



PHYTOCHEMICAL CONSTITUENTS AND MICROBIAL ACTIVITIES OF SYZYGIUM AROMATICUM PLANT-A REVIEW**KUMAR S**

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The experiment was conducted to explore the phytochemical constituents and antibacterial association of *Syzygium aromaticum* extract against some food borne pathogen. Fluid and methanol removes from *Syzygium aromaticum* buds were readied, examined for phytochemical investigation & tried for antibacterial action against 6 pathogenic microscopic organisms (*Klebsiella pneumoneae*, *Salmonella typhi*, *Shigella spp*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*). The Phytochemical screening of the concentrates demonstrated that *Syzygium aromaticum* bud extricates contained Alkaloid, Anthraquinone, saponin, tannin, phenol, steroid, and flavonoid, terpenoid while glycoside and diminishing sugar were missing. Then again, alkaloids were seen as the most inexhaustible phytochemical (9.6%) trailed by tannin, saponin and flavonoids. Factual investigation of the outcome indicated that methanol remove showed most elevated antibacterial action with normal zone of restraint of 14.18±2.47 mm among the secludes than watery concentrates (12.33±1.82 mm). In view of the weakness of the living beings to the concentrates, *E. coli* was seen as the most elevated helpless creatures with normal range of restraint of 14.79±1.97 mm, followed *Salmonella typhi* (13.84±2.52 mm), *S. aureus* (13.7±1.65 mm), *Shigella* (13.44±1.12 mm), *Pseudomonas* (12.27 ±0.98mm) while least normal zone of hindrance is appeared by *Klebsiella* (11.55±1.60 mm). The MIC and MBC of the concentrates ranges from 3.125 to 50 mg/ml There is not a noteworthy diverse on the helplessness of the living beings against the concentrates at p< 0.05. The consequences of current investigation has given defense to remedial capability of ginger and furthermore utilized as dietary enhancement for food seasoning and safeguarding.

Keywords: Syzygium aromaticum; Pathogenic bacteria; Antibacterial activity; Phytochemicals

INTRODUCTION

Many spices have been used as a food preservative as well as medicinal purpose from many decades. These spices are clove, ginger, mint, thyme and cinnamon which have antioxidant and antimicrobial activities. These spice plants have anti-bacterial, anti-fungal, anti-viral and anti-carcinogenic properties [1]. Cloves are also used as an anodyne anthelmintic, antiseptic, aromatic, carminative, stimulant; used to treat arthritis, asthma, bronchitis, bruises, burns, cholera, colds, colic, coughs, diarrhoea, digestive disorders, earaches, gum diseases, headaches, hypertension, impotence, inflammation of the pharynx, intestinal worms, nausea, rheumatism, toothache, ulcers, vomiting and wounds [2].

It is used in the flavor of food [3]. The antimicrobial activity of clove is caused by eugenol, oleic acid and lipid found in its essential oils [4].

Significant spices in particular clove attract attention due to its effective antioxidant and antimicrobial activity between other spices [5]. It is one of the most cherished spices used as a food preservative and for many remedial purposes. Clove is an important plant, a wide range of pharmaceutical effects that

have been associated from customary use for centuries.

Antimicrobial activities of *Syzygium aromaticum* have been proven against several viral and fungal infections. Sofia, *et al.* tested for the antimicrobial action of various sub-continent spices such as *mentha*, *Cinnamomum verum*, *Brassica*, *Zingiber officinale*, *Allium sativum* and *Syzygium aromaticum*. The main example that indicated a total bactericidal impact against all food-borne pathogens attempted in *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* was a wet wash of *Syzygium aromaticum* at 3% [6].

Extract of clove at 1% concentration proved excellent inhibition effects. The antibacterial activity of *Syzygium aromaticum* essential oil was also experienced against *E. coli* O157:H7, the outcome exhibited diverse grades of inhibition for the essential oils [7]. The purpose of the study is responsibility of the bioactive ingredients and antibacterial activity of aqueous and methanol extract of *Syzygium aromaticum* against food-borne pathogens.

MATERIALS AND METHODS

Clove (*Syzygium aromaticum*) buds were used in this study. **Figure 1** is a sample of *Syzygium aromaticum* bud.



Figure 1: *Syzygium aromaticum* buds

Bacterial Strains

For the experiment six (6) bacterial strains were obtained from Laboratory of Science Lab Technology, School of Technology Kano. These bacterial strains were dominant for spoilage of food comprising of *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella sp*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. The spoiled food were the source of bacteria which was isolated from different laboratory procedures and biochemical tests which also identified species level through gram stain culture.

