller-Hinton agar to determine their antimicrobial susceptibility pattern. The phytochemical analysis of the plants' juices were also determined. **Results:** At 100% (neat) the juices of *G. kola*, *B. pinnatum* and *Garcinia kola* showed substantial zones of inhibition against the pathogens with a zone diameter of about 22.0 mm and above. At 75% concentrations, the juices inhibited the pathogens tested against them. *A. sativum* (garlic) inhibited *K. pneumonia*, *P. mirabilis*, and *S. aureus* even at 50% concentration. *C. albicans* isolates were 60% susceptible to *G. kola* juice, 40% at 100% concentration. At 75% concentration of the juice, *C. albicans* isolates were also 60% susceptible to the juices. At 50% - 100% concentrations, *C. albicans* isolates were 100% sensitive to *A. sativum* extract. **Conclusions:** The medicinal plant juices tested against the pathogens possess some potentials worth exploiting as potent antimicrobial agents on gram-positive, gram-negative bacteria and the fungus.

Keywords

Antimicrobial, Phytochemical, Plant Juices, Pathogens

1. Introduction

The increasing rates of antimicrobial resistance among bacteria of clinical importance have given rise to the emergence of microorganisms with multiple-drug resistance. The unavailability and high cost of antimicrobial drugs with limited effect on microbes have increased morbidity and mortality [1]. There is a need to search for more alternative sources of antimicrobial agents with high potency. Consequently, this has led to more search for valuable antimicrobial agents from plants, with the hope to uncover potentially active plants, and or their components that may serve as sources for the production of new antimicrobial drugs [2] [3] [4]. *Bryophyllum pinnatum*, also known as the air plant, cathedral bells, life plant, miracle leaf and regeneration plant is a succulent plant native of Madagascar, which is a popular house plant and has become adapted to tropical and subtropical regions of the globe. *B. pinnatum* belongs to the plant family *Crassulaceae*. *B. pinnatum* is a crassulescent herb of about 1 meter in height, with opposite, glabrous leaves (with 3 - 5 deeply crenulated, fleshy leaflets) [5] [6]. This plant is used in ethnomedicine for the treatment of many diseases such as earache, cough, diarrhoea, dysentery, abscesses, ulcers etc. [7] [8]. In Southern Nigerian, *B. pinnatum* is used to facilitate the dropping and healing of placenta wounds of the new born babies [8]. The leaves of *B. pinnatum* contain bryophyllin, malate, potassium, ascorbic acid, citric acid and malic [8] [9]. The plant is rich in macro and microelements, vitamins, calcium, phosphorus, ascorbic acid, inulin [8] [10] [11]. Other compounds such as saponin, flavonoids, anthraquinones, xanthones, bryophyllin A and B might be identified [8] [11]. It is also said to have anti-inflammatory, hypoglycaemic, anti-diabetic and anti-cancer properties [7]. It has been accepted as herbal remedy in almost all parts of the world [6] [12] [13] [14]. The plant is distributed worldwide but growing primarily in the rainforest [6] [15]. It is used as folk medicine in most other countries of the globe [6] [16] [17].

Garcinia kola is a species of flowering plant in the Clusiaceae or Guttiferae family. It is used in most parts of West Africa as a social beverage and offered to guests as kola in many Nigerian cultural settings [18]. *G. kola* is valued for its medicinal properties. It is used in traditional medicine for the treatment of laryngitis, general inflammation, bronchitis, viral infections and diabetes. It is also used as rejuvenating agents, adaptogen and general antidotes [18]. The combination of aqueous extract of *G. kola* mixed with honey has demonstrated synergistic effects against some bacterial pathogens, such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella specie* and *Pseudomonas aeruginosa* [19]. Methanol and water-soluble fractions of *G. kola*

and *Cola nitida* possess antibacterial activity. *G. kola* was more active against some members of Enterobacteriaceae, namely, *E. coli* and *Salmonella typhi*, whereas, methanol extracts of *C. nitida* showed greater activity against *S. aureus*. Thus, the plants may possess the potentials for the manufacture of a potent drug for the treatment of infections caused by these organisms associated with typhoid fever, gastroenteritis, urogenital infections, boils etc. It was also observed that the zones of inhibition of the extracts are comparable to those of orthodox antimicrobial drugs used as control [19].

Studies on the antimicrobial activity of *A. sativum* (garlic) juice against some selected pathogenic bacteria had been reported. The antimicrobial activity of the water and ethanol extracts of *A. sativum* are shown to be effective against *Proteus mirabilis*, *Salmonella typhi* and *S. aureus*, but ineffective against *E. coli*, *B. subtilis* and *P. aeruginosa*. While the ethanol extracts of *A. sativum* were effective against four of the organisms tested except *Escherichia coli* and *Bacillus subtilis* [20]. [21] reported the antibacterial activities of *A. sativum* on some pathogenic bacterial strains. The aqueous extract of garlic (*A. sativum*) was shown to inhibit the growth of both Gram-positive and Gram-negative bacteria with maximum inhibitory activity noted against *K. pneumoniae*, *B. cereus*, *E. coli* and *S. mutans*. Autoclaved *A. sativum* (garlic) sample did not exhibit antibacterial activity on the test organisms [22].

