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ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *ALPINIA CALCARATA, BAUHINIA TOMENTOSA* AND *CURCUMA ZEDOARIA*

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ABSTRACT

Traditional system of medicine including Ayurvedha,Siddha, Unani were practised globally to treat bacterial infections. To make herbal medicine more viable these medicinal plants were screened for evolution of new antibacterial drugs.The antibacterial activity of ethanol, petroleum ether and aqueous leaf extracts of *Curcuma zedoaria, Alpinia calcarata,* and *Bauhinia tomentosa* on *Staphylococcus aureus and Pseudomonas aeruginosa* was evaluated by well-diffusion method. The ethanolic solvent extract of these medicinal plants showed the significant zone of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethanolic extract of *Alpinia calcarata*, *Curcuma zedoaria* and *Bauhinia tomentosa* profoundly inhibited the growth of *S.aureus* and *Pseudomonas aeruginosa* with inhibition zone diameter between 11-14mm. This revealed that these medicinal plants have possessed significant antimicrobial activity and may probably used as potential drug to treat bacterial infections associated with these microorganisms and used for the development of industrial product.

INTRODUCTION

In India, infectious diseases accounts for high proportion of health problems. Morbidity and mortality due to these infections continues to be a major problem, especially amongst children. Infections due to a variety of bacterial agents, such as pathogenic *Escherichia coli, Staphylococcus aureus, Shigella* sp., *Salmonella* sp., *Enterobacter* sp.and *Pseudomonas aeruginosa* are most common (Mukherjee *et al.*, 1998). With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products because of the emergence of resistant pathogens. Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. In the present time multiple drug resistance in microbial pathogens become a serious health problem to humankind worldwide (Peng *et al.*, 2006). Therefore, there is need to search new infection fighting strategies to control microbial infections. The use of medicinal plants in the world and especially India, contributes significantly to primary health care (Giri *et al*., 2010). Plants even though are rich sources of pharmaceutically important compounds, many plant species are undiscovered and their medicinal properties unknown; and even the medicinal remedies past down from generations are being lost (Mamta *etal*., 2013). It is necessary to analyse the bioactive compounds of these plants and study their antimicrobial activity in laboratory condition.

So in the present study, three traditionally important medicinal plants are taken for evaluation of their phytochemical constituents and their antibacterial properties.The three plants are *Alpinia calcarata, Bauhinia tomentosa* and *Curcuma zedoaria*.

ALPINIA CALCARATA

Scientific classification

Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Liliopsida*
Order	*Zingiberales*
Family	*Zingiberaceae*
Genus	*Alpinia*
Species	*Calcarata*

Alpinia calcarata Roscoe (Zingiberaceae) commonly known as Snap ginger is a rhizomatous perennial herb which is commonly used to stimulate digestion, treating colds to relieve stomach ache and reducing swelling.

BAUHINIA TOMENTOSA

Scientific classification

Kingdom	*Plantae*
Order	*Fabales*
Family	*Fabaceae*
Subfamily	*Caesalpinioideae*
Genus	*Bauhinia*
Species	*Tomentosa*

Bauhinia tomentosa commonly known as St.Thomas Tree, Yellow Bauhinia and Butterfly tree, is a medium shrub or a small tree that can grow up to 4m in height. Yellow Bauhinia is native to tropical Africa and can be found as far as India and Sri Lanka. The dried leaf, flower bud and a decoction of the root and bark of *Bauhinia tomentosa* are used medicinally by the African doctors. In India and Sri Lanka, the root bark is used internally for conditions of the large intestine, while the flower is used as a remedy for dysentery and diarrhoea (Rhama *etal.,2012)*

CURCUMA ZEDOARIA

Scientific classification

Taxon	Value
Kingdom	*Plantae*
Class	*Magnoliopsida*
Order	*Zingiberales*
Family	*Zingiberaceae*
Genus	*Curcuma*
Species	*Zedoaria*

Curcuma zedoaria, also known as Zedoary, is an herb that grows up to 1.2 m in height. This plant has both vertical aerial stems (pseudostems) and horizontal underground stems known as rhizomes, which allow the plant to spread so this species often grows in large clumps.

The rhizome is considered to aid digestion, to purify the blood, to provide relief for colic, and for the treatment of colds and infections. The essential oil is an active ingredient in antibacterial preparations. In India the rhizome is chewed to alter a sticky taste in the mouth, and in both Java and India a decoction of the root is used to treat weakness resulting from child birth(Kamanashi *etal*., 2012).