EXTRACT FORMULATION

For the preparation of extracts, fresh buds of clove were washed and dried in the air for a period of 14 days. Then buds were grounded into fine powder. After that add 500 ml of distilled water in 50 g powder and kept in the laboratory for about three days. Flasks were shaken with intervals and filter this solution with whatman filter paper. At 50°C evaporation of methanol extract and at 70°C aqueous extract was evaporated. Then add this dried extract into 10% DMSO for final solution of

200 mg/ml as stock concentration. For storage put it at 4°C before usage.

Qualitative- HPLC Test

For the analysis of phytochemicals in the plant material standard procedure was adopted according to Sofowora and Trease and Evans [8, 9].

Quantitative – SFE Test

Spectrophotometry method was also used to determine phytochemicals using standard procedure adopted by Adeniyi, *et al.* [10].

Antibacterial Susceptibility Test

The sensitivity of each extract is determined using a well diffusion method with the changes outlined by Ahmad and Beg [11]. The bacterial suspension prepared by the equivalent of 0.5 McFarland standard (1.5×10^6 CFU) was inoculated in a clean petri-dish in a clean Mueller - Hinton agar medium. 6 mm diameter sterile corn bore is used to mine 5 medium wells into the agar medium. The wells then being diluted to 25, 50, 75 and 100 mg / mL at a rate of approximately 0.1 mL of extract solution to prevent it from spreading to the outside of the agar medium. The plates are permitted to remain on the research center seat for 1 hour to permit legitimate scattering of the concentrate into the medium, trailed by plates brooded for 24 hours at 37 °C, and afterward the plates were incubated with inhibition. Areas

examined and measured for areas. Ciprofloxacin 50 mg / mL from Micro Lab Ltd was used as a positive control.

Determination of Minimum Inhibitory Concentration

The MIC of the extract is determined using the broth dilution method. Twice serial dilutions of the extract was been prepared by mixing 2 ml of nutrient broth in 100 ml / ml 2 ml test tube, thereby generating a solution containing 50 mg / ml of the extract. The procedure of testing the tube number 5 is continuous, so giving the following concentrations; 50, 25, 12.5, 6.25 3.125 mg / ml. There is no extract in test tube number 6 and serves as a negative control. 0.5 McFarland of the test organisms were inoculated into 0.5 ml test tubes in equal standards and incubated for 24 h at 37 ° C. An increase in probe tubes after incubation was observed when checking for turbulence [11].

Determination of Minimum Bactericidal Concentration (MBC)

For minimum bactericidal conc. each tube that showed unobservable growth in the MIC, transfer of 0.1ml aseptically into extract free Mueller Hilton agar plates. For 24 hrs put these plates at 37°C. The minimum bactericidal conc. was noted as the lowermost conc. of the extract that had less than 99% growth on the agar plates [11].

Statistical Analysis

The data was analyzed by using one way anova through SPSS software at 5% probability level

RESULTS

Phytochemical Screening

The subjective and quantitative phytochemical screening of *Syzygium aromaticum* extract is introduced in (Table 1). The outcome showed the nearness of Alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, saponin and tannin while lessening sugars and glycoside are missing. Quantitatively, Alkaloid was seen as the plenteous constituent creation about 9.6%, trailed by Tannin and saponin comprising 4.8% and 3.7% individually.

Antibacterial Activity of Aqueous Extract

Activity of aqueous *Syzygium* showed by *E. coli* (17.4 mm) depends on the type of bacterial isolates & centralization of the concentrates. Most elevated zone of restraint is at 100 mg/ml. The aromaticum separate is introduced in (Table 2). The outcomes range of limitation of the control (Ciprofloxacin 50 mg/ml) demonstrated that zones of hindrance recorded by the segregates ranges from to 19-22 mm.

Antibacterial Activity of Methanol Extract

Activity of methanol separate is introduced in (Table 3). The outcomes demonstrated

that zones of control (Ciprofloxacin 50 mg/ml) ranges from to 19-22 restraint recorded by the disengages rely upon the sort of bacterial separates and convergence of the concentrates. The Most noteworthy range of hindrance is shown by *Shigella sp* (19.7 mm) at 100 mg/ml.

MIC and MBC of the Extract

Least restrictive. grouping of watery and MBC of methanol extricate extends between

12.5 - 50mg/ml methanol concentrate of *Syzygium aromaticum* is spoken to while the MBC of *Shigella sp* and *Pseudomonas aeruginosa* in (Table 4). The outcome demonstrated weakenings of different was not found in watery concentrate. groupings of watery and methanol concentrates can repress as well as murder the separates. Lower MIC (3.125 mg/ml) was observed in methanol remove than fluid concentrateza.