The extract from *A. sativum* had been reported to show good antimicrobial activities against most human pathogens such as intestinal parasites, fungi, bacteria and viruses. This plant might be a good candidate for use as an antimicrobial agent in terms of its ability to inhibit the growth of pathogens, safe to handle, affordable and very easy to obtain [23]. *A. sativum* had been reported to inhibit the growth of multidrug-resistance bacteria of both Gram-negative and Gram-positive bacteria. The authors showed the antimicrobial properties of garlic oil against human enteric bacteria in comparison with garlic oil sulfides and garlic powder and observed a large zone of inhibition using 100% aqueous extract of *A. sativum* superior to Neomycin sulphate which is commercially used against *S. aureus*, *P. aeruginosa*, and *E. coli* [24].

Several plant species have shown promising microbiostatic and microbiocidal activities against a range of enteric pathogenic microbes and these are attributed to the presence of minute doses of bioactive contents referred to as phytochemicals [25]. These phytochemicals include alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, anthraquinones, among others. Alkaloids are the largest group of secondary plant metabolites comprising basically nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen. These nitrogenous compounds which act in plants' defense against pathogenic organisms are widely exploited as pharmaceuticals, psycho-stimulants, narcotics, and poisons due to their renowned biological activities [26]. This study is aimed at determining the antimicrobial activities of *G. kola*, *B. pinnatum* and *A. sati-*

vum juices and the combined juices on some selected pathogens and the phytochemical components of the plant juices.

2. Materials and Methods

2.1. Sources of Materials

Fresh leaves of *B. pinnatum* was obtained from Mgbuoduohia community, Rumuolumeni in Obia/Akpor Local Government Area of River state, while *Garcinia kola* was obtained from the tree source in Obiohia community in Omuma Local Government Area of Rivers State, Nigeria. *Allium sativum* (Garlic) 1 kg weight of montage garlic was bought from Mile III market in Diobu Port Harcourt, Rivers State, Nigeria.

2.2. Preparation of Extracts

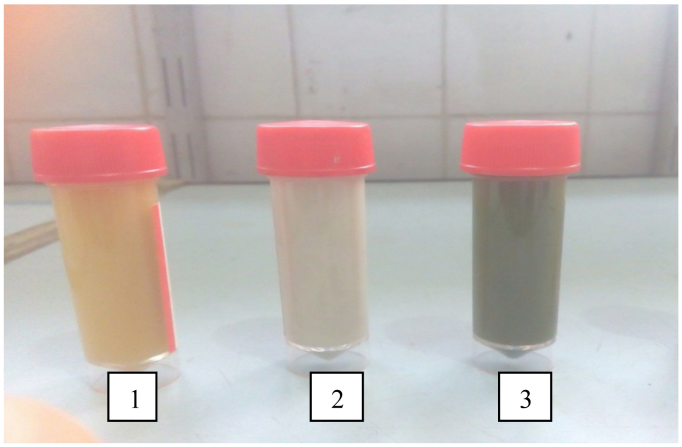
The fresh leaves of *B. pinnatum* obtained from the plant were properly washed in clean running tap water and air-dried. The air-dried leaves were squeezed to press out the fluid content, the greenish fluid was decanted into a universal bottle and preserved in a refrigerator for further uses. *G. kola*, the brown covers of the seeds were peeled using table knife, the seeds were chopped into smaller pieces. The pieces were further ground using a mechanical blender. The crushed *G. kola* was placed in a fine sieve clot and sieved-squeezed to obtain sticky, milky juice. This was decanted into a bottle and preserved in the refrigerator. *A. sativum*, the upper cover (bulbs) was peeled off exposing the inner compartments of the garlic. The soft inner parts were ground with a manual blender and the milled product was sieved using fine cloth material to obtain the liquid content. The amber coloured fluid obtained was filtered into a universal bottle and kept in a refrigerator for subsequent uses.

A syringe filter (Minispike syringe filters) consisting of a plastic housing with a membrane which serves as a filter, the pore size of a filter of 0.45 μm was used to filter the juice. The unsterilized juice was drawn into a sterile syringe before connecting the wheel like a filter to the syringe. Then the unfiltered juice was forced out, through the filter into a sterile bottle. To ascertain the sterility of the juice, it was cultured on blood agar and Muller-Hinton agar, incubated for 24 hrs at 37˚C, and there was no growth.

Mini-UniPrep syringe filter.

