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Phytochemical and antibacterial potential of lime (*Citrus aurantifolia)* and lemon (*Citrus limon*) juices as bioactivators of charcoal

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Abstract

Citrus juices are widely consumed because of their many health benefits and antimicrobial properties. Biological activators are preferred to the conventional chemical activators for safety and less health hazards thus the need to search for available indigenous biological materials for charcoal activation. Hence, the study on the potentials of lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juices as biological activators for charcoal. Freshly harvested lime and lemon juice were extracted using juice extractor and sterilized through filtration with membrane filter (0.45µl pore size). The phytochemical of the juices was determined and results revealed the presence of (carbohydrates, flavonoids, cardiac glycosides, saponins, terpenoids, ketose(fructose) and reducing sugars). The lime and lemon juices were subjected to antibacterial screening by agar well diffusion method against the test bacterial isolates (*Staphylococcus aureus* and *Salmonella enterica subsp. entericaserovar*) at 75%, 50%,25% and 12.5% concentrations respectively. The lime and lemon juice inhibition effects decreased with decrease in concentration. However, lime juice demonstrated higher inhibition across all concentrations in both test organisms with zones of inhibition (12-30mm) in *Salmonella* and (10-28 mm) in *S. aureus* while lemon juice demonstrated a low antibacterial activity across the different concentrations with zones of inhibition ranging from (12-17mm) in *Salmonella* and (9-24mm) in *S. aureus*. The phytochemical and antibacterial activities of lime and lemon juice revealed their potential utilization as bio activators.

Keywords: Biological activator; Lime; Lemon; Phytochemical; Antibacterial screening

1. Introduction

Lime (*Citrus aurantifolia*) and Lemon (*Citrus limon*) are citrus fruit which are either sweet or sour. Sour limes possess a greater sugar and citric acid content than lemons with an acidic and tart taste. Their nutritional information includes carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids amongst others [1].

In Nigeria, herbalists use lime and lemon juice in the treatment of diarrhea, dysentery, typhoid fever, wound infections, urinary tract infection [2]. It is also believed to be effective and potent against diphtheria, upper respiratory tract infection and other bacterial infections [2]. The cholera epidemic in Venezuela in 1885 was claimed to be resolved by massive consumption of lime and lemon juice [3].

Lime and lemon contain unique flavonoid compounds which are linked to antioxidant and anticancer properties, these flavonoids have been reported to stop cell division in cancer cells and exhibit antimicrobial properties [4,5]. The major organic acid in lime and lemon juices is citric acid.

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Staphylococcus aureus is one of the important pathogenic bacteria that is associated with toxin and its resistance to antibiotics [6]. Its infection has been reported to cause skin soft tissue, respiratory, bone, joint and endovascular disorders [7]. *Salmonella spp* which includes *Salmonella enterica subsp. entericaserovar* are pathogenic organisms that causes enteric fever, which is systemic in humans [6].

The conventional method of activating charcoal is the use of chemicals,this process is expensive and has been reported to have toxic effect on both humans and the environment. This led to the search for affordable, safe and environmentally friendly biological activators for our local charcoal. This study was aimed at screening for phytochemical and antimicrobial potentials of lime and lemon juice against *Staphylococcus aureus* and *Salmonella enterica subsp. entericaserovar* potential biological activators, an alternative to the toxic chemical activators.

2. Materials and Methods

Freshly harvested lime and lemon fruits were purchased from New market, Enugu state, Nigeria.

The typed bacterial strains; *Staphylococcus aureus* and *Salmonella enterica subsp. entericaserovar* were obtained from the Department of Applied Microbiology & Brewing, Enugu State University of Science & Technology, Enugu.

2.1. Preparation of samples

Lime and lemon fruits were washed several times with distilled water to remove soil and dirt on the surface and cleaned with 70% ethanol, it was cut open with sterile knife and the juice was extracted using manual juice extractor. The extracted juice was kept in a sterile container, and sterile filtered using sigmur membrane filter paper (0.45 milipore). The crude juice was freshly used without refrigeration using the method of [8].

2.2. Determination of pH

The pH of the extracted fruit juices were determined using Jenway 3305 digital pH meter.

2.3. Preparation of Innoculum

The method of [9] was adopted. Each of the stock organisms were subcultured on nutrient agar plates and incubated at 37 ^{o}C for 24h to obtain a pure culture. A loopful of 24h culture each was diluted in 0.9% sodium chloride to obtain a density comparable to 0.5 0f McFarland standard turbidity scale corresponding to about 1.5x 10^{8}Cfu/ml .

2.4. Phytochemical screening

The crude juices of lime and lemon were screened for the presence of phytochemical compounds such as carbohydrates, flavonoids, saponins, terpenoids, cardiac glycosides, alkaloids, ketoses, amino acids, proteins, phenols , reducing sugars, resins, steroids, tannins, oil and polysaccharide using the method of [10].

