IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2019 Vol.:16, Issue:2

Phytochemical Screening and Antibacterial Activity of Extracts of *Curcuma longa* Rhizomes and *Bougainvillea glabra* Flowers

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Submission: 26 August 2019
Accepted: 31 August 2019
Published: 30 September 2019


www.ijppr.humanjournals.com

Keywords: Antibacterial activity, *B. glabra, C. longa, K. pneumoniae, MIC, S. aureus.*

ABSTRACT

Introduction: Nowadays, the development of bacterial resistance to the available antibiotics and increasing the availability of traditional medicine has led the researchers to investigate the antibacterial compounds in plants. Medicinal plants comprise of many phytochemicals (secondary metabolites). Apart from their defence property on the plants, these phytochemicals have also a potential to protect humans against a variety of diseases. The extraction of these secondary metabolites from plants has been found to act against growth of microbes like bacteria, fungi and even viruses. Turmeric (*curcuma longa*) which belongs to the ginger (Zingiberaceae) and *Bougainvillea glabra* which belongs to (Nyctaginaceae) are among these traditional medicines against many ailments. **Objective:** The main objective of my research was screen phytochemical constituents and to test antibacterial activity of extracts of turmeric (*C. longa*) rhizomes and *Bougainvillea glabra* flower on bacterial isolates (*Staphylococcus aureus* and *Klebsiella Pneumoniae*). **Methods:** Simple maceration method and evaporation on water bath was employed for extraction and concentration of phytochemicals from rhizomes of Turmeric and flower of bougainvillea. Then the preliminary phytochemical screening had conducted by using standard procedures and antibacterial activity was determined by using disc diffusion method. The minimum inhibitory concentration (MIC) of the extracts was determined by two-fold serial dilution method. **Results:** The phytochemical screening of ethanolic extract of *C. longa* rhizomes revealed 9 phytoconstituents like alkaloids, saponins, anthocyanins, coumarins, flavonoids, terpenoids, cardiac glycosides and the aqueous extraction of it contains 10 phytoconstituents like alkaloids, saponins, steroids, tannins, anthocyanin, flavonoids, diterpenes, phenols, terpenoids and cardiac glycosides. The phytochemical screening *of* ethanolic extraction of *B. glabra* flower revealed the presence of 8 phytoconstituents such as alkaloids, saponins, steroids, tannins, flavonoids, diterpenes, cardiac glycosides and carbohydrates. The MIC of ethanolic extract of *C. longa* against *S. aureus* and *K. pneumoniae* was 50 mg/ml and 25 mg/ml respectively, while the MIC of ethanolic extract of *B. glabra* flower was 25 mg/ml against both *S. aureus* and *K. pneumoniae.* The mean inhibition zone diameter of the ethanolic extract of *C. longa* rhizomes against *S. aureus* was 23mm, 22mm and 11mm at 200mg/ml,100mg/ml and 50mg/ml respectively, while the mean inhibition zone of this extract against *K. pneumoniae* was 27mm, 26.5mm and 11.5 mm at 200mg/ml, 100mg/ml and 50mg/ml respectively. The mean inhibition zone diameter of ethanolic extract of *B. glabra* flower against *S. aureus* was 22 mm, 24 mm and 21 mm at 200mg/ml,100mg/ml and 50mg/ml respectively, while it was 28 mm, 28 mm and 25.5 mm at 200mg/ml, 100 mg/ml and 50 mg/ml respectively against *K. pneumoniae*. The mean inhibition zone of chloramphenicol was 34 mm and 35 mm against *S. aureus* and *K. pneumoniae* respectively. **Conclusions:** The result of the present study shows that the extracts of *Curcuma longa* rhizomes and *Bougainvillea glabra* flower contains potential antimicrobial components that may be a great use for the development of medicines by pharmaceutical industries as therapy against various diseases.

INTRODUCTION

Plant oils and extracts have been used for a wide variety of purposes for thousands of years. Around the world, there are many thousands of plant species have been reported to be used for medicinal purposes in various human cultures and one of their purposes is as a supply of drugs since they contain organic compounds with therapeutic values. Majority of people depend on traditional medicine as their primary healthcare and about 80% of people in this world depend on herbs for health. In most developing countries, the use of medicinal plants which is a basis for maintenance of good health has been discovered. Besides serving as medicinal purposes, the plant extracts are also used as spices and these spices are considered safe and effective against certain ailments (Ching W.Y. *et al*., 2014).