2.3. Dilutions of Extracts

Sterile distilled water was used for diluting the different concentrations of each extracts 2 ml of working solution were prepared from the stock of each extract. The 100% extract constitute the neat without dilution, 75% was prepared by adding 0.5 ml of distilled water to 1.5 ml of each extract from the neat, 50% dilutions of the working solution of each extract were prepared by adding 1ml of distilled water to 1 ml of the neat of each extract, 25% made by 0.5 ml extract to 1.5 ml of distilled water and 10% dilutions were made by adding 0.2 ml of each extract to 1.8 ml of distilled water respectively.



Juices of *Allium sativum* (1), *Garcinia kola* (2) and *Bryophyllum pinnatum* (3).

2.4. Test Organisms

Pure cultures of the microorganisms were obtained from the microbiology laboratory of Braithwaite Memorial Specialist Hospital, Port Harcourt. They were further subjected to chemical and biochemical tests for confirmation. The isolates were *Escherichia coli*, *Klebsiella pneumoniae Proteus mirabilis*, *Staphylococcus aureus* and *Candida albicans*.

2.5. Antimicrobial Susceptibility Testing

Agar well diffusion method was used for susceptibility testing of the extracts on the microorganisms. The pure culture of each organism was aseptically inoculated into a bijou bottle containing 3 ml of peptone water. The inoculums were standardized by matching the turbidity with 0.5 McFarland standards. The suspensions were incubated at ambient temperature. Then the suspensions were poured onto already prepared and dried Muller-Hinton agar in a petri dish. This was immediately decanted after allowing it to spread through the surface of the agar plate by gentle rotation. Using sterile a cork borer with a diameter of 10 mm, agar wells were made on the seeded Muller-Hinton agar plates at positions already marked at the back of the petri dish. Then 100 µl of the extracts were dispensed into each well using a sterile micropipette. The first batch contains 100% of the juice (neat) of *G. kola*, *B. pinnatum* and *A. sativum* respectively.

The plates were incubated in an upright position, at a temperature of 37˚C for 18 - 24 hrs. The diameters of the zones of inhibition were measured in millimeters (10 mm is equivalent to zero) and the results were recorded. The processes were repeated for the different dilutions or concentrations 75%, 50%, 25%, and 10% for each organism separately. The inhibition zones are compared with that of gentamicin (CN).

2.6. Phytochemical Analysis

Phytochemical analysis was carried out on three juices (*A. sativum*, *B. binnatum* and *G. kola*). They were conducted at the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt. The metabolites tested for were: alkaloids, flavonoids, tannins, anthraquinone, triterpenoid, steroids, carbohydrates, cardenolide, cyanogenic glycosides and saponin.

2.7. Data Analyses

Results were presented as percentages and *p*-value was calculated using Chi-Square (GraphPad Prism Software Version 5.03, San Diego, CA). Statistical significance was defined as a *p*-value of less than 0.05 at a 95% confidence interval.

3. Results

3.1. Antimicrobial Potential of Herbal Extracts against *Escherichia coli*

Of the ten (10) clinical isolates of *E. coli* used for susceptibility testing, 60% was sensitive to gentamicin (CN), 80% were sensitive to sparfloxacin and 60% were sensitive to ofloxacin. The effect of plant juices, *E. coli* was 90% susceptible to *G. kola* juice neat (100%) concentration), at 75% concentration of the juice, *E. coli* was 70% susceptible to *G. kola* juice (Table 1). At a concentration of 100% (neat) *E. coli* was 80% sensitive to *B. pinnatum* and at 75% concentration of *B. pinnatum* juice, *E. coli* was 60% susceptible. At concentrations of 100% and 75%, *E. coli* was 100% susceptible to *A. sativum* juice and 70% susceptible to a 50% concentration of *A. sativum*. *E. coli* was totally sensitive to 100% concentration of the combined juices of *G. kola* + *B. pinnatum*, *G. kola* + *A. sativum*, *B. pinnatum* + *A. sativum* and G. kola + *B. pinnatum* + *A. sativum*. At 75% concentration

Table 1. Percentage comparison of antimicrobial activity of control antibiotics and medicinal plant juice against *E. coli*.

Zone of Inhibition (mm)	Control Drugs (%)			*G. kola* (%)					*B. pinnatum* (%)					*A. sativum* (%)				
	Gen	Spa	Ofl	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
R (≤12)	20	20	20	100	100	100	0	0	100	100	100	20	0	100	100	10	0	0
I (13 - 14)	20	0	20	0	0	0	30	10	0	0	0	20	20	0	0	20	0	0
S (≥15)	60	80	60	0	0	0	70	90	0	0	0	60	80	0	0	70	100	100

of the juices *E. coli* was 100% sensitive to the combined juices, *G. kola* + *B. pinnatum*, 90% sensitive to *G. kola* + *A. sativum*, *B. pinnatum* + *A. sativum* and a combination of the three juices.