MATERIALS AND METHODS

Plant materials

The leaves of *Alpinia calcarata, Bauhinia tomentosa* and *Curcuma zedoaria* were collected from (Foundation for Revitalization of Local Health Tradition), Yelahanka, (Bangalore, India) at an altitude of 949 meters (3113 ft.). Plant samples were authenticated and preserved in the Herbarium, Department of lifesciences, kristu jayanti college ,Bangalore .

Crude extraction

Fresh plant leaves was collected; shade dried and powdered in a mixer. A known weight of the leaves of each plant material (20 gms) was packed into soxhelet apparatus for extraction using three different solvents namely: aqueous, ethanol and petroleum ether.The temperature is set to between 70-100˚c. After extraction the plant samples were reconstituted using the respective solvent at a particular concentration of 1g/50ml of the respective solvent(Prashant *etal* 2011).

Phytochemical Screening

The phytochemical analyses of the leaves of *Alpinia calcarata, Bauhinia tomentosa* and *Curcuma zedoaria* were carried out for screening the presence of various constituents(Girendra Kumar *et al*.,2012)

Alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Tannins: To 5ml of the extract, a few drops of 1% lead acetate were added. A yellow precipitate was formed, which indicates the presence of tannins.

Phenols: About 2ml of the extract was added to 2ml of ferric chloride solution and appearance of deep bluish green colour solution indicates the presence of phenols.

Steroids: One ml of the extract was dissolved in 10 ml of chloroform and equal volume of conc. Sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric layered showed yellow with green fluorescence, which indicates the presence of steroid.

Reducing sugar: To 1ml of the extract, 5-8 drops of Fehling's Solution was added and boiled and observed for brick red precipitate.

Flavonoids: To 1ml of extract, a few drops of dilute sodium hydroxide were added. An intense yellow colour was produced in the plant extract, which became colourless on addition of a few drops of dilute acid. This indicates the presence of flavonoids.

Terpenoid: The extract was dissolved in 1ml of chloroform,1ml of acetic anhydride was added following the addition of 2ml of conc. sulphuric acid. Formation of reddish violet colour indicates the presence of terpenoid.

Glycosides: To the solution of the extract in glacial acetic acid, few drops of ferric chloride and conc. Sulphuric acid were added and observed for the reddish brown at the junction of two layers and the bluish green colour in the upper layers shows the presence of cardiac glycoside.

Amino acid: The extract were treated few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Antibacterial activity

Antibacterial assay - Well diffusion method:

Two bacterial *Staphylococcus aureus, Pseudomonas aeruginosa* were used for assessing the antimicrobial activity of the test extracts. The microorganisms were obtained from Research centre, Kristujayanti College, Bangalore.

Antimicrobial activity of extracts was determined by Agar well Diffusion method using Muller Hinton agar media. Muller Hinton agar was prepared in conical flask and autoclaved at 121˚C for 45 minutes. Glass wares like petriplates, beaker and well borer cork were autoclaved. After autoclaving is over, it was cooled and then poured on petriplates under sterile conditions. One day before the start of experiment to revive them. 0.1 ml of the culture were pipette out by micropipette using sterile tips and spread using sterile L-shaped glass rod. Well is made using sterile well borer. Stock is maintained at particular concentration and different volume were pipetted out using sterile micropipette into the well to form a concentration of 500µg, 600µg, 700µg, 800µg, 900µg, 1000µg, 1100µg, 1200µg and 1300µg.

The plates were incubated overnight for around 16 hours and zone of inhibition were measured using zone of inhibition scale. The results were observed and recorded (Mythreyi *etal* 2012 and Adriana *etal* 2007).

RESULTS AND DISCUSSION

The yield percentages of the three plant extracts using different solvents were showed in the (Table.1) Ethanol extract showed significant yield on *Curcuma zedoaria* with 27.16%. It was also observed that aqueous solvent give a slightly better yield than petroleum ether.

TABLE 1: Percentage yield of the three leave extracts in different solvents.

