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Comparative Phytochemical screening and Antibacterial activity of leaf and flowering bud of *Syzygium aromaticum* L.

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Abstract
Syzygium aromaticum (L.) is an important plant medicinal plant. It is a precious and valuable spices of the world. It is an unopened flower bud growing on a tree belonging to the family Myrtaceae. The present study involves comparative phytochemical analysis of leaves and buds of *Syzygium aromaticum* (L.). Phytochemical analysis of leaves shows that presence of alkaloids, flavonoids, phenols, tannins, terpenoids and glycosides in ethanolic, methanolic and aqueous extracts and Phytochemical analysis of buds shows that presence of alkaloids, flavonoids, phenols, tannins, terpenoids and glycosides in ethanolic, methanolic and aqueous extracts. Antibacterial activity done by agar well diffusion method. The antibacterial activity of both parts of plant showed positive results against *E-coli* but comparatively buds showed higher zone of inhibition as compare to leaves.

Keywords: *Syzygium aromaticum*, phytochemicals, antibacterial activity

Introduction
Spices are very rich in their chemical nature. Mostly they are used in kitchen for various purposes including flavoring food items as well as used as medicine. Due to their highly percentage of chemical compound, they are including in spices. They are very beneficial in different disease and health problems like diabetes, pyria, fever, digestive problems, asthma, skin disease etc. Some spices have antiseptic and antibiotic properties which helps into preparation of drugs. In the international market, the highest spices are exported by India. Spices are not considered as a whole plant but they are different part of plant like leaf, bark, bud, flower, seed, and root. These parts of plants having rich number of phytochemicals by which they are having different pharmacological activities. In India cardamom, clove, cumin, mustard, cassia bark, coriander seed, fenugreek, turmeric, cinnamon are highly cultivated for different purposes.

Materials and methods
Plant material: *Syzygium aromaticum* (L.) is an important plant medicinal plant. It is a precious and valuable spices of the world. It is an unopened flower bud growing on a tree belonging to the family Myrtaceae. Cloves are the aromatic dried flower buds, which are commonly used in salads and garam masala. Clove is indigenous to the Maluku Island of Eastern Indonesia. The essential oil extracted from the dried flower buds of cloves is used for acne, warts, scars and parasites. Clove buds possess brown colour and burning taste. In India, it is traditionally used in all spicy rich dishes.
Pathogenic Bacteria: *Escherichia coli*

Methodology for phytochemical screening
Extract preparation method
The dried plant material (flowering bud and leaves) were crushed with help of mixture machine. 10gm powder material was taken and kept in 100ml solvent (Ethanol, Methanol and Aqueous or Distil Water) in the beaker. Cover the beaker with help of aluminium foil and put it on the shaker for 24 hours so all the phytochemicals are dissolve gradually. After that, the solution was filtered with the help of whatmann filter paper no.1. Weighed six petri plates were selected and transfer the filtered liquid form in to the petri plates. Than allowed it to evaporate the solvent for 24 hours (also used incubator). After that, we got a dried or crude extract.

Qualitative screening of secondary metabolites
(A) Alkaloids
3mg extract were dissolved individually in 3ml ethanol, 3ml methanol and 3ml distil water. Then 3ml 1N HCL was added then filtered with whatmann filter paper no.1. The filtrates were used to test the presence of alkaloids. There were three tests used to check the presence of the alkaloids which were Mayer's test, Wagner's test and Dragendroff's test.

(B) Flavonoids
1. **Lead acetate test:** 1ml liquid extract treated with 1ml 10% lead acetate solution; formation of yellow precipitation indicates the presence of flavonoids.
2. **H_2SO_4 test:** 1ml extract was treated with few drops of H_2SO_4; orange colour precipitation indicates the presence of flavonoids.
3. **Alkaline reagent test:** 1ml of extract was treated with few drops of dilute NaOH and few drops of dilute HCL; yellow colour turns into colour less solution, indicates the presence of flavonoids.
4. **Zinc hydrochloride reduction test:** 1ml of extract was treated with zinc dust and conc. HCL; formation of red colour indicates the presence of flavonoids.

5. **Pew test:** 1ml of extract was treated with pieces of metallic magnesium and 2-3 drops of conc. HCL were added; formation of brownish colour indicates the presence of flavonoids.