3.2. Antimicrobial Potential of Herbal Extracts against *Klebsiella pneumoniae*

K. pneumoniae were 60% susceptible to gentamicin, 50% were susceptible to sparfloxacin, whereas for ofloxacin 60%. *K. pneumoniae* were 100% susceptible to the neat (100%) juices of *G. kola*, *B. pinnatum* and *A. sativum* (Table 2). At 75% concentration of the juices, *K. pneumoniae* were 70% sensitive to *G*, *kola*; 60% sensitive to *B. pinnatum* and 100% sensitive to *A. sativum*. At 50% concentration of *A. sativum*, *K. pneumonia* was 50% susceptible to *A. sartivum* juice. *K. pneumoniae* were completely susceptible to 100% concentration of the combined juices of *G* + *kola B. pinnatum*, *G. kola* + *A. sativum*, *G. kola* + *A. sativum* and *B. pinnatum* + *A. sativum* and *G. kola* + *B. pinnatum* + *A. sativum*. At 75% concentration *K. pneumoniae* were 100% susceptible to the combined juices, *G. kola* + *B. pinnatum*, 90% sensitive to *G. kola* + *A. sativum* and 80% susceptible to *B. pinnatum* + *A. sativum* 80%. At 100% concentration of the combined juices of *G. kola* + *B. pinnatum* + *A. sativum K. pneumoniae* were 100% susceptible and 80% susceptible to the three combined juices at 75% concentration (Table 3).

3.3. Antimicrobial Potential of Herbal Extracts against *Proteus mirabilis*

The antimicrobial susceptibility of control drugs on *P. mirabilis* showed 70% of

Table 2. Percentage comparison of antimicrobial activity of control antibiotics and medicinal plant juice against *K. pneumoniae*.

Zone of Inhibition (mm)	Control Drugs (%)			*G. kola* (%)					*B. pinnatum* (%)					*A. sativum* (%)				
	Gen	Spa	Ofl	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
R (≤12)	20	20	20	100	100	100	10	0	100	100	100	10	0	100	100	10	0	0
I (13 - 14)	20	30	20	0	0	0	20	0	0	0	0	30	0	0	0	40	0	0
S (≥15)	60	50	60	0	0	0	70	100	0	0	0	60	100	0	0	50	100	100

Table 3. Percentage comparison of antimicrobial activity of control drug with combined juices of medicinal plants on *K. pneumoniae*.

Zone of Inhibition (mm)	Control Drug (%)			*G. kola* + *B. pinnatum* (%)					*G. kola* + *A. sativum* (%)					*B. pinnatum* + *A. sativum* (%)					*G. kola* + *B. pinnatum* + *A. sativum* (%)				
	Gen	Spa	Ofl	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
R (≤12)	20	20	20	100	100	100	0	0	100	100	100	0	0	100	100	100	0	0	100	100	100	0	0
I (13 - 14)	20	30	20	0	0	0	0	0	0	0	0	10	0	0	0	0	20	0	0	0	0	0	0
S (≥15)	60	50	60	0	0	0	100	100	0	0	0	90	100	0	0	0	80	100	0	0	0	100	100

clinical to both gentamicin and sparfloxacin and 80% susceptible to ofloxacin (Table 4). *P. mirabilis* were 100% sensitive to *G. kola* juice at 100% concentration (neat). At 75% concentration of the juice *P. mirabilis* was 50% susceptible to *G. kola*. *P. mirabilis* were 90% sensitive to *B. pinnatum* juice neat (100%) concentration. At 75% concentration of the *B. pinnatum* juice, *P. mirabilis* was 40% susceptible. *P. mirabilis* isolates was 100% sensitive to *A. sativum* juice at 100% and 75% concentrations. At 50% concentration, the isolates were 80%. *P. mirabilis* were also sensitive completely susceptible to 100% concentration of the combined juices of *G. kola* + *B. pinnatum*, *G. kola* + *A. sativum*, and *B. pinnatum* + *A. sativum* and *G. kola* + *B. pinnatum* + *A. sativum*. At 75% concentration *P. mirabilis* were 100% sensitive to the combined juices, *G. kola* + *B. pinnatum*, 90% sensitive to *G. kola* + *A. sativum*, 100% to *B. pinnatum* + *A. sativum* and 80% to the combination of the three juices (Table 5).