Plants	Yield % (Ethanol)	Yield % (Aqueous)	Yield % (Pet. Ether)
Alpinia calcarata	12.4%	11.15%	10%
Bauhinia tomentosa	10.3%	16.6%	10%
Curcuma zedoaria	27.16%	4.4%	0.25%

PHYTOCHEMICAL CONSTITUENTS

Phytochemical analysis of the these plant extracts revealed the presence of phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids(Table.2).Ten different phytochemicals were evaluated and it was observed that steroid, cardiac glycoside and amino acids were present in all the three plants. Phenol and terpenoid are present in both *Alpinia calcarata* and *Bauhinia tomentosa* while reducing sugar and flavonoid are present in both *Alpinia calcarata* and *Curcuma zedoaria.* It was also observed that alkaloid is present in both *Bauhinia tomentosa* and *Curcuma zedoaria* except *Alpinia calcarata* .

TABLE 2: Phytochemical constituents of *Alpinia calcarata* , *Bauhinia tomentosa* and *Curcuma zedoaria* leaf extracts.

Sl no.	Phytochemicals	*Alpinia calcarata*	*Bauhinia tomentosa*	*Curcuma zedoaria*
1.	Alkaloid	-	+	+
2.	Tannins	-	-	+
3.	Phenol	+	+	-
4.	Steroids	+	+	+
5.	Reducing sugar	+	-	+
6.	Monosaccharide	+	-	-
7.	Flavonoids	+	-	+
8.	Terpenoids	+	+	-
9.	Cardiac glycosides	+	+	+
10.	Amino acids	+	+	+

+: Present; -: Absent

ANTIBACTERIAL ACTIVITY

The antibacterial activity of ***Alpinia calcarata* , *Bauhinia tomentosa* and *Curcuma zedoaria* leaf extracts possess significant activity against** *Staphylococcus aureus and Pseudomonas aeruginosa.* The ethanolic extract of *Alpinia calcarata, Curcuma zedoaria* and *Bauhinia*

tomentosa profoundly inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with inhibition zone diameter between 11-14 mm(Table.3)

TABLE 3:Antibacterial activity of *Alpinia calcarata* on *S. aureus, P. aeruginosa*

Conc. of extract (µg)	*Staphylococcus aureus (mm)*	*Pseudomonas aeruginosa (mm)*
500	14	10
600	13	11
700	13	12
800	12	12
900	12	12
1000	11	14
1100	11	13
1200	11	12
1300	11	12

Alpinia calcarata have a significant antibacterial activity on the two experimental microorganisms. It was observed that the ethanolic extract showed a maximum zone of inhibition on *S. aureus* at 500µg concentration. The activity slightly decreases as the concentration increases and remain constant from 1000µg to 1300µg concentration. This shows that the ethanolic extracts of the leaf of the plant have a significant antibacterial activity against a highly resistant and opportunistic pathogen, *S. aureus.* The ethanolic extracts of the leaf of *Alpinia calcarata* also showed significant antibacterial activity on *Pseudomonas aeruginosa* with maximum zone of inhibition of 14mm at 1000µg concentration. This shows that the ethanolic extract of the leaf of *Alpinia calcarata* have a significant antibacterial activity on highly resistant bacterial *Pseudomonas aeruginosa.*

the concentration of the extract increased the antibacterial activity also was found to increase but however when the concentration increased beyond 900µg the antibacterial significantly decreased only with *S. aureus*. The extract was found to exhibit broad spectrum activity against both *S. aureus* and *P. aeruginosa* which is an ideal feature of any antibacterial agent that is employed for treating any infection. The maximum zone of inhibition for *S. aureus* was ranging between 12-14mm diameter at an very minimal concentration of 500µg, while the maximum zone of inhibition for *P. aeruginosa* was found to be 14mm in diameter at a concentration of 1000µg.

The antibacterial efficacy exhibited by the ethanolic extract can be directly correlated to the presence of pyhtochemcials. The present phytochemical study and the earlier phytochemical study conducted reveals that the ethanolic extract of *A.calcarata* contains many pharmacologically active substances such as phenolic compounds, flavanoids.

TABLE 4: Antibacterial activity of *Bauhinia tomentosa on S.aureus and P. aeruginosa*

Conc. of extract (µg)	*Staphylococcus aureus (mm)*	*Pseudomonas aeruginosa (mm)*
500	10	11
600	12	13
700	13	13
800	10	13
900	13	13
1000	14	13
1100	12	14
1200	12	15
1300	12	15