2.5. Antibacterial screening

The antibacterial activities of the lime and lemon crude juices were determined by agar well diffusion methods as described by [11]. One millimeter each of the prepared bacteria inoculums were spread on previously prepared sterile Muller-Hinton agar (Oxoid) plates using sterile swab sticks and allowed to dry at room temperature for 10m. Three wells were bored in each agar plate using 6mm sterile cork borer, 0.1ml of the crude juice was introduced into each of the three holes in the medium using sterile syringe. It was allowed to stand on the bench for 1hr for proper diffusion after which they were incubated at 37 ^{0}C for 24h and zones of inhibition (mm) were recorded afterwards.

3. Results

3.1. Result of Ph of lime and lemon juices

- pH of lime juice -1.00
- pH of lemon juice- 1.85

Table 1 Results Of Phytochemical Screening of Lime and Lemon Juices

Constituents	**Lime juice**	**Lemon juice**
Alkaloids	ND	ND
Amino acids	ND	ND
Proteins	ND	ND
Carbohydrates	+++	+++
Ketose (fructose)	ND	+++
Flavonoids	++	++
Cardiac glycosides	+++	+++
Phenols	ND	ND
Reducing sugars	ND	+++
Resins	ND	ND
Saponins	+	++
Steroids	ND	ND
Tannins	ND	ND
Terpenoids	+	+++
Oil	ND	ND

Key:+ == present in low quantity; ++ == present in moderate quantity; +++ == present in large quantity; ND==not detected.

Table 2 Result of Antibacterial Screening Of Lime Juice against *Staphylococcus aureus* and *Salmonella enterica subsp. entericaserovar*

Concentrations (%)	**Zones of inhibition (mm)**	
Lime juice	***S.aureus***	***Salmonella***
75	28	30
50	25	28
25	15	20
12.5	10	12

Table 3 Result of Antibacterial Screening of Lemon Juice against *Staphylococcus aureus* and *Salmonella enterica subsp. entericaserovar*

Concentrations (%)	**Zones of inhibition (mm)**	
Lemon juice	***S.aureus***	***Salmonella***
75	*24*	*27*
50	*20*	*23*
25	*12*	*18*
12.5	*9*	*12*

4. Discussion

In this study, the phytochemical revealed the presence of carbohydrates, flavonoids, cardiac glycosides, saponins, terpenoids in both lime and lemon juices while ketose (fructose) and reducing sugars are present in large only in lemon juice. This phytochemical result is in accordance with the findings of [12, 13]. The varaiation in the constituents of the fruit juices aligns also with the findings of [14] which is suspected to be as a result of difference in species and geographical location. However, this analysis focused only on the qualitative constituents and not quantitative.

The antimicrobial susceptibility of the crude fruit juices against gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Salmonella enterica subsp. entericaserovar*) by agar well diffusion method as shown in table 2 and 3. Lime juice showed highest antibacterial activities on both *Staphylococcus aureus* and *Salmonella spp* with zones of inhibition ranging from 30-12mm at 75%, 50%, 25%, 12.5% concentrations against *Salmonella enetrica subsp. entericaserovar* and 28-10mm at same concentration against *Staphylococcus aureus*. At the concentrations studied, the juices had lower activity (lower zones of inhibition) which is in contrast with the findings of [15] where grape and tangerine juices had higher activity against *S. aureus* (higher zones of inhibition >25mm) while it had lower activity on *Salmonella spp* (zones of inhibition <12mm). This variation is likely to be as a result of the difference in the citrus juices and strains of the test microorganisms. *Citrus limon* (lemon) juice had inhibitory effect on both *S. aureus* and *Salmonella spp* with zones of inhibition (12-27mm) for *Salmonella* and (9mm-24mm) for *S. aureus* at 75%,50%,25% and 12.5% concentrations. *Staphylococcus aureus* and *Salmonella enterica subsp. entericaserovar* are among the commonest bacteria implicated in food borne infection and intoxication and lime and lemon juices showed good inhibitory effects against them with zones of inhibition (30 and 27mm) respectively at 50% against *Salmonella spp* while against *S. aureus* at same concentration the zones of inhibition were (28 and 24mm) respectively. At the lowest concentration studied (12.5%) the inhibitory activity of the juices were low for both test organisms with zone of inhibition (12mm) for both lime and lemon against *Salmonella spp* and (10 and 9mm) against *S. aureus*. It was observed that the susceptibility of the test organisms increased with increase in concentration which is similar to the findings of [16, 4]. The differences in the susceptibility of gram positive and gram negative bacteria to lime and lemon juices is as a result of the differences in their cell wall composition [17]. In the present study, *Citrus aurantufolia* (lime) juice showed higher antibacterial activity than *Citrus limon* (lemon) juice. However, this difference has been reported to be as a result of their pH as acidic pH affects the amino acid that constitute the peptidoglycan which may affect the active sites of enzymes leading to defect in their activity [18].

5. Conclusion

The results of this study reveal that lime and lemon juices possess substantial antimicrobial properties and are effective against some food borne pathogens which also indicates they have the potentials to be utilized as acidic bioactivators for charcoal. However, there is need for further study on the separation of the different components of the phytochemicals to determine the particular component that possesses the antimicrobial property.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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