3.4. Antimicrobial Potential of Herbal Extracts against *Staphylococcus aureus*

For the control drugs, 80% showed sensitivity to gentamicin and 60% was sensitive, 60% sensitive to sparfloxacin while 50% was sensitive to erythromycin (Table 6). *S. aureus* was 100% resistant to *G. kola* juice at all concentrations but was 100% sensitive to *B. pinnatum* neat. At 75% concentration of the juices, *S. aureus* isolates were 80% sensitive to *B. pinnatum*, while *A. sativum* juices at 100%, 75% and 50% concentration completely inhibited *S. aureus* (100%). *S. aureus* isolates were 100% sensitive to *G. kola* + *B. pinnatum* at 100% and 75% concentrations. At 100% and 75% concentrations of *G. kola* + *A. sativum S. aureus* isolates were 100% sensitive to the juices. *S. aureus* isolates were 100% sensitive *B. pinnatum* + *A. sativum* juices at 100% and 75% concentrations (Table 7).

3.5. Antimicrobial Potential of Herbal Extracts against *Candida albicans*

Table 8 is showing the antifungal activity of control drugs and the medicinal plant juices against *C. albicans*. For the control drugs, 30% were resistant to fluconazole, 20% SDD and 50% sensitive. *C. albicans* isolates were 60% sensitive to *G. kola* juice, 40% SDD at 100% concentration. At 75% concentration of the juice, *C. albicans* isolates were 40% resistant and 60% SDD. *C. albicans* were

Table 4. Percentage comparison of antimicrobial activity of control antibiotics and medicinal plant juice against *P. mirabilis*.

Zone of Inhibition (mm)	Control Drugs (%)			*G. kola* (%)					*B. pinnatum* (%)					*A. sativum* (%)				
	Gen	Spa	Ery	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
R (≤12)	20	30	30	100	100	100	100	100	100	100	100	0	0	100	100	0	0	0
I (13 - 14)	20	10	20	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0
S (≥15)	60	60	50	0	0	0	0	0	0	0	0	80	100	0	0	100	100	100

Table 5. Percentage comparison of antimicrobial activity of control drug with combined juices of medicinal plants on *P. mirabilis*.

Zone of Inhibition (mm)	Control Drug (%)			*G. kola* + *B. pinnatum* (%)					*G. kola* + *A. sativum* (%)					*B. pinnatum* + *A. sativum* (%)					*G. kola* + *B. pinnatum* + *A. sativum* (%)				
	Gen	Spa	Ofl	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
R (≤12)	30	30	20	100	100	100	0	0	100	100	100	0	0	100	100	100	0	0	100	100	100	0	0
I (13 - 14)	10	10	20	0	0	0	10	0	0	0	0	10	0	0	0	0	0	0	0	0	0	20	0
S (≥15)	60	60	50	0	0	0	90	100	0	0	0	90	100	0	0	0	100	100	0	0	0	80	100

Table 6. Percentage comparison of antimicrobial activity of control antibiotics and medicinal plant juice against *Staphylococcus aureus*.

Zone of Inhibition (mm)	Control Drugs (%)			*G. kola* (%)					*B. pinnatum* (%)					*A. sativum* (%)				
	Gen	Spa	Ofl	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
R (≤12)	30	30	30	100	100	100	20	0	100	100	100	10	0	100	100	10	0	0
I (13 - 14)	10	10	20	0	0	0	30	0	0	0	0	50	10	0	0	10	0	0
S (≥15)	60	60	50	0	0	0	50	100	0	0	0	40	90	0	0	80	100	100

Table 7. Percentage comparison of antimicrobial activity of control drug with combined juices of medicinal plants on *Staphylococcus aureus*.

Zone of Inhibition (mm)	Control Drug (%)			*G. kola* + *B. pinnatum* (%)					*G. kola* + *A. sativum* (%)					*B. pinnatum* + *A. sativum* (%)					*G. kola* + *B. pinnatum* + *A. sativum* (%)				
	Gen	Spa	Ery	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
R (≤12)	20	30	30	100	100	100	0	0	100	100	100	0	0	100	100	100	0	0	100	100	100	0	0
I (13 - 14)	20	10	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S (≥15)	60	60	50	0	0	0	100	100	0	0	0	100	100	0	0	0	100	100	0	0	0	100	100

Table 8. Percentage comparison of antimicrobial activities of control antifungal drug with medicinal plant juice against *C. albicans*.

Zone of Inhibition (mm)	Control Drugs (%)	*G. kola* (%)					*B. pinnatum* (%)					*A. sativum* (%)				
	Flu	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
R (≤14)	30	100	100	100	40	0	100	100	100	100	100	100	100	0	0	0
SDD (>14 <19)	20	0	0	0	60	40	0	0	0	0	0	0	0	0	0	0
S (≥19)	50	0	0	0	0	60	0	0	0	0	0	0	0	100	100	100

100% resistant to *B. pinnatum* juice at all concentrations. At 100%, 75% and 50% concentration, *C. albicans* isolates were 100% sensitive to *A. sativum* juice but resistant to lower concentrations. *C. albicans* isolates were 100% sensitive to the mixed juice *G. kola* + *B. pinnatum* at 100% concentration. At 75% concentration of the mixed juice, the isolates were 40% sensitive and 60% SDD. *C. albicans* isolates were100% sensitive to combined juices *G. kola* + *A. sativum* at 100% and 75% concentrations. At 100% concentration, *C. albicans* isolates were 100% sen-

sitive to the combined juices of *B. pinnatum* + *A. sativum* and at 75% concentration. *C. albicans* were 40%, sensitive, 50% SDD. *C. albicans* were 100% sensitive to combined juices of *G. kola* + *B. pinnatum* + *A. sativum* at 100% and 75% concentrations (Table 9).