Antibacterial activity of leaf extract of *Bauhinia tomentosa*

showed a maximum zone of inhibition of 14 mm on *Staphylococcus aureus* at 1000µg concentration. This shows that the ethanolic extracts of the leaf of the plant have a significant antibacterial activity against a highly resistant and opportunistic pathogen, *Staphylococcus aureus.* The ethanolic extracts of the leaf of *Bauhinia tomentosa* also showed significant antibacterial activity on *Pseudomonas aeruginosa* with maximum zone of inhibition of 15mm at 1200µg and 1300µg concentration. This shows that the ethanolic extract of the leaf of *Alpinia calcarata* have a significant antibacterial activity on highly resistant bacterial *Pseudomonas aeruginosa.*

Maximum zone of inhibition of about 14 mm diameter was found at a concentration of 1000 µg for *S.aureus* and around 15 mm diameter of zone of inhibition was found *P.aeruginosa* at a concentration of 1200 µg of the plant extract.

The active phytochemicals in the ethanolic extract are possibly responsible for the antimicrobial efficacy exhibited by the extract. The antimicrobial efficacy is directly proportional to the zone of inhibition that was observed(Table .4)

TABLE 5: Antibacterial activity of *Curcuma zedoaria* on *S. aureus* and *P. aeruginosa*

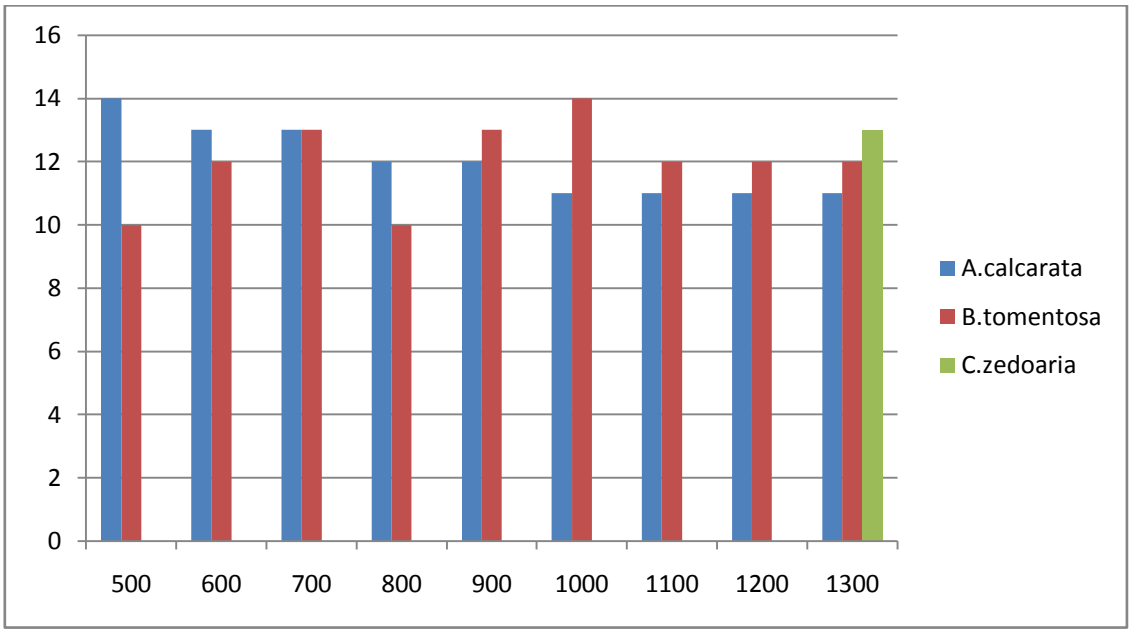
Conc. of extract (µg)	*Staphylococcus aureus (mm)*	*Pseudomonas aeruginosa (mm)*
500	-	11
600	-	11
700	-	13
800	-	13
900	-	15
1000	-	13
1100	-	13
1200	-	14
1300	13	12

Antibacterial activity of leaf extract of *Curcuma zedoaria* was observed at 1300µg concentration with zone of inhibition of 13mm. This shows that the ethanolic extracts of the

leaf of the plant have a poor antibacterial activity against a highly resistant and opportunistic pathogen, *Staphylococcus aureus* as compare to the other two plants.

