Bima Journal of Science and Technology, Vol. 6 (3) Dec, 2022 ISSN: 2536-6041

DOI: 10.56892/bima.v6i03.42

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *Zingiber officinale* AND *Syzygium aromaticum* ON BACTERIA PATHOGENS ISOLATED FROM KUNUN-AYA

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ABSTRACT

The increased use of antibiotics in agricultural practices is accompanied with the development of resistant foodborne pathogens. Spices and herbs have been reported to be potent source of natural antioxidants. This study aimed to assess the antibacterial activity of *Zingiber officinale* (Ginger) and *Syzygium aromaticum* (Clove) against bacterial food pathogens. Fresh ginger and clove were collected, identified, air dried and extracted the bioactive compound using distilled water. The extract was used for phytochemical analysis of flavonoids, tannins, saponins, steroids, glycosides, alkaloids and total phenols and determination of inhibition zone. Bacteria and fungi were isolated and identified from locally prepared Pap (Kunun aya) and later used for the antimicrobial analyses. Phytochemical screening shows clove contain flavonoids, tannins, saponins, steroids, glycosides, alkaloids and total phenols whereas glycosides and tannins were absent in ginger. Ginger has more antibacterial activity with the highest zone of inhibition observed in *E coli* at 16.73mm at 2000µg and the lowest in Salmonella at 13.83mm at 2000µg, clove also shows antibacterial activity with the highest zone of inhibition (14.8mm) in *S. aureus* at 2000µg and the lowest 11.5mm in *E. coli* at 2000µg. Thus, from the result obtained, it can be concluded that *Zingiber officinale* and *Syzygium aromaticum* extracts possesses effective antimicrobial agents against food pathogens.

Keywords: Spices, Herbs, Phytochemicals, Antibacterial, Ginger, Clove, Kunun-aya

INTRODUCTION

Spices have been important to mankind since the beginning of history. Several mythological evidences including “Epic of Gilgamesh” and the “Bagavad Gita” suggest their use for several purposes. Spices have been used as food and flavors since ancient times, and as medicine and food preservatives in recent decades. Ginger (*Zingiber officinale*), because of its antimicrobial ability against different microbial pathogens, it has been used as naturopathy (Callixte *et al.,* 2020). Ginger has been against number of pathogenic microorganisms in its natural state, without transforming it which is a proof of using it as a strong medicine in treatment of infectious diseases (Callixte *et al.,* 2020). Many spices such as clove, oregano, thyme, cinnamon, and cumin have been applied to treat infection and bacteria. The secondary metabolites of these spices are known as antimicrobial agents, the majority of which are generally recognize as safe materials for food with insignificant adverse effect. Ginger is used in many countries as a spice and condiment to give a pungent taste to food (Park and piz-zuto, 2002). Ginger rhizome has also been used in traditional medicine, because of its diversity in terms of phytochemicals. Ginger and its constituents show antioxidant activity and

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prevent damage of macromolecules caused by free radicals/oxidative stress. Ginger belongs to Zingiberaceae family, the Zingiberaceous plants are naturally with strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes. Ginger is available and accessible at low cost for everyone to use it, it is universally acceptable and well tolerated by the most people (Callixte *et al.,* 2020). Ginger also shows antimicrobial and other biological activities due to gingerol and paradol, shagoals and zingerone (Giriraju and Yunus, 2013).

Cloves are aromatic flower buds of a tree in the family myrtaceae (*Syzygium aromaticum*). Clove is native of Indonesia but nowadays cultured in several parts of the world and is commonly used as a spice. Clove has been used for centuries as a primary preservative as well as flavoring agent for preserved foods. It has high antimicrobial, antifungal, antioxidant and antilarval properties. Cloves contains eugenol which makes it a great preserving food is (Chaeib *et al*., 2007b). The study aimed to assess the antibacterial activity of Zingiber *officinale* (Ginger) and *Syzygium aromaticum* (Clove) against bacterial food pathogens.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh Ginger *(Zingiber officinale)* and Clove (*Syzygium aromaticum*) were purchased from Janguza Market, Kano. All the plant materials were identified in accordance with Demetrio *et al*., (2015) at the Department of Plant Biology, Bayero University Kano. Herbarium accession number was given to each of the plants used and *Syzygium aromaticum* identified as BUKHAN 0342 and *Zingiber officianale* as BUKHAN 0296.