Table 1: Qualitative and quantitative phytochemical screening of *Syzygium aromaticum* extract

S/N	Phytochemical	Qualitative analysis	Quantitative analysis (%)
1	Alkaloids	+	9.60±0.12
2	Flavonoid	+	3.00±0.09
3	Glycosides	-	0.00±0.00
4	Reducing sugar	-	0.00±0.00
5	Saponin	+	3.70±0.00
6	Steroids	+	1.80±0.04
7	Phenols	+	0.10±0.01
8	Terpenoid	+	1.70±0.01
9	Anthraquinones	+	1.20±0.03
10	Tannin	+	4.80±0.00

Table 2: Antibacterial activity of *Syzygium aromaticum* aqueous extract

Isolates	Concentration (mg /ml)/zone of inhibition (mm)				
	25	50	75	100	Control
<i>Klebsiella pneumoniae</i>	9.2±0.00	9.8±0.00	10.5±0.11	12.1±0.13	22
<i>Salmonella typhi</i>	11.3±0.15	12.4±0.13	13.7±0.22	16.5±0.26	21
<i>Shigella sp</i>	9.7±0.17	11.2±0.20	13.8±0.09	13.6±0.31	22
<i>Pseudomonas aeruginosa</i>	8.3±0.00	10.2±0.26	12.4±0.14	12.6±0.21	20
<i>Escherichia coli</i>	12.3±0.20	13.5±0.12	14.7±0.17	17.4±0.36	22
<i>Staphylococcus aureus</i>	9.3±0.15	11.8±0.20	14.3±0.23	15.0±0.12	19

Table 3: Antibacterial activity of *Syzygium aromaticum* methanol extract

Isolates	Concentration (mg /ml)/zone of inhibition (mm)				
	25	50	75	100	Control
<i>Klebsiella pneumoniae</i>	10.8±0.20	11.4±0.12	13.2±0.17	15.4±0.17	22
<i>Salmonella typhi</i>	12.8±0.12	13.5±0.17	14.4±0.25	16.2±0.20	21
<i>Shigella sp</i>	11.7±0.32	12.8±0.25	15.1±0.32	19.7±0.37	22
<i>Pseudomonas aeruginosa</i>	12.0±0.12	12.2±0.36	13.9±0.15	16.0±0.23	20
<i>Escherichia coli</i>	13.3±0.32	14.2±0.20	15.8±0.12	16.9±0.32	22
<i>Staphylococcus aureus</i>	12.3±0.17	12.5±0.32	16.1±0.20	18.3±0.47	19

Table 4: Minimum inhibitory concentration (MIC) and MBC of the extract

Isolates	Aqueous extract		Methanol extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
<i>Klebsiella pneumoniae</i>	12.5	50	6.25	25
<i>Salmonella typhi</i>	6.25	12.5	6.25	25
<i>Shigella sp</i>	12.5	NF	6.25	50
<i>Pseudomonas aeruginosa</i>	12.5	NF	12.5	50
<i>Escherichia coli</i>	6.25	25	3.125	12.5
<i>Staphylococcus aureus</i>	12.5	50	6.25	25

Key: NF = Not found

DISCUSSION

From present study it is seen that clove bud extract contains many phytochemicals which can be correlate that this can reduce the activity of bacterial strains. Phytochemicals give plants their shading, flavor, smell and are a piece of a plant's normal guard framework and ensure them against herbivorous bugs and vertebrates, growths, pathogens, and parasites [12]. The phytochemicals alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, saponin and tannin were available in *Syzygium aromaticum* extricates as indicated by this examination. The outcomes are as per the discoveries of different creators who have considered this plant [13]. As per this study, Alkaloid is available in the concentrates. Alkaloids containing a huge gathering of nitrogenous mixes are generally utilized as disease chemotherapeutic specialists, sedatives and Focal Anxious Energizers [14]. Alkaloids are said to give some metabolic jobs and command advancement in living framework [15]. It additionally meddles with cell division, consequently the nearness of alkaloids in clove could represent their