Products of natural plant have been used all over human history for numerous purposes. Since they have co-evolved with animal life, most of the plants from which these natural products are derived are billions of years old. Many thousands of these products are produced as secondary metabolites by higher plants as they are used as natural defence mechanism against any ailments and infection. Most of these natural products have biological or pharmacological action that can be oppressed in pharmaceutical drug discovery and drug development design. Medicaments obtained from plants have played a crucial role in the disease management of many cultures in both prehistoric and modern. For example, the Indian system of general medicine with historical root known as “Ayurveda” uses mainly plant-based drugs or formulations to treat various illnesses, including cancer. As some literatures indicates, between 1981 and 2002 at least 877 small molecule drugs have been introduced to this world; out of which around 61% can be traced to natural products in origin (Prasad S. and Aggarwal B.B., 2011).

Herbal plant extracts used as medicine are nowadays used as a spare for synthetic drugs. These plants are used as raw materials for herbal medicinal products and supplements due to their low cost, effectiveness and they have no much side effects when related to those of synthetic formulations. Turmeric (*Curcuma longa)* is one of the herbal medicines. It is an annual plant belongs to the Zingiberaceae family (Louay L., 2014; Azwanida N.N., 2015; Oghenejobo *et al*.,2017). *et al*.,2017).

The family of Zingiberaceae contains many genera of medicinal and aromatic plants such as Alpinia, Zingiber and Kaempferia and one of the plants that commonly found in Asia is

Curcuma longa Linnaeus or turmeric. This *Curcuma longa* is sometimes called *Curcuma domestica* as it is constantly used in the kitchen for preparing dishes. *Curcuma longa* has been used for making ancient Indian curries for many years as a flavour, colour, and preservative. Annually, India harvests about 400,000 tons fresh weight or about 80% of the world's supply of marketable turmeric (Ching W. Y *et al*., 2014).

Phytochemicals are the non-nutritive chemical constituents of plants that facilitates to guard the plant for defence and stop them from being infected by diseases. Turmeric comprises many phytochemicals (secondary metabolites) like alkaloids, tannins, steroids, saponin, flavonoids and many others. Apart from their defensive property on the plants, these phytochemicals have also a potential to protect humans against variety of diseases (Oghenejobo *et al.*, 2017).

Most of the phytochemicals from plant bases such as phenolics and flavonoids have been stated to have positive effect on health and cancer prevention (Azwanida N. N., 2015). The extraction of these secondary metabolites from plants has been found to act against growth of microbes like bacteria, fungi and even viruses. Turmeric (*Curcuma longa*) also comprises of nutritive components like fats, protein, carbohydrate, minerals and moisture. Essential oils present in Turmeric include sabinene, borneol, α-phellandrene, cineol, sesquiterpenes, zingiberene and curcumin (Oghenejobo *et al*., 2017). The yellow characteristic of turmeric is due to the presence 3–5% of curcuminoids (Mottahedin *et al; 2016*).

Turmeric has been used in traditional medicine, but in recent years the interest for this rhizome increased due to its potential use as a food colorant (Mottahedin *et al; 2016*). It is also considered as prosperous and is a part of religious rituals and in old Hindu medicine, it is extensively used for the treatment of wound and swelling caused by injury. Even In recent times, traditional India medicine uses turmeric powder for the treatment of anorexia, biliary disorders, coryza, diabetes, cough, wounds, hepatic disorders, rheumatism and sinusitis *etc* (Rathaur *et al*., 2012).

In Indian and Chinese medicines, turmeric was used as anti-inflammatory agents to treat gas, colic, toothaches, chest pains, menstrual difficulties, used to treat stomach and liver problems, to heal wounds and lighten scars and also used as a cosmetic. Turmeric has been shown to own a good a spectrum of biological actions like antioxidant, ant-carcinogenic, anti-inflammatory, ant-mutagenic, anticoagulant, antifertility, anti-diabetic, antibacterial,

antifungal, antiprotozoal drug, antiviral, anti-fibrotic, antiulcer, hypotensive and hypercholesteraemic activities (Rathaur *et al*., 2012; Louay L., 2014). Its rhizome is used as one of the most important medical plants since curcumin possesses antibacterial property against a number of Gram positive and Gram-negative bacteria and also, its anti-inflammatory properties are well documented. In recent years, the extraction and purification of natural pharmaceuticals from medical plants is notable due to less side effects on human body (Rathaur *et al*., 2012).