The Clove and Ginger were washed with distilled water to remove all extraneous materials and adhering particles, the Ginger and Clove were later dried under shade environment for 3 weeks before grounding into fine powder using motor and pestle. The powder was then stored in a container until required for use. Ginger has both gingerols and shogaols that are rich in antimicrobial effects helps in resolving stomach infection and other health outcomes. It has strong antimicrobial properties and active constituents of ginger inhibit the replication of colon bacteria. It inhibits the growth of *E. coli, Staph, spp.,* and more (Callixte *et al.,* 2020). Ginger may be due to phenolic compounds considered traditionally, Ginger was used in treatment of intestinal infections, especially those related to digestive health outcomes. It is against this background that this study is going to assess antimicrobial activity of ginger against bacteria most commonly found in digestive tract (Callixte *et al.,* 2020).

Preparation of Aqueous and Phytochemical Screening of Ginger *(Zingiber officinale)* and Clove (*Syzygium aromaticum*):

Test for Tannins

The extract was weighed (0.5g) and mixed thoroughly with 10 ml distilled water and then filtered; five milliliters of the filtrate was added to 1 ml of 5% Ferric chloride solution. The appearance of blue black, greenish or blue green precipitate indicates the presence of tannins (Adwan and Mhanna, 2008).

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. The Immediate development of a red colour indicates the presence of flavonoids (Dawoud *et al.*, 2013).

Test for Saponins

The powdered plant material (0.1g) was boiled with 10 ml of water for 5 minutes then

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filtered. After cooling, 5 ml of filtrate was then diluted with water and shaken vigorously. The formation of persistent foam indicates the presence of saponins (Adwan and Mhanna, 2008)

Test for Steroids

One mililitre solution of the extract was added to 1ml sulphuric acid, the appearance of red colour indicates the presence of steroids (Adwan and Mhanna, 2008)

Test for Alkaloids

The extract (0.5g) was stirred with 5 ml of 1% hydrochloric acid on a steam bath and filtered. 1 ml of the filtrate was then treated with few drops of Mayer's reagent. A white or creamy white precipitate was considered to be an indication for the presence of alkaloids (Dawoud *et al.*, 2013).

Isolation and Identification of Some Spoilage Bacteria from Locally Made Pap (Kunun aya)

Samples of kununaya drink sold around Dorayi, Kabuga and in Bayero University Kano campus premises were collected and kept at room temperature for 24 hours to spoil. The sample of kununaya collected was appropriately labeled at the point of collection as A, B, and C respectively. The samples were immediately carried to the laboratory of Department of Microbiology, Bayero University where the analysis was carried out as described by Jideani *et al* (2006).

From One litre (IL) of Kunun Aya, 10ml was transferred into 100mL capacity conical flask. Ninety milliliter (90ml) of sterilized distilled water was added into the 100mL capacity conical flask to arrive at 10^{-1} dilution. A six-fold serial dilution (10^{6}) was made as described by Bristone *et al*., (2015). Aliquot portion of the serially diluted samples was inoculated onto freshly prepared, surface-dried nutrient agar (NA), MacConkey agar (MCA) and potato dextrose agar (PDA) using spreading method with plates made in triplicates. Nutrient agar and MacConkey agar plates was inoculated for 24hrs at 37^{o}C (Cheesbrough, 2006), while potato dextrose agar was incubated for 3-5days at ambient room temperature. Morphological, gram reaction and biochemical reactions were used for identification of the isolates (Cheesbrough, 2006)

Determination of Antimicrobial Activity of the Extracts on the Isolated Organisms:

Preparation of various concentrations of Ginger and Clove extracts

Four grams (4000mg) of each plant extract was reconstituted individually in 10ml dimethyl sulphoxide (DMSO) extract to obtain a 400mg/ml concentration (Stock solution). A five milliliter (5mL) of the 400mg/ml solution was diluted with an equal volume of distilled water to obtain a 200mg/ml solution. The double dilution procedure was applied further to obtain lower concentration of the extracts (100mg/ml, 50mg/ml and 25mg/ml as described by Taura and Oyeyi (2009).