4. Discussion

The results of this study showed that the juices of *G. kola*, *B. pinnatum* and *A. sativum* exhibited antimicrobial action on the pathogens tested against them, but the degree of susceptibility of the pathogens to the juices varies with the plant and concentrations or dilutions used. Antimicrobial activities were observed mostly at concentrations 75% and 100% (neat) for *G. kola* and *B. pinnatum*, but for *A. sativum* antimicrobial susceptibility was exhibited even at 50% concentration. In some instances, the antimicrobial susceptibility of the plant juices on the test organisms performed better than the conventional antimicrobial drugs used as control e.g. *E. coli* was 80% sensitive to gentamicin (10 mg) and 60% sensitive to Ofloxacin (5 mg).

There was a significant difference in the susceptibility pattern of the juice extracts compared to the control drugs against *E. coli* ($p = 0.009$). This result is in agreement with the reports of [20] who also reported the inhibitory activity of *G. kola* against *E. coli.* This was in line with the research of [27] who reported that *K. pneumoniae* was susceptible to *G. kola* extract. The results also agreed with other researchers that *G. kola* extract was effective against both gram-positive and gram-negative bacteria such as *Bacillus anthracis* and *E. coli* etc. It was also in conformity with the finding of [19] that *B. pinnatum* has mild antimicrobial activity against *E. coli.* [28]. It was also noted that garlic had a broad range of antimicrobial activities against Gram-positive and Gram-negative bacteria such as *Clostridium* sp., *E. coli* etc. [29] [30]. The reports were concomitant with the findings of this study on the sensitivity of *G. kola*, *B. pinnatum* and *S. sativum* juices against Gram-positive and Gram-negative bacteria. *G. kola* may be of use in treating infections caused by some pathogens and infections associated with these bacteria in other body sites. *G. kola* has inhibitory action against *E. coli*, and *C. albicans* [31]. In conformity with the studies [32] [33] reported that methanol and aqueous extract of *G. kola* were active against *E. coli*, *S. aureus* and *C.*

Table 9. Percentage comparison of antimicrobial activity of control drug with combined juices of medicinal plants on *C. albicans*.

Zone of Inhibition (mm)	Control Drug (%)	*G. kola* + *B. pinnatum* (%)					*G. kola* + *A. sativum* (%)					*B. pinnatum* + *A. sativum* (%)					*G. kola* + *B. pinnatum* + *A. sativum* (%)				
	Flue	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100	10	25	50	75	100
R (≤12)	30	100	100	100	0	0	100	100	100	0	0	100	100	100	10	0	100	100	100	0	0
SDD (≤14 <19)	20	0	0	0	60	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0
S (≥19)	50	0	0	0	40	100	0	0	0	100	100	0	0	0	40	100	0	0	0	100	100

albicans and concluded that *G. kola* might be useful in the management of infections caused by this microorganism. Although in this research, *S. aureus* was resistant to *G. kola* at all concentrations this might be because *G. kola* is used in most parts of West Africa as a social beverage and offered to guests as a sign of reception in many Nigerian cultural settings [18], *S. aureus* may have developed resistance due to constant exposure to it. This bacterium is the commonest normal flora of humans inhabiting the mouth, nostril, skin etc. hence prone to sub-inhibitory concentrations of *G. kola* that may stimulate resistance. It could be that the alcohol used as an extract for *G. kola* against [28] may have acted as an adjuvant to aid the active ingredients in *G. kola* extract or the alcohol complementing the antimicrobial activity.

There was a significant difference between the susceptibility pattern of the control drugs (gentamicin) against *K. pneumoniae* and the plant juices (p = 0.003). [34] reported that *B. pinnatum* extracts have strong activity against *K. pneumoniae*, which is in accord with the results of this study. The observation here was also in harmony with the report of [28] that *A. sativum* has a broad spectrum of antimicrobial activities against Gram-negative bacteria such as *K. pneumoniae* and *E. coli* even at 50% concentration. It was observed that the plant juice (neat) were more effective at higher concentration against *E. coli* compared to the control drugs. This was also noted in a previous report by [21] that 100% aqueous garlic extract showed a higher inhibition zone compared to the inhibition zones of commercially prepared antibiotics discs e.g. gentamicin. Almost all the plant juices neat exhibited greater antimicrobial activity against *K. pneumoniae* at (100%) concentration compared to the control drugs.