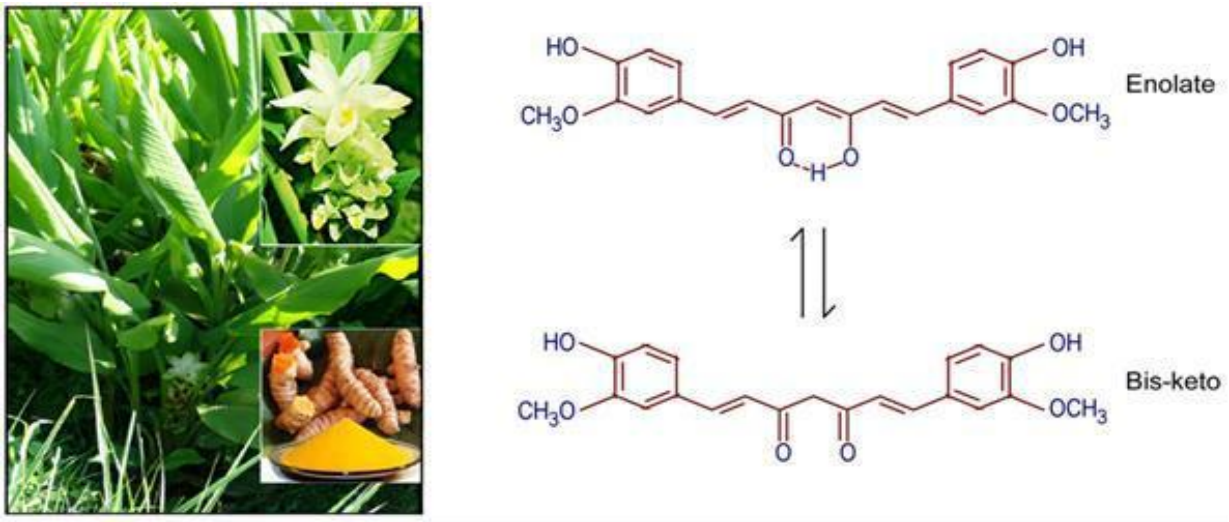


Figure 1: Turmeric plant with rhizome and Curcumin chemical structures.

The genus Bougainvillea is also a medicinal plant and widely spread group of plant throughout the world (Rodolfo A. V. and Vera L. P., 2018). This ornamental flowering plant from the genus Bougainvillea and Nyctaginaceae family is widely distributed in Southeast Asian countries. Due to the pharmacological effects of this plant, it is widely used in traditional and modern medicine for cough, bronchitis, respiratory infection, gastritis, hyperacidity, gastroduodenal ulcer, colic, fever, diarrhoea, injury, diabetes, and stomach ache and it is also used as expectorant (Figueroa *et al.*, 2014).

Bougainvillea has 18 species and horticulturally important species are *Bougainvillea spectabilis* (*B. spectabilis*), *B. glabra* and *B. peruviana* (Adebayo *et al.*, 2005; Bhat *et al*., 2011; Nadia S. *et al.*, 2013). The leaves of *B. spectabilis* are reported to have antidiabetic, hepatoprotective, antiviral, antibacterial and insecticidal properties. The constituents responsible for these activities were flavonoids, betacyanin, alkaloids and tannins which are used as a medicine for variety of disorders (Nadia S. *et al.*, 2013).

Bougainvillea glabra which is also called as paper flower is a climbing evergreen woody ornamental shrub which inhabited to warmer climates is a native to Brazil and now also seen in areas like Middle East, Bangladesh, India, Pakistan, North America *etc*. Its stems are thin, with recurred prickles and leaves covered with small hairs. It produces abundant flowers with white and purple bracts and there are many varieties with different colours: red, orange, yellow, violet etc. (Zahidul I. *et al.*, 2016).

Figure 2: Bougainvillea plant

OBJECTIVES

GENERAL OBJECTIVE

The main objective of my research was screen phytochemical constituents and to test antibacterial activity of extracts of turmeric (*C. longa)* rhizomes and *Bougainvillea glabra* flower on bacterial isolates (*Staphylococcus aureus* and *Klebsiella Pneumoniae*).

SPECIFIC OBJECTIVES

To extract turmeric rhizome and Bougainvillea flower by ethanol.