Standardization of Inoculum

Few colonies of the bacterial isolates were emulsified in normal saline of 4mls in test-tubes to match the 0.5 Mcfarland Standard for sensitivity test as described by Cheesbrough, (2006). The McFarland standard was prepared by mixing 0.6 mL of 1% (w/v) dihydrate barium chloride solution with 99.4 mL of 1% (v/v) sulphuric acid solution (Cheesbrough, 2000).

Sensitivity testing

Agar well diffusion test was used for antibacterial bioassay according to Kirby Bauer (2011). Pure cultures of the isolates were inoculated in nutrient broth and incubated at 37^{0}C for 24 hrs. The growth was

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standardized by diluting the culture with 4ml of sterile normal saline to match 0.5 McFarland turbidity standards (Cheesebrough, 2006). The solidified Mueller-Hinton agar plates surface of each plate was streaked using bent glass rod with the bacterial isolate under aseptic conditions. Agar plate was punched with a sterile Cork borer of 6mm size and 0.1ml of each spice extracts at different concentrations of 2000µg/ml, 1000µg , 500µg/ml, 250µg/ml was poured with micropipette in each bored hole. The control used was gentamicin. Plates were allowed to standby for 30min. The plates were incubated at 37°C for 48hrs. After the incubation period, the diameter of the growth inhibition zones was measured using veiner caliper and recorded in mm.

Determination of Minimum Inhibitory Concentration (MIC)

The spice extracts that showed significant antibacterial activity by agar well diffusion method was subjected to MIC assay, Equal volume of 1ml of Muller Hilton broth were prepared according to manufacturers' instruction then dispensed in test tubes and sterilized by autoclaving. Equal volume (1ml) of the different extracts were added to 1ml Muller Hinton broth and 0.1ml standardized inocula of the test organism adjusted to McFarland turbidity standard was added to each test tube and incubated at 37ºC for 24 hours for bacteria. Tubes containing broth and Ginger/Clove extract without inocula serve as extract control while the tube containing broth and inocula serves as positive control. The least concentration with no detectable bacteria growth was considered as (MIC) minimum inhibitory concentration as reported by Akinyemi *et al.* (2005); Khurram *et al.,* (2009).

Minimum Bactericidal Concentration (MBC)

Sterile Mueller Hinton agar plates were inoculated with samples from the MIC tubes that shows no visible bacterial growth and incubated at 37^0C for 24hrs. The lowest concentration in which no growth occurred in the medium was taken as the MBC. This was done in accordance with the national committee for clinical laboratory standard (2004).

Statistical Analysis

Data obtained were analyzed by Microsoft Excel 2016. The mean values were subjected to analysis of variance (ANOVA) to ascertain whether there's significant difference between the treated samples and the control using GraphPad Prism Software (GraphPad Inc., San Diego, CA, USA) (*6*) at $p<0.05$.