(C) Phenols

1. **Ferric chloride test:** 1ml extract was treated with few drops of 5% ferric chloride solution; formation of bluish black colour indicates the presence of phenols.

(D) Tannins

1. **Lead acetate test:** 1ml extract was treated with 1ml 10% lead acetate solution; white colour precipitation indicates the presence of tannins.
2. **Ferric chloride test:** 2ml extract was treated with 5 drops of 0.1% ferric chloride solution; brownish green or blue black colour indicates the presence of tannins.

(E) Terpenoids

1. **Salkowski 's test:** Few mg of extract mixed with 2ml of chloroform and 3ml of conc. H_2SO_4 was carefully added to form a layer; an appearance of reddish brown colour ring indicates the presence or terpenoids.
2. **Copper acetate test:** Extract was dissolved in water and treated it with 5% copper acetate solution; formation of emerald green precipitation indicates the presence of terpenoids.

(F) Saponins

1. **Frothing test:** About 0.5mg of extract was shaken with 5ml distil water; formation of froth (appearance of creamy small bubble) shows the presence of saponins.

(G) Glycosides

1. **Keller kiliani test:** 2ml of test solution was treated with few drops of glacial acetic acid and 1% ferric chloride solution mixed, conc. Sulphuric acid was added and observed for the formation of two layers; lower reddish brown and upper acetic acid layer which turns bluish green indicates a positive test for glycosides.
2. **Bromine water test:** 1ml of test solution was dissolved in bromine water; formation of yellow colour precipitation indicates the presence of glycosides.

3.3 Methodology for Antibacterial activity

Agar well diffusion method was selected for study of antibacterial activity. 3.9mg nutrient broth and 9mg agar-agar were mixed with 300ml distil water in conical flask. After that, the media was sterilized with the help of autoclave. After 30 minutes the flask was carefully taken out from the autoclave. 25 to 30 ml of nutrient agar media was poured into sterilized petri plates. This whole process was done in laminar air flow cabinet and between two spirit lamps. Then after, it was allowed to solidify at room temperature for 24 hours. The *Escherichia coli* bacteria were inoculated on nutrient agar plate with help of inoculating loop. Nutrient agar plates were prepared for ethanolic, methanolic and aqueous extract of leaf and flowering bud of *Syzygium aromaticum* (L.). Using the cork borer several wells of 2.5mm in diameter were punched. The equal volume (100µl) of ethanolic, methanolic and aqueous extracts with particular concentration were poured into the wells. Then the plates were incubated at 37°C for 24 hours. Inhibition zone was measured with zone scale.

Results and Discussion

Result of qualitative analysis of secondary metabolites

Table 1: Showing the result of qualitative screening (+ indicates presence and – indicates absence)

Phytochemicals	Biochemical Test	Solvents					
		Ethanol		Methanol		Aqueous	
		Leaf	Bud	Leaf	Bud	Leaf	Bud
Alkaloids	Mayer's test	-	+	+	+	+	-
	Dragendroff's test	-	-	-	-	-	-
	Wagner's test	-	-	-	-	-	-
Flavonoids	Lead acetate test	+	+	+	+	+	+
	H_2SO_4 test	+	+	+	+	-	-
	Alkaline reagent test	+	+	+	+	+	+
	Zinc hydrochloride reduction test	-	-	-	-	-	-
	Pew test	-	-	-	-	-	-
Phenols	Ferric chloride test	-	+	+	+	+	+
Tannins	Lead acetate test	+	+	+	+	-	-
	Ferric chloride test	+	+	+	+	+	+
Terpenoids	Salkowski's test	+	+	+	+	-	-
	Copper acetate test	+	-	+	+	+	+
Saponins	Frothing test	-	-	-	-	-	-
Glycosides	Keller kiliani test	+	+	+	+	-	-
	Bromine water test	+	-	+	-	+	+