There was no significant difference in the susceptible pattern of plant juices and the control drugs against *P. mirabilis* (p = 0.28). [35] showed that *A. sativum* inhibited reasonably the growth of Gram-positive bacteria such as MSSA and MRSA *S. aureus*, *E. faecalis* and *L. monocytogenes.* For the Gram-negatives, *E. coli* and *P. aeruginosa* from clinical isolates were susceptible [36]. [35] also noted that the methanol extract of *A. sativum* showed high antimicrobial activity against *S. aureus* and *E. coli*. [37] observed that all isolates of *H. pylori* tested against *A. sativum* extract were sensitive to 20 - 400 mg of the extract with better zone diameter compared to amoxicillin, ciprofloxacin, clarithromycin, metronidazole and tetracycline.

There was also a significant difference in the susceptibility pattern of control drugs against *S. aureus* (p = 0.005). [38] reported that the extract of *B. pinnatum* was effective in the treatment of typhoid and other bacterial infections especially those caused by *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, *K. aerogenes*, *K. pneumoniae* and *S. typhi*. [39] found that 5% v/v of *B. pinnatum* has antimicrobial activity against *B. subtilis*, *S. aureus*, *S. pyogenes*, *S. faecalis*, *E. coli*, *Proteus sp*, *Klebsiella sp*, *Shigella sp*, *Salmonella sp*, *S. marcescens* and *P. aeruginosa* including the clinical isolates possessing multiple drug resistance [40] [41]. In their findings were also in agreement with our results.

This was in accord with other researchers [32] [33]. It was observed that the

methanol extract of *B. pinnatum* inhibited *C. albicans* [42]. From this research, *C. albicans* was resistant to the juice of *B. pinnatum*. The result was in agreement with this study because *C. albicans* were resistant to *B. pinnatun* juice [43].

The combination of drugs, plant extracts and plant juices that show synergy has been in use for the treatment of infections such as the extracts of *G. kola* with honey which was recommended as a better option than individual use for the treatment of infections caused by the bacteria tested against it; such as *P. aeruginosa*, *E. coli*, *S. aureus*, *Salmonella*, *K. pneumoniae* and *B. subtilis* [19]. The combination of plant extracts such as *T. catappa* with tetracycline showed 70% synergistic action against Gram-positive bacteria and 100% synergism with nystatin and amphotericin B [44] [45]. A combination of *B. pinnatum* and *S. kurz* extract have been shown to synergistically act against pathogens such as *K. pneumoniae*, *S. aureus* and *Salmonella typhi* compared to their extracts alone [33]. It was demonstrated that the combined extracts of *G. kola* and *A. sativum* have antimicrobial activity suggesting the presence of one or more soluble constituents with antifungal properties [46].

The combination of *Citrus aurantifolia* and *B. pinnatum* demonstrated synergistic activity against some Gram-positive, Gram-negative bacteria and fungi. The combination was noted to have higher zones of inhibitions compared to the single extracts [42]. The combination of *Zingiber officinale* and *A. sativum* extracts combined showed greater inhibition zones on the microbial growth tested against it than the individual extracts alone [47]. In their work noted that the combined extracts of *A. sativum* and *Gongronema latifolium* on the bacteria tested against it were not effective due to antagonism between the plant juices. *Allium sativum* and *Gongronema latifolium* extract individually demonstrated antimicrobial activity with zone inhibition > 16 mm [48]. In a combination of *S. hispidus* and *A. melaguta* extracts, no synergy was observed against the tested bacteria species; although the effect differed according to species (antagonism) [49]. In this study, the combinations of the plant juices demonstrated inhibitory activity on the bacteria tested against them just as the individual juices on tested bacteria. It may be that the antimicrobial effects of the plants might be more prominent in the extracted forms (alcohol extracts) than the juice. It was noted that the combination of *A. sativum* with other plant juices tested decreased the efficacy of its antimicrobial activity in some cases compared with the singular application. This is in procession with the findings of [49] [50].

Phytochemical analysis of *G. kola* showed the presence of some secondary metabolites which might be assumed as part of the active ingredient responsible for the antimicrobial activities. These include alkaloids, flavonoids, tannins, triterpenoid, steroids, some of these active components were reported by other researchers to be present and to possess antimicrobial activities [31] [32]. Alkaloids have been proposed to possess antimicrobial activities against microorganisms, a tertiary antimicrobial agent against was noted to inhibit gram-positive and gram-negative, acid-fast bacteria, filamentous bacteria and yeast-like fungi.