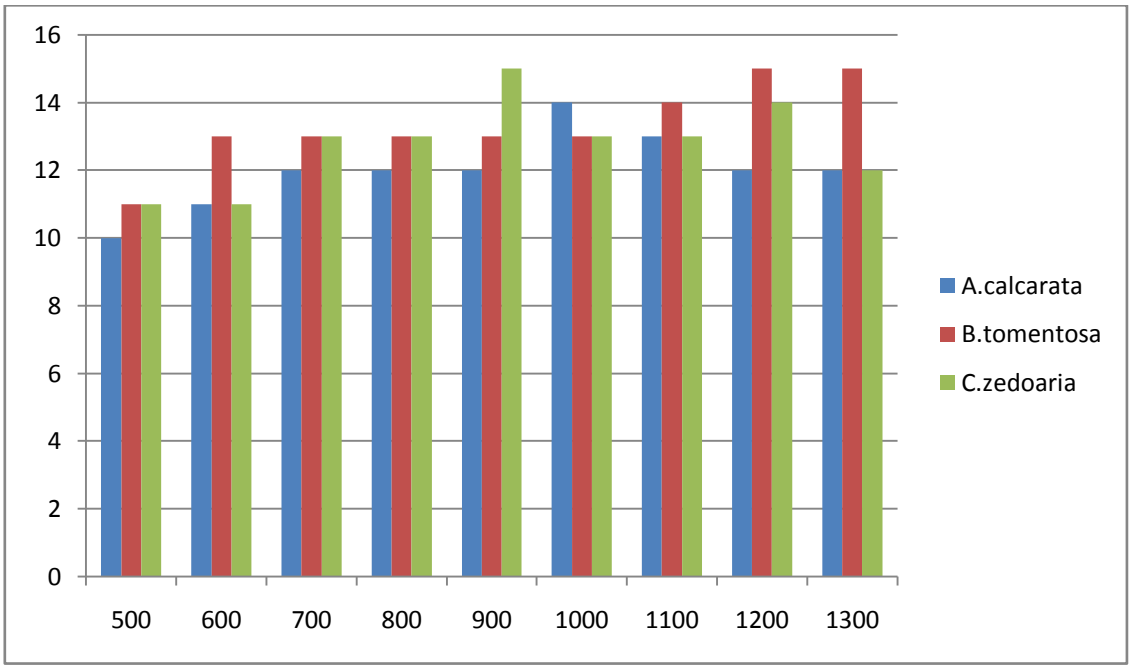
The ethanolic extracts of the leaf of *Curcuma zedoaria* also showed significant antibacterial activity on *Pseudomonas aeruginosa* with maximum zone of inhibition of 15mm at 900µg concentration(Table.5)The phytochemical studies of ethanolic extract also shows the presence active pharmacognostic substances such as flavonoids, alkaloids and phenolic compounds. In accordance, from the table it can be seen that the ethanolic extract of *C. zedoaria* is highly effective against gram negative bacilli such as *P. aeruginosa* while not much activity was found against the gram positive cocci such as *S. aureus*. This difference in the antimicrobial efficacy can be due to the difference in structure between the two different organisms. Maximum zone of inhibition of 15 mm diameter was recorded against *P. aeruginosa* at a concentration of 900 µg, whereas maximum zone of inhibition of 13 mm diameter was found against *S. aureus* at a concentration of 1300 µg. It can also be noted that concentration below 1300 µg did not show any significant antibacterial activity.

Figure.1 Antibacterial activity of leaf extracts on *Staphylococcus aureus.*



From the graph it was observed that *Curcuma zedoaria* have a minimum antibacterial activity on *S. aureus* while *Alpinia calcarata* and *Bauhinia tomentosa* have a maximum activity at 500µg and 1000µg respectively(Fig.1)

Figure.2.Antibacterial activity of leaf extracts on *Pseudomonas aeruginosa.*



From the graph it is understood that *Alpinia calcarata* have a comperatively low antibacterial activity on *P. aeruginosa* while *Bauhinia tomentosa* and *Curcuma zedoaria* have a maximum activity with a zone of inhibition of 15 mm at 1200µg and 900µg respectively(Fig.2)

CONCLUSION

The Phytochemical screening of plant extracts showed the presence of 6-8 different Phytochemical constituents including flavanoids,alkaloids are richest source of pharmacologically active substances and many of these components are structural analogue of many chemical substituents used in manufacture of medicines but are highly efficient in their action when compared to the synthetic medicines. These active components vary in their constituents and as well as their concentrations from one plant to another. The active phytoconstituents of the plants can be selectively extracted from a plant based on their relative polarity. With further studies, these active pharmacognostic substances can be employed in treating potent pathogens that are known to cause many infections, these active compounds can also circumvent the prevalence of multi drug resistant bacteria that have emerged due to profuse and continuous use of antibiotics.

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