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Article

Phytochemical Analysis and Antimicrobial Activity of Seed Oils of *Parkia Biglobosa and Syzygium Aromaticum* on *Escherichia Coli* and *Staphyloccocus Aureus*

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Abstract: Nature-based products, including plant secondary metabolites are employed in a bid to combat bacterial resistance, which is a major cause of failure in the treatment of infectious diseases. This study was designed to test the effectiveness of oils of *Parkia biglobosa and Syzygium aromaticum* seeds against *Staphylococcus aureus* and *Escherichia coli* in order to ascertain suitability of the oils as food preservatives for the control of the organisms on cooked food. The study involves the antibacterial activity of the oils on cooked rice spiked with *E. coli* 0157:H7 and *S. aureus*. Oil extracted from *P. biglobosa* seeds was subjected to qualitative phytochemical analysis using standard methods. This revealed the presence of saponins, flavonoids, terpenoids, steroids and glycosides. Antibacterial activity of oils of *P. biglobosa* and *S. aromaticum* were carried out using agar diffusion and broth dilution methods. The test organisms were laboratory isolates of *E. coli* and *S. aureus*. Results revealed that both oils possess significant anti-*E. coli* and anti-*S. aureus* activity at various concentrations (25%, 50% and 100%). Oil of *P. biglobosa* seeds showed stronger anti-*E.coli* activity at 50% concentration and strong anti-*S. aureus* at 25 % concentration. Oil of *S. aromaticum* with bud showed strong anti-*E. coli* at 100 % concentration and showed no anti-*S. aureus* activity. The oil of *S. aromaticum* without bud showed strong anti-*E. coli* activity at 100% concentration and anti-*S. aureus* at 50% concentration. This study revealed medicinal value addition to the traditional uses of the oils of *P. biglobosa* and *S. aromaticum*

seeds. These oils have veritable potential for treatment of common food-borne diseases caused by *E. coli* and *S. aureus*.

Keywords: Phytochemical analysis; antimicrobial activity; *P. biglobosa; S. aromaticum; S. aureus; E. coli*

1. Introduction

Synthetic food preservatives have been known to cause adverse reactions and also increase cancer risk (Gultekin et al, 2015). Many plant oils and extracts, such as tea tree, myrrh and clove, have been used as topical antiseptics and antimicrobial agents (Aiyegoro, 2005; Lawless, 1995). Various publications have documented the antimicrobial activity of essential oils and plant extracts including those of rosemary, peppermint, bay, basil, tea tree, celery seed and fennel (Orchard, 2017; Lis-Balchin, 1997; Gherraf, 2017; Oraby, 2013; Swamy, 2016). Oils such as sweet almond, carrot and mandarin were shown to possess little or no antimicrobial activity (Gherraf, 2017; Lopez-Romero, 2015). *S. aromaticum* are the aromatic flower buds of a tree in the family Myrtaceae. The *S. aromaticum* tree is evergreen and grows up to 8–12 m tall, with large leaves and sanguine flowers grouped in terminal clusters. The essential oil of *S. aromaticum* is used as an anodyne (painkiller) for dental emergencies, in aromatherapy when stimulation and warming are needed, especially for digestive problems (Balchand, 2000). It acts as an antimicrobial agent, killing parasites and bacteria in the digestive tract (Alqareer et al, 2012). *P. biglobosa* is a dicotyledonous angiosperm belonging to the family Fabaceae – Mimosoideae. It is categorized under spermatophytes, vascular plants (Thiombiano, 2012). It is a deciduous perennial that grows to between 7 and 20 m high, in some cases up to 30 metres (Ntui, 2012). The tree is characterized by a thick dark gray-brown bark. The pods of the tree, commonly referred to as locust beans, are pink in the beginning and turn dark brown when fully mature. Each pod can contain up to 30 seeds (worldagroforestrycentre.org, 2013). It is found in a wide range of environments in Africa. Various parts of the tree are used for medicinal purposes. The yellow pulp, which contains the seeds, is naturally sweet and is processed into a valuable carbohydrate food known as *iru* and *daddawa* among the Yoruba and Hausa people of Nigeria, respectively (Olaniyan, 2013). *P. biglobosa* is one of the highest cited plants used for treating hypertension (Karou, 2013). It is a potential source of compounds used in the management of bacterial infections (Abioye, 2013). It helps to promote good sight and reduces the odds of hypertension and diseases like stroke and diabetes; it has also shown potential benefit for enhancing weight loss and controlling blood sugar levels (Ikhimalo, 2019).