Result of antibacterial activity against *Escherichia coli* bacteria

Table 2: Showing result of antibacterial activity

	Extracts	Zone of inhibition (mm)				
		Control	5mg/2ml	10mg/2ml	15mg/2ml	20mg/2ml
Leaf	Ethanol	0.0mm	4.5mm	5.5mm	6.0mm	6.5mm
	Methanol	0.0mm	1.5mm	2.5mm	2.5mm	4.5mm
	Aqueous	0.0mm	4.5mm	5.5mm	5.5mm	12.5mm
Flowering bud	Ethanol	0.0mm	4.5mm	5.5mm	7.5mm	8.5mm
	Methanol	0.0mm	4.5mm	6.5mm	7.0mm	8.5mm
	Aqueous	0.0mm	6.5mm	7.5mm	8.5mm	10mm

Graphical representation of antibacterial activity

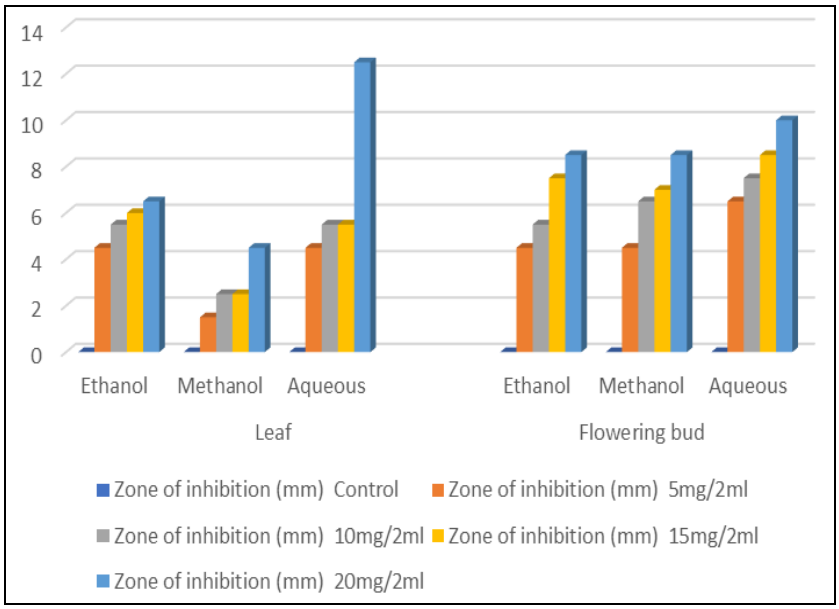

Fig 1: Shows that when concentration of solvent increases, the zone of inhibition also increases