RESULTS AND DISCUSSION

The extract of ginger has the highest with 16.6% and the clove with 14.7%. The texture of the extract of ginger was gummy with pungent odor and that of clove was sticky solid. Color of the extract also varies which shows extract of ginger to be brown in color while extract of clove to be dark coffee brown (Table 1)

Table 1: Physical properties of the extract of ginger and clove

Physical properties	Ginger Extract	Clove Extract
Weight of plant material(g)	100g	100g
Weight of extract recovered (g)	16.6g	14.7g
Percentage recovery (%)	16.6(%)	14.7(%)

The results of the phytochemical composition of ginger and clove extracts. Phytochemical screening carried out on the extract of clove showed the presence of flavanoids, tannins, saponins, steroids, glycosides, alkaloids and total phenols. While in ginger, alkaloids,

DOI: 10.56892/bima.v6i03.42

flavonoids, phenols, saponins and steroids were present, but tannins and glycoside were absent (Table 2)

Table 2: Phytochemical composition of the extracts of ginger *Zingiber officianelle* and clove *Syzigium aromaricum*

Phytochemicals	Ginger	Cloves
Flavanoids	+	+
Tannins	_	+
Saponins	+	+
Steroids	+	+
Glycosides	_	+
Alkaloids	+	+
Total phenols	+	+

The cultural, morphological and biochemical characteristics of the bacteria isolated. Two of the isolates were gram negative rod while one is gram positive cocci. Isolate one appears circular convex, green metallic sheen on EMB agar and circular convex, smooth pink on MacConkey agar. Isolate one was Catalase, Indole and MR positive but VP, Oxidase, Citrate, Urease and Coagulase negative which was suspected to be *Escherichia coli*. Isolate 2 appears circular low convex smooth, and colorless on MacConkey agar, circular convex smooth red with black center on *Salmonella Shigella* agar. Isolate 2 was Catalase, MR and Citrate positive but VP, Indole, Oxidase, Urease and Coagulase negative which was suspected to be *Salmonella* spp. Isolate 3 shows a smooth shiny surface colony and appears opaque and are often pigmented, golden yellow on mannitol salt agar. Isolate 3 was Indole and Oxidase negative but Catalase, VP, MR, Citrate, Urease, and coagulase positive which was also suspected to be *Staphylococcus aureus.* (Table 3).

Table 3: Cultural, morphological and biochemical test results of the bacteria isolated from Kununaya

	Cultural characteristic	Gram Reaction	Catalase Test	VP Test	Indole Test	MR Test	Oxidase Test	Citrate Test	Urease Test	Coagulase Test	Suspected organism
Isolate I	CC, Smooth, GMS on EMB. CC, Smooth pink on MA.	-ve Rod	+	-	+	+	-	-	-	-	*E. coli*
Isolate II	CLCS, Colorless on MA, CC, Smooth, RWBC on SSA.	-ve Rod	+	-	-	+	-	+	-	-	*S.* Typhi
Isolate III	SSS colonies and appear opaque and are often pigmented, golden yellow on Mannitol salt agar	+ve cocci	+	+	-	+	-	+	+	+	*S. aureus*

KEY; CC-circular convex, GMS- Green metallic sheen, EMB- Eosin methylene blue, MA- MacConkey agar, CLCS- Circular low convex smooth, RWR

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The result of fungi isolated from this study; two strains of fungi were isolated and identified based on colonial morphology and microscopic characteristics. The upper surface of the colony of the first isolate was yellow green with edge, granular surface and green coloration on the reverse side. The isolate was then observed under the microscope where the conidiosphores were thick walled, hyaline and slightly roughed, erect, long aseptate with a vesicle with short conidial chains. This isolate was suspected to be *Aspergillus flavus.*

The colonies of the second isolate were widely spread, black with smooth white edges and spongy surface densely packed and brown on reverse side. It was observed under the microscope and its conidiospore was long, erected from the base of the vesicle, smooth walled, hyaline with globes conidial head. The isolate was suspected to be *Aspergillus niger* (Table 4)

Table 4: Macroscopy and microscopy of the fungal isolates

Fungal species	Macroscopy	Microscopy
Aspergillus flavus	The upper surface of the colony was yellow-green with edge, granular surface and green coloration on the reverse side.	The conidiophores were thick walled, hyaline and slightly roughed, erect, long aseptate with a vesicle with short conidial chains.
Aspergillus niger	The colonies were widely spread black with smooth white edges and spongy surface densely packed and brown on reverse side.	The conidiospore was long, erected from the base to the vesicle, smooth walled, hyaline with globes conidial head.
Fungal species	Macroscopy	Microscopy