The diseases transmitted by foods have remained a major concern for public health and an important economic problem in many countries over the past two decades. *S. aureus* and *E. coli*

poisoning are the most common examples of food-borne diseases (Ikhimalo, 2019). *S. aureus* is a gram-positive cocci bacterium in the Firmicutes phylum of bacteria, and is frequently found in human and animal respiratory tract, skin and hair. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning (Kluytmans , 1997; Cole, 2001). *S. aureus* infection can occur in manually prepared food that requires no additional cooking. *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. *E. coli* as germ or bacterium lives in the digestive tracts of humans and animals. However, some types of *E. coli*, particularly *E. coli* 0157:H7 can cause intestinal infection (Singleton, 1999) such as bloody diarrhoea and urinary tract infections. Some strains of *E. coli* bacteria (such as the O157:H7 strain) may also cause severe anaemia or kidney failure, which can lead to death. Some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (Vogt, 2005). The bacterium can be grown easily and inexpensively in a laboratory setting. Optimal growth of *E. coli* occurs at 37 °C but some laboratory strains can multiply at temperatures of up to 49 °C (Fotadar, 2005). Foods that can be infected with *E. coli* include cooked food, raw milk or dairy products. Bacteria can spread from cow udders to her milk, raw fruits and vegetables, or other unpasteurized juices that have come in contact with infected animal faeces. Human or animal faeces infected with *E. coli* sometimes get into lakes, pools, and water supplies.

This study was undertaken to screen phytochemically for secondary metabolites in the oils of *P. biglobosa* and *S. aromaticum* as well as to profile the antimicrobial properties of the oils. Results of this study will provide useful suggestions to the nagging problem of food-borne diseases caused by *E. coli* and *S. aureus* poisoning.

2. Materials and Methods

2.1. Sample Preparation

Dry *P. biglobosa* (17 kg) seeds were collected at Doma, Doma Local Government Area of Nasarawa State, Nigeria. Dry *S. aromaticum* (358 g) flower buds were purchased at Modern market in Lafia, Lafia Local Government Area of Nasarawa State, Nigeria. The dried pulp of *P. biglobosa* seeds were removed by prolonged contact with ash using mortar and pestle before it was milled manually. Dry *S. aromaticum* without bud (150 g) was manually isolated, leaving behind the ones with bud (208 g), which were washed, air dried and powdered using an electronic blender. Powdered *P. biglobosa* weighed 16 kg while *S. aromaticum* with bud weighed 207 g and the ones without bud weighed 93 g. 207 g of *S. aromaticum* with bud was extracted with 1610 mL of *n*-hexane for 24 hours, using Soxhlet apparatus. 93 g of *S. aromaticum* without bud was extracted with 340 mL of *n*-hexane for 12 hours. The oil of *P.*

biglobosa was extracted by cold press method using manual oil presser machine. A little quantity of hot water (80 ºC) was sprinkled on 16 kg powdered *P. biglobosa* and mixed thoroughly to form a paste. The paste was pre-heated in a large covered pan on a heating source. The pre-heated paste was transferred into delicate sheer muslin cloth tied properly and placed on the pressing machine. A reasonable weight of pressure was applied on the tied paste for six hours. Extracted oil was collected, weighed and stored in the refrigerator at 4 ºC until needed.

2.2. Phytochemical Analysis of Oil of P. biglobosa

The oil of *P. biglobosa* was subjected to phytochemical tests for the presence of saponins, alkaloids, tannins, phenols, flavonoids, terpenoids, phlobatannins, anthraquinones, glycosides, carbohydrates and steroids using standard procedures (Sofowora, 1993; Trease, 1989).

2.3. Antimicrobial Analysis

Antimicrobial analyses of the oils were determined using pathogenic microbes such as *E. coli* and *S. aureus*. Nutrient agar and Macconkey agar were the growth media used for the bacteria. All media were prepared according to the manufacturer instructions**.** One gram each of cooked rice was placed in eighteen 50 mm Petri dishes. 1 mL of each of the broth culture of *E. coli* and *S. aureus* were separately added to each of the weighed food sample in the Petri dishes. 1 mL of 100%, 50% and 25% concentration of each oil samples (*P. biglobosa*, *S. aromaticum* with bud and *S. aromaticum* without bud) were separately introduced into the dishes respectively. The oils were diluted with ethanol and left to stand for 24 hours. After the incubation, the plates were observed for turbidity.

Antibacterial activity was assayed by disc diffusion and borehole methods. For all bacteria strains, overnight culture containing few colonies was made. Few colonies of each bacterium were separately spread on 20 mL of sterile agar plates by using a sterile cotton swab. Sterile 5 mm filter paper discs impregnated with different test oils (100% concentrations) were placed on the surface of inoculated agar plates. Two holes were made in the inoculated agar plates and impregnated with the different oils (100% concentrations) using 30 µg/disc Ceftroaxone as positive control.

Each oil was also added drop wise to different inoculated agar plates containing the different tests bacteria that were sub-cultured from the inoculated food sample at different concentrations (100%, 50% and 25%). These were incubated at 37 °C for 18 hours after which microbial growth for some plates were determined by measuring the diameter of the inhibition zone (mm) using a transparent scale (meter rule) while some were observed for the presence or absence of growth.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the oils were determined. The lowest concentrations that resulted in complete inhibition of the bacterial

growth were recorded as MIC while the least concentration that showed no bacterial growth were recorded as MBC (Oliveira , 2012).