Photo plate of antibacterial activity
(A) Control plates

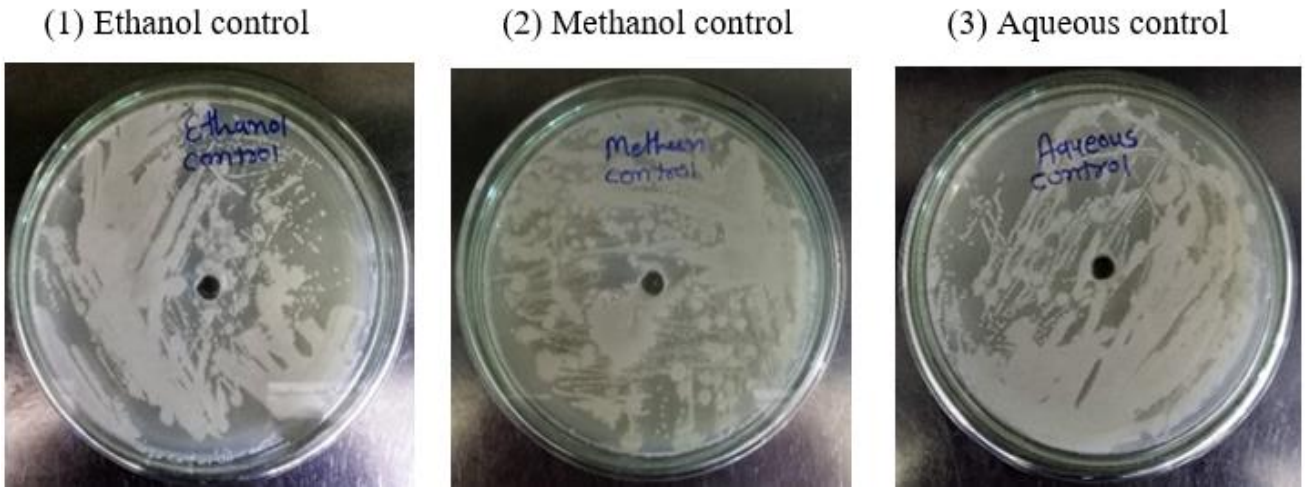

Fig 2: Showing control plates with absence of antibacterial activity

(B) Plate of leaf with different extracts with different concentrations

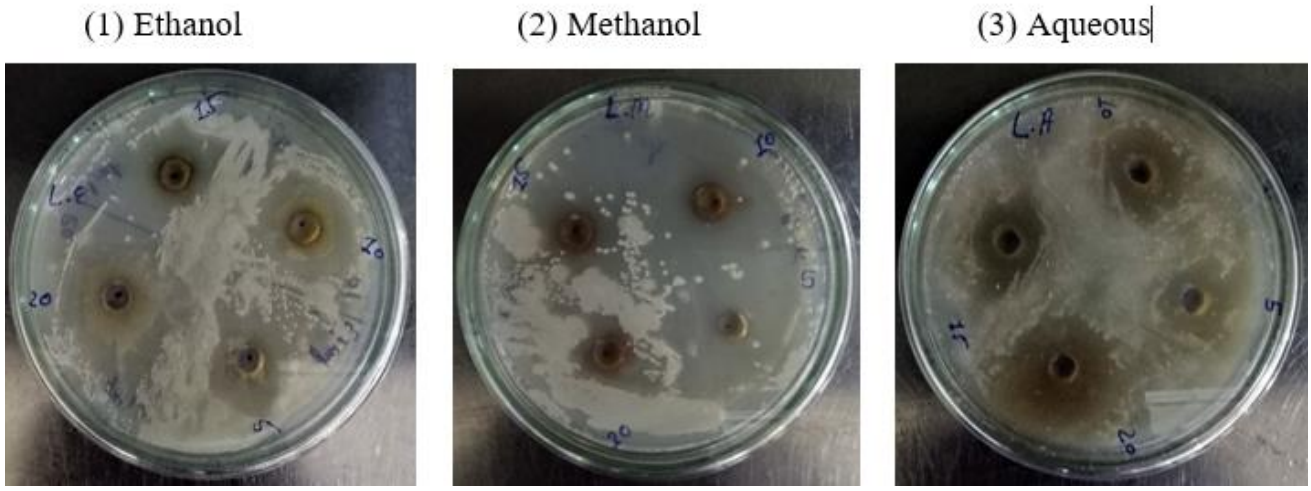

Fig 3: Showing the presence of antibacterial activity

(C) Plate of flowering bud with different extracts with different concentrations

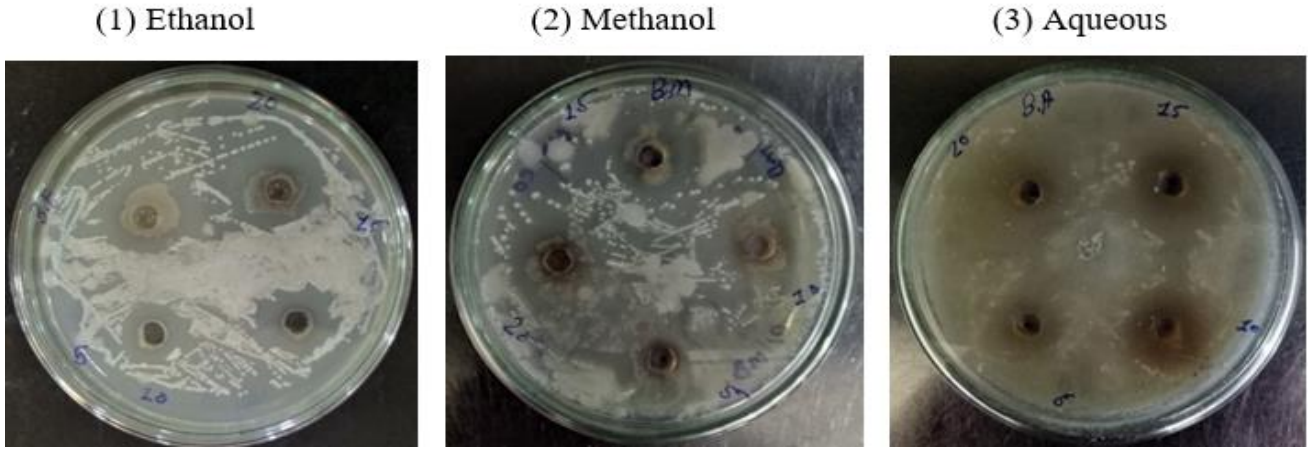

Fig 4: Showing the presence of antibacterial activity

Discussion

After the phytochemical screening various phytochemicals are present in different extracts of flowering bud and leaf. According to Simit olanike jimoh *et al,* 2017 [3] observed that ethanolic and methanolic bud extracts showed presence of alkaloid but in this study, the result obtained shows that presence of alkaloids in ethanolic and methanolic extracts. In leaf, Paul madubuike *et al.*, 2018 observed that ethyl acetate, aqueous, N-Hexane and methanolic extracts showed presence of alkaloids and this study also showed the presence of alkaloids in Methanolic and Aqueous extracts. According to Shailesh, 2015 [4] observed that benzene, acetone and aqueous bud extracts showed presence of flavonoids but this study shows the presence of flavonoids in ethanolic, methanolic and Aqueous bud extracts. In leaf, methanolic, ethanolic and aqueous extracts shows the present of flavonoids but flavonoids were found to be present in only methanolic extract in work done by Paul madubuike *et al.*, 2018. In this study phenols shows the presence in all extracts of bud but phenols were present in methanolic, ethyl acetate and acetone extracts of bud in work done by Duraisamy kayal vizhi *et al*, 2016 [6]. Phenols shows the presence in methanolic and aqueous extracts of leaf in high amount. In this study, Tannins shows the presence in all extracts of bud and leaf but according to Emmanuel ola oshomoh *et al.*, 2015 [5] in bud only aqueous extracts showed the presence of phenols. In leaf only, N-Hexane extract showed the presence of phenols according to Emmanuel ola oshomoh *et al.*, 2015 [5]. Terpenoids shows the presence in all all extracts of bud and leaf. According to Duraisamy kayal vizhi *et al.*, 2016 [6], terpenoids were present in ethyl acetate, acetone and methanolic extracts of bud. In leaf, terpenoids showed presence in methanol and aqueous extracts in work done by Paul madubuike *et al.*, 2018. In this study, saponins shows the absence in all extracts of leaf and flowering bud. According to Simit olanike jimoh *et al.*, 2017 [3], saponins showed presence in aqueous and methanolic extracts of bud and in leaf saponins also showed presence in methanol and aqueous extracts in work done by Paul