The diameter of zones of inhibition (mm) of *Zingiber officianelle* and *Syzygium aromaticum* against *S. aureus, Salmonella* and *E. coli* at various concentrations (µg/ml). *Zingiber officianelle* (Ginger) extract showed more antibacterial effect on all the isolates as compared to *Syzygium aromaticum* (clove) extract. Highest zone of inhibition 16mm at 2000µg/ml was shown by ginger against *E. coli*while the lowest effect of ginger was shown on *Salmonella* at 13.5mm at 2000 µg/ml. Zone of clove extracts was shown on *S. aureus* with zone of inhibition 15mm at 2000µg/ml and lowest zone was shown on *E.coli* with zone of inhibition of 11mm at 2000µg/ml (Table 5).

The MIC and MBC of *Zingiber officianelle* and *Syzigium aromaricum* extract against the test organisms. The minimum inhibitory concentration of *Zingiber officianelle* extract against *Salmonella* and *E. coli* is 125µg/ml while 250µg/ml for *S. aureus*. The minimum inhibitory concentration of *syzigium aromaricum* against *Salmonella* and *E. coli* is 500 µg/ml and 250 µg/ml for *S. aureus.*

The minimum bactericidal concentration of *Zingiber officianelle* extract against *S. aureus* is 500µg/ml, against *E. coli* is 250µg/ml and against *salmonella* is 1000µg/ml. The minimum bactericidal concentration of *Syzigium aromaricum* extract against *S. aureus* 500 µg/ml, against *E. coli* is 1000 µg/ml against salmonella 1000µg/ml. (Table 6)

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Table 5: Mean diameter of zones of inhibition (mm) of *Zingiber officianelle* and *Syzigium aromaricum* extracts against *E. coli, S. aureus* and *Salmonella* spp at various concentrations (µg/ml)

			Ginger Extract				
Organism	2000	1000	500	250	125	62.5	Control
E.coli	16.73±0.37	15.2±0.2	12.5±0.28	11.76±0.39	9.1±0.58	6±0.00	21
S. aureus	15.33±0.33	14.1±0.1	11.6±0.30	9.1±0.58	6±0.00	6±0.00	25
			Clove Extract				
Organism	2000	1000	500	250	125	62.5	Control
E.coli	11.5±0.28	8.83±0.16	6.66±0.33	6±0.00	6±0.00	6±0.00	21
S. aureus	14.8±0.4	12± 0.00	8.3±0.3	6.6±0.6	6±0.00	6±0.00	25
Salmonella	12.5±0.5	9.6±0.33	7±0.00	6±0.00	6±0.00	6±0.00	23
Salmonella	13.83±0.44	12.9±0.49	12.1±0.59	11.1±0.1	8±0.29	6±0.00	23

Key: Values and means of three replications

Table 6: MIC and MBC of *Zingiber officianelle* and *Syzigium aromaricum* extracts against the test organisms

	MIC (ug)	**MBC (ug)**
G/E	125	250
G/ST	250	500
G/SL	125	1000
C/E	500	1000
C/ST	250	500
C/SL	500	1000

KEY; G/E– Ginger against *E.coli*, G/ST-Ginger against *S.aureus*, G/SL-Ginger against *Salmonella*, C/E-Clove against *E.coli*, C/ST-against *S.aureus*, C/SL- Clove against *Salmonella,* ug-microgram

CONCLUSION

The result of this study showed that the extract of *Zingiber officinale* and *Syzygium aromaticum* has ntimicrobial effect on gram negative and gram-positive bacteria and fungi. Thus, from the result obtained, it can be concluded that *Zingiber officinale* and *Syzygium aromaticum* extracts possess effective antimicrobial agents against food

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pathogens. Hence, future studies should be directed towards the development of purified bioactive compounds and quantitative determination of safe concentrations that can be used to improve existing drugs on these pathogens.

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