3. Results and Discussion

3.1. Phytochemical Analysis of Oil of P. Biglobosa

Phytochemical analysis of oil of *P. biglobosa* indicated the presence of secondary metabolites saponins, flavonoids, terpenoids, glycosides and steroids. Tannins, phenols, phlobatannins, anthraquinones and carbohydrates were completely absent (Table 1). The identified phytochemicals are found to be antimicrobial substances against a wide array of microorganisms in vitro (Salah, 1995; Ene-Obong, 2018).

Table 1: Phytochemical analysis of oil of *P. biglobosa*

Test	**Result**
Saponins	+
Alkaloids	-
Tannins	-
Phenols	-
Flavonoids	+
Terpenoids	+
Phlobatannins	-
Anthraquinones	-
Glycosides	+
Carbohydrates	-
Steroids	+

+ = present, - = below detectable levels

3.2. Anti-microbial Analysis of Oils of P. biglobosa and S. aromaticum

The seed oils of *Parkia biglobosa* have been analysed for their possible edible utility and to provide some physical data. The fatty acid composition of the oils was identified (Aiyelaagbe, 1996). Proximate compositions and physicochemical characteristics have been determined (Olowokere, 2018). In this research, the antimicrobial activity of the oil of *S. aromaticum* was confirmed for *E. coli* and *S. aureus*.

Results of antimicrobial activity of oil of *S. aromaticum* with bud against *E. coli* established that the oil had anti-*E.coli* activity at concentrations of 100% and 50% and showed resistance at 25% concentration. *S. aureus* was not sensitive to the oil at all concentrations (Table 2).

Result of antimicrobial activity of oil of *S. aromaticum* without bud proved that the oil had significant anti-*S. aureus* activity at concentrations of 100% and 50%. *E. coli* showed significant response to the oil only at 25% concentration (Table 2)

Oil of *P. biglobosa* showed significant microbial activity on *E. coli* at concentrations of 50 % and 25 % and at 25 % on *S. aureus* (Table 2). This result revealed that the oil must be diluted before use; this is because the anti-microbial activity was higher at 25% concentration.

Table 2: Antimicrobial analyses of *S. aromaticum* and *P. biglobosa* against *E. coli* and *S. aureus*

		% Concentration of Oil		
Bacteria	**Source of oil**	**100**	**50**	**25**
E. coli	*S. aromaticum* with bud	S	S	R
	S. aromaticum without bud	S‡	S‡	S
	P. biglobosa	R	S	S
S. aureus	*S. aromaticum* with bud	R	R	R
	S. aromaticum without bud	S	S	-
	P. biglobosa	R	R	S

S= Sensitive, S*= Insignificant growth (sensitive), R= Resistant

Determination of MIC and MBC of the oils showed that oils of *P. biglobosa* and *S. aromaticum* with bud can inhibit the growth and completely kill *E. coli* while that of *S. aromaticum* without bud can only weaken and inhibit growth of *E. coli* (Table 3). This indicated that only oil of *P. biglobosa* and *S. aromaticum* with bud had positive antimicrobial activity on *E. coli.*

Table 3: Determination of MIC and MBC on *E. coli* and *S. aureus*

Oil	**MIC (*E.coli*)**	**MBC (*E.coli*)**	**MIC (*Staph.*)**	**MBC (*Staph.*)**
P. biglobosa	25 %	50 %	25 %	R
S. aromaticum with bud	50 %	100 %	R	R
S. aromaticum without bud	100%	-	50 %	50 %

R = Resistant; - = Not determined

From the determination of MIC and MBC of the oils against *S. aureus* (Table 3), it could be said that only oil of *S. aromaticum* without bud could be used for the control of *S. aureus* since it showed both bacteriostatic (at 50%) and bactericidal (at 50%) effects.

4. Conclusion

Results of phytochemical analysis of oils of *S. aromaticum and P. biglobosa* suggested that the identified secondary metabolites are a valuable reservoir of bioactive compounds of veritable anti-bacterial activity.

Results of the antimicrobial analysis against test organisms suggest that the oil of *S. aromaticum* could be used to control both *E. coli* and *S. aureus*; specifically, that of *S. aromaticum* with bud (at 100% concentration) against *E. coli* while *S. aromaticum* without bud could control *S. aureus* at 50% concentration. Oil of *P. biglobosa* may be used for the control of *E. coli* at diluted concentration of 50%. Results of this research support the application of the seed oils as potential antimicrobial agents to help reduce *E. coli and S. aureus* in cooked foods.

Authors' contributions

A. B. Solomon carried out the research and wrote the first draft of the manuscript. T.A. Toranyiin managed the analyses of the study. L. A. Nnamonu designed the study, wrote the protocol, managed literature searches and wrote the final draft of the paper. All authors read and approved the final manuscript.

The authors declare no conflict of interest

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