To screen phytochemical constituents like alkaloids, saponins, tannins, coumarin, flavonoids, diterpenes, phlobatannins, cardiac glycosides, phenols, steroids, terpenoids, Amino acids, carbohydrates and anthocyanin.

To find the minimum inhibitory concentration (MIC) of the extracts.

To perform antibacterial activity of ethanolic extracts of *C. longa* rhizomes and *B. glabra* flower on bacterial isolates (*Staphylococcus aureus* and *Klebsiella Pneumoniae*).

MATERIALS AND METHODS

STUDY DESIGN, SAMPLING AND METHODS

An experimental type of study design was conducted for extraction of Turmeric (*Curcuma longa*) by using ethanol and water; and extraction of Bougainvillea by using ethanol and the comparison of preliminary phytochemical constituents and antibacterial activity of the extracts against bacterial isolates (*Staphylococcus aureus* and *Klebsiella Pneumonia*e). Simple maceration method and evaporation on water bath was employed for extraction and concentration of phytochemicals from rhizomes of Turmeric and flower of Bougainvillea. Then the preliminary phytochemical screening was conducted by using standard procedures and antibacterial activity was determined by using disc diffusion method. The minimum inhibitory concentration (MIC)of the extracts was determined by two-fold serial dilution method.

Procedures for Collection of plat materials and extraction of turmeric: (Oghenejobo *et al*, 2017)

The fresh turmeric rhizomes of Indian origin were purchased from a local market (Greater Noida) in March 2019. These turmeric rhizomes were soaked in sterile distilled water and washed and then rinsed again with distilled water. The rhizomes were air dried for two hours and then they were grated using a sterile grater. 200 grams of turmeric was macerated with 1400ml of ethanol in a clean container for one week (7 days). After 7 days the mixture was sifted by using clean sterile muslin. The resultant filtrate was concentrated by evaporating on water bath until semi solid form of the extract was left and then it was air dried. Finally, the air-dried extracts were stored in refrigerator at 4ºC prior to use.

Procedures for Collection of plat materials and extraction of Bougainvillea: (Yolwin J. P. and Merlyn L., 2012)

Mature and intact flowers of the plant were cleaned and one hundred grams (100g) of the flower part were weighed using the digital balance. These were then chopped into small pieces and were placed in conical flasks. Ninety-five percent (95%) ethanol was added to

flask until the plant parts were completely submerged. The plant materials were soaked for 24 hours and extraction was done by filtering the mixture through a vacuum filtrator. Plant residue was then discarded properly. The filtrate was concentrated by evaporating on water bath. The concentrates of plant part were stored in properly labelled vial, and refrigerated until use.

Procedures for preparation of extracts for serial dilutions:

A reconstitution of plant extracts was done as follows according to (Oghenejobo *et al*, 2017):

400 mg of the extracts were dissolved in 2 ml of ethanol in a test tube as stock. Then two-fold serial dilutions of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.125mg/ml and 3.125 mg/ml was prepared aseptically from it. Then these dilutions were used for antibacterial assay.

Procedures for phytochemical screening of extracts

Phytochemicals are the non-nutritive chemical compositions of plants which used to protect the plant for defence and prevent them from being infected by diseases. In this research, the individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. The phytochemicals tests were carried out adopting from standard procedures with some modifications (Behar N. *et al*., 2013; Sawant R.S. and Khandwal K. R. and Sethi V. K., 2016; Oghenejobo *et al.*,2017; Godghate A. G. and Sawant R. S., 2013).

Alkaloids: A quantity (3 ml) of concentrated extract was taken into a test tube and 1 ml HCl was added, then the mixture was heated gently for 20 minutes cooled and filter, the filtrate was used for following tests.

a) Wagner's test: 1ml of the extract was added with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.

b) Dragendoff's test: - 2 drops of Dragendroff's reagent was added to 1ml of the extract. The development of a creamy precipitate was indicative of the presence of alkaloids.

c) Hager's test: 1ml of the extract was treated with Hager's reagent, presence of alkaloids was confirmed by the yellow colored precipitate.

Saponin: 5 ml the extract was mixed with 20 ml of distilled water then shaked in graduated cylinder for 15 minutes and formation of foam indicates Saponin.

Steroid: 1ml of the extract was dissolved in 10 ml of chloroform & equal volume of concentrated H_2SO_4 acid was added to it from the side of test tube. Then, if the upper layer turn to red and H_2SO_4 layer shows yellow with green fluorescence, it indicates the presence of steroid.