madubuike *et al.*, 2018. Glycosides shows the presence in all extracts of leaf and bud. According to Paul madubuike *et al.*, 2018, glycosides showed the presence in N-Hexane and methanolic extracts of leaf.

In this study, the different extracts of leaf and bud shows the antibacterial activity against *Escherichia coli* bacteria. The control plates show absence of antibacterial activity.

According to Muhammad Saeed *et al.*, 2013 [9], ethanolic and aqueous extracts of bud showed antibacterial activity against *Escherichia coli* bacteria but in this study, all extracts of leaf and bud shows antibacterial activity against *Escherichia coli* bacteria. In this study, at 5mg concentration leaf ethanolic and aqueous extract shows good results as compare to methanolic extract and bud shows highest results in aqueous extract. At 10mg concentration ethanolic and aqueous extract of leaf shows good results and bud shows highest result in aqueous extracts as compare to ethanolic and methanolic extracts. At 15mg concentration methanolic extracts of leaf shows lowest result and bud shows highest result in aqueous extract. At 20mg concentration aqueous extract of leaf shows highest result as compare to other extracts of bud extracts. According to *Amit Pandey and Parul Singh (2011) [7] Eugenol, carvacrol and thymol are the phenolic compounds which are responsible for antibacterial activity.

Conclusion

Thus, the results obtained in present study indicates that the flowering buds of *Syzygium aromaticum* (L.) are rich in production of phytochemicals as compare to leaves. Both parts of *Syzygium aromaticum* (L.) have the potential to act as a source of useful drugs because of presence of various phytochemical components such as alkaloids, flavonoids, phenols, tannins, terpenoids, glycosides. Because of presence of phytochemicals the flowering buds and leaves of *Syzygium aromaticum* (L.) have showed the antibacterial activity against *Escherichia coli* bacteria.

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