Dimeric alkaloids have been reported to possess strong antibacterial activity [30] [51]. This was not in agreement with the findings of this research, the different could subsist in methods of obtaining the plant extracts that were used for testing, while juice from the sample plants was used for this research, other researchers used aqueous and ethanol extracts possibly the concentration of the alkaloids in the *G. kola* juice was not enough to inhibit the growth of *S. aureus* being the only Gram-positive bacteria employed in the study [35] [52]. Several mechanisms of action of alkaloids had been proposed by other researchers based on the type or class of the alkaloid, it was stated that several antimicrobial alkaloids such as sanguinarine, quinine or berberine intercalate with microbial DNA or bind to it, the resulting compounds inhibits DNA replication and RNA transcription which are vital for the bacterial cell [53].

Another target point of action of the alkaloids (steroidal alkaloids) such as solanine and tomatine interfere with the stability of the bacterial cell membrane by forming a complex with phospholipid bilayer of the cell membrane, this may result in pore on the cell membrane thereby causing cell lysis and death [54]. Protein biosynthesis in the ribosome was stated to be another point of interaction of some groups of alkaloids to the bacterial cell. Alkaloids bind to DNA, which results in inhibition of CDR 1 protein, this induces fungal apoptosis.

Flavonoids which were also present in *G. kola* juice were believed to contribute to the antimicrobial activities of *G. kola* against the pathogens in this research. Many researchers have previously reported the antimicrobial activities of flavonoids. It was proposed that flavonoids inhibit nucleic acid synthesis in the bacterial cells, in a study using radioactive precursors, it was noted that the DNA synthesis was strongly inhibited by flavonoids in *Proteus* sp. while RNA synthesis was most affected in *S. aureus*. The authors suggested that the binding of flavonoids may play a role in intercalation or hydrogen bonding with the stacking of nucleic acid bases and this may explain the inhibitory action on DNA and RNA synthesis [55] [56]. Sophoraflavonone G interferes with the fluidity of outer and inner layers of the bacterial cell membrane [57] [58].

Flavonoids have also been reported to possess antifungal activities and the ability to inhibit spore germination [59].

Tannins are polyphenols that suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism. Triterpenoids have shown some level of antimicrobial activity against Gram-positive bacteria and *C. albicans* [50]. They showed that 6-oxophenolic triterpenoid inhibits cell wall synthesis that results in cell lysis and changes in cells morphology. These compounds (secondary metabolites) may have contributed greatly to the antimicrobial activity of *G. kola* against the pathogens used for this research. However, the antimicrobial activity and mode of action of cardenolide are yet unclear.

B. pinnatum juice exhibited a certain level of inhibitory activity to all the bacterial pathogens tested with 100% (neat) of the juice and at 75% concentration. The antimicrobial activity of *B. pinnatum* might be due to the presence of secondary metabolites identified in the juice which their mode of action has been

explained above. *C. albicans* were resistant to *B. pinnatum* juice at all concentrations. The absence of triterpenoid steroids and cardenolide which are trace may be associated or the concentration of some active components in juice might not be enough to elicit antifungal activity. The inhibition of growth against bacterial pathogens is in agreement with the previous report by [34] [40] that *B. pinnatum* extract has good antimicrobial activity against Gram-positive and Gram-negative bacteria. [4] reported that there was no antimicrobial activity observed against *C. albicans* with extracts of *B. pinnatum*, this is also in union with the finding of this research.

Allium sativum juice exhibited good antimicrobial activities against the pathogens of both the Gram positive and Gram-negative bacteria as well as against *C. albicans*. The antimicrobial activity of *A. satium* extracts has been reported by several authors. [27] [60] reported that garlic has a broad spectrum of antimicrobial activities against Gram positive and Gram negative bacteria, the report is in agreement with the finding of this research work. The antimicrobial activity of *A. sativum* was linked to the presence of allicin which is the major active component of *A. sativum*. The chemical structure of allicin plays a key role in the antimicrobial property of *A. sativum* [58] [59]. According to Cavallo's hypothesis, allicin may attack cysteine residues on the elongating amino acid chains. In this case, proteins synthesis will be inhibited at a certain stage. The wide range of inhibitory activity against *C. albicans* by *A. sativum* could be attributed to the ability of allicin to inhibit even spore germination. This synchronized with previous studies by [50] [60] that allicin shows toxic effects on fungal cells. In the phytochemical analysis, alkaloids were positive in *A. sativum* juice; triterpenoid/steroids positive. Other phytochemical compounds may have augmented the antimicrobial activity of allicin content in *A. sativum* juice to achieve the broad spectrum of activity observed against the pathogens.

5. Conclusion

The three medicinal plant juices tested against the selected pathogens were very promising candidates for use as antimicrobial agents. The active ingredient or ingredients responsible for the susceptibility of pathogens could be singled out and exploited for good efficacy. The studies could not pin down the culprit or culprits responsible for antimicrobial actions against the test bacteria and fungus from the plant juices.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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