Tannin: 4ml of the extract was treated with 4 ml $FeCl_3$ and formation of green colour indicates that presence of condensed tannin.

Anthocyanin: 2 ml of extract was added to 2 ml of 2N HCl & NH_3, the appearance of pink red turns blue violet indicates presence of Anthocyanin.

Coumarin: 3 ml of 10% NaOH was added to 2 ml of aqueous extract and formation of yellow colour was and indicative of coumarins.

Amino acids

Ninhydrin test: To 2 ml of the extract was added to 2 ml of ninhydrin reagent and then boiled for few minutes and the formation of blue colour indicates the presence of amino acid.

Flavonoid

a. Alkaline reagent test: the extract was treated with 10 % of NaOH solution. Then, formation of intense yellow colour indicates presence of Flavonoid.

b. Zn test: 2 ml extract was treated with Zn dust and concentrated HCl. Then, development of red colour indicates presence of Flavonoid.

Diterpenes

Copper acetate test: the extracts were dissolved in water and treated with 10 drops of copper acetate solution; the formation of bright green colour was indicative for the presence of diterpenes.

Phenol

Ferric Chloride test: 1 ml of the test extract was treated with 4 drops of Alcoholic $FeCl_3$ solution. Then, formation of bluish black colour indicates the presence of Phenol.

Terpenoids: To 1 ml of the extract, 1 mL of chloroform along with 4-5 drops of concentrated H_2SO_4 was added. Then, the formation of a reddish-brown interface indicates the presence of Terpenoids.

Phlobatannins: Deposition of red precipitates when aqueous extract of each plant sample was boiled with 1% Aqueous HCl was taken as evidence for the presence of Phlobatannins.

Cardiac Glycosides

Legal's Test: To the extract 1ml of pyridine and few drops of freshly prepared sodium nitroprusside solution was added, and the appearance of pink to red colour indicates presence of glycosides.

Keller-Killani Test: the plant extract was treated with 2 ml of glacial acetic acid containing a drop of $FeCl_3$. Then, the formation of brown colour ring indicates the presence of positive test.

Carbohydrate: Extract were dissolved individually in 5ml of distilled water and filtered. The filtrates were used for the following tests.

a) Molisch's Test: The filtrates were treated with 2 drops of alcoholic-naphthol solution, formation of violet ring at the junction indicates the presence of carbohydrate.

b) Barfoed's Test: 1ml of test solution was taken and 1ml of Barfoed's reagent was added in a test tube, then this test tube was kept in boiling water bath. If brick red colored precipitate is formed at the bottom it indicates the presence of carbohydrate.

c) Benedict's test: The filtrates were treated with Benedict's reagent and heated gently; orange red precipitate indicates the presence of reducing sugar.

Procedures for preparation of bacterial isolates: (Oghenejobo *et al.*, 2017)

Two bacterial isolates (*Staphylococcus aureus* and *Klebsiella Pneumonia)* were used for this study. The medias for the investigation are the nutrient broth, MacConkey broth, MacConkey

agar, nutrient agar and Muller- Hinton agar for susceptibility test. The test organisms were inoculated in to nutrient broth for *S. aureus* and MacConkey broth for *K. pneumonia* and incubated for 24 hours at 37 ^{o}C. The bottles containing the broth organisms were matched and compared with the McFarland standard, to give approximately 0.5×10^{8} CFU/ml.

***Procedures for antibacterial sensitivity test*:** (Oghenejobo *et al.*, 2017; Somayeh R. *et al*., 2014).

The antibacterial sensitivity was carried out using the agar disc diffusion technique. A stock solution of extract was prepared by dissolving 4 gm of extract with 20ml of their respective solvents (ethanol) to produce a final concentration of 200mg/ml. This stock solution was then, diluted by two-fold serial dilutions to make different concentrations (200mg/ml, 100mg/ml, and 50mg/ml). The overnight prepared broth cultures of organisms were matched with McFarland standard, and they were swabbed on the muller Hinton agar plate in duplicates by using cotton swab sticks. Then, 20µl of each dilutions of the extracts were impregnated in to sterile blank discs of 6mm in diameter. Ethanol loaded discs were used as negative controls, while chloramphenicol antibiotic disc was used as positive control. All the discs were fully dried before application on bacterial lawn. After application, it was incubated 18-24 hours at 37^{o}C. At the end of incubation period, the inhibition zone Diameter (IZD) were measured using a transparent meter ruler.