utilization as antimicrobial specialists. Aboaba, et al. had revealed that the antimicrobial properties of substances are alluring devices in food decay and sanitation [16]. This recommends the *Syzygium aromaticum* extricates which have been affirmed to contain alkaloids may likewise be helpful as additives in food. Terpenoids have been seen as helpful in the avoidance and treatment of a few illnesses, including malignant growth. Terpenoids are likewise known to have antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, insect hyperglycemic, mitigating and immunomodulatory properties [17]. Flavonoids are additionally present in the concentrate as an intense water-solvent cancer prevention agent and free extreme forager, which forestall oxidative cell harm and furthermore have solid anti-cancer movement [18, 19]. It additionally helps in overseeing diabetes incited oxidative pressure. Steroids are significance in drug store as they have mixes like sex hormones and can be utilized for medicate creation [20]. Tannin and saponin were available in the concentrate. Saponins ensure against

hypercholesterolemia and anti-infection agents properties [21]. What's more, it has been discovered that saponins have antitumor, cancer prevention agent and hostile to mutagenic exercises and can bring down the danger of human diseases by hindering the development of malignant growth cells [22, 23]. The development of numerous growths, yeast, microscopic organisms and infections was restrained by tannins [24]. The phytochemical substance of the concentrate of *S. aromaticum* uncovered that the Alkaloids was seen as the richest phytochemical (9.6%) trailed by tannin, saponin and flavonoids.

The aftereffects of antibacterial movement of *Syzygium aromaticum* extricates against food borne pathogens are surrendered (**Tables 2 and 3**) which shows that the methanol separates are more successful against the tried disengages than fluid concentrates. *E. coli* and *Salmonella* were additionally increasingly defenseless to the concentrates in examination with the rest 14.97 and 13.84 mm separately. The consequence of antimicrobial action of *Syzygium aromaticum* in this examination was inconformity with the investigation led by numerous analysts [6, 7]. The concentrate of *Syzygium aromaticum* indicated most elevated zone of restraint (11.67±1.53mm) in case of *Salmonella* spp. what's more, most minimal range of hindrance (8.0±1.73mm) against *Escherichia*

coli. *Syzygium aromaticum* remove additionally indicated lower zone of restraint (8.67±2.52mm) in case of *Staphylococcus aureus* contrasted with the Gram-negative microorganisms. In view of the powerlessness of the life forms to the concentrates, *E. coli* was seen as the most elevated vulnerable life forms with normal range of hindrance of 14.97±1.97 mm, followed *Salmonella typhi* (13.84±2.52 mm), *S. aureus* (13.7±1.65 mm), *Shigella* (13.44±1.12 mm), *Pseudomonas* (12.27 ±0.98mm) while least normal zone of hindrance is appeared by *Klebsiella* (11.55±1.16 mm).

The antibacterial exercises of the concentrates are relied upon because of the nearness of mixes, for example, alkaloid, flavonoids and tannin. The outcomes got in this examination verify with the report of Nzeako, et al. which found that clove remove had a wide range of antimicrobial action displayed for the two microorganisms and growths [24]. The aftereffect of this advocated of Sofia *et al.* [6] who tried the antimicrobial movement of various Indian flavor plants as mint, cinnamon, mustard, ginger, garlic and clove, the outcome indicated total bactericidal impact against all the food-borne pathogens tried *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* was the fluid concentrate of clove at 3%. At the grouping of 1% clove

extricate likewise demonstrated great inhibitory activity.

The consequence of MIC and MBC of the concentrates demonstrated that weakenings of different groupings of watery and methanol concentrates of *Syzygium aromaticum* can hinder as well as destroy the isolates. Lower MIC (3.125 mg/ml) was appeared by methanol remove than fluid concentrate. MBC of methanol remove goes between 12.5 - 50mg/ml while the MBC of *Shigella sp* and *Pseudomonas aeruginosa* was not found in watery concentrate. This is in accordance with the study of Gislene, *et al.*, who found that the plant withdraw the contained specialists that executed *Salmonella*, *C. albicans*, *P. aeruginosa* and *E. coli* [3].

CONCLUSION

Taking everything into account, this research uncovered that *Syzygium aromaticum* separates have restorative properties and antibacterial movement that repress bacterial development. The consequences of the current study show that *Syzygium aromaticum* ethanol containing extracts are more viable against all tried bacterial strains than fluid concentrates. *E. coli* and *Shigella* were likewise progressively powerless to the concentrates while *Klebsiella* was the least defenseless. The antibacterial exercises of the concentrates are relied upon maybe because of the present of bioactive mixes like Alkaloid,

Terpenoid, Saponin, Tannin, flavonoids and Anthraquinones which are isolated in the solvents. The eventual outcomes of present assessment has given the legitimization to remedial capability of *Syzygium aromaticum* and furthermore utilized as dietary enhancement for food protection likewise pharmacological qualities.

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