Procedures for determination of minimum inhibitory concentration (MIC): (Oghenejobo *et al.*, 2017)

Minimum inhibitory concentration is the highest dilution or the least concentration of antimicrobial agent that will inhibit the growth or kill the micro-organisms.

Various concentration for the MIC was 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. Then 1 ml of each concentration was displayed in sterile petri-dishes and 19 ml of Muller Hinton agar was poured for MIC test against *S. aureus* and *K. Pneumoniae*. Then the mixture was swirled thoroughly to mix well and allowed to solidify and the plates were divided in to two segments according to the organisms and labelled accordingly. Then, using the nichrome wire loop, each segment of the plate was aseptically streaked with the overnight broth of organisms. Then incubate the plates for 24 hours at 37 ^{o}C and observe it. The least concentration for each organism that do not show growth was recorded as the MIC for the organisms.

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENINGS

The extraction of *Curcuma longa* and *Bougainvillea glabra* were screened to determine the presence of the following metabolites through preliminary phytochemical screening. These were Alkaloids (determined by Dragendroff's reagent test, Hager's reagent and Wagner's reagent), Saponins (determined by froth test), steroids, Tannins (determined by Ferric chloride test), Anthocyanin, Coumarin, Amino acids (Ninhydrin test), Flavonoids (determined by alkaline reagent test and zinc test), Diterpenes, Phenols (by Ferric chloride test), Terpenoids, Cardiac glycosides (determined by Legel's test and keller kellani test), Carbohydrates (determined by Molisch's test, Barford's test and Benedict's test) and Phylobatannins (determined by Hydrochloric acid).

Phytochemical Screening of turmeric rhizome extracts: In this study, Phytochemical screening carried out on the ethanolic and aqueous extract of Turmeric rhizomes revealed the presence of 9 phytochemicals (alkaloids, saponin, anthocyanins, coumarin, flavonoids, terpenoids, cardiac glycosides, phlobatannins and carbohydrates) in ethanolic extract and 10 phytochemicals (alkaloids, saponins, steroids, Tannins, anthocyanin, Flavonoids, Diterpenes, Phenols, Terpenoids and cardiac glycosides) in aqueous extraction as shown in (Table 1) below. Another study conducted by Oghenejobo *et al*., 2017, showed the presence of 14 phytochemicals such as alkaloids, saponin, tannins, coumarin, flavonoids, diterpenes, phlobatannins, cardiac glycosides, phenols, steroids, anthraquinones, reducing sugars, anthocyanins and terpenoids.

According to the research conducted by Oghenejobo *et al*., 2017, the phytochemicals found in ethanolic extract of turmeric were alkaloids, saponins, tannins, coumarins, flavonoids, diterpenes, phlobatannins, cardiac glycosides, phenols, steroids and anthraquinones. Sawdhini S. P. *et al*., 2011, determined the presence of six phytochemicals (alkaloids, flavonoids, tannins, saponins, cardiac glycosides and phenols) in aqueous extract of turmeric. Rajesh *et al.*, 2013, also had reported the presence of six phytochemicals such as tannins, alkaloids, saponins, flavonoids, terpenoids and cardiac glycosides in methanolic extract of turmeric rhizomes. Saxena Jyoti and Sahu Rajeshwari, 2012, screened ten phytochemicals (Carbohydrates, Proteins, Starch, Amino acids, steroids, glycosides, flavonoids, tannins, and saponins) from methanolic extract of rhizomes of three different species of curcuma

(*Curcuma longa*, *Curcuma amada* and *Curcuma caesia*). The result of this study revealed the presence of four phytoconstituents (proteins, amino acids, steroids and flavonoids) in *Curcuma longa* species.

Table 1. Results of phytochemical screening of ethanolic and aqueous extract of turmeric rhizome

Serial no.	Phytochemical constituents	Ethanolic extract	Aqueous extract
1	Alkaloids		
	a. Dragendoff's test	+	-
	b. Wagner's test	+	-
	c. Hager's test	+	+
2	Saponins	+	+
3	Steroids	-	+
4	Tannins	-	+
5	Anthocyanin	+	+
6	Coumarin	-	-
7	Amino acids		
	Ninhydrin test	-	-
8	Flavonoids		
	8a. alkaline reagent test	-	+
	8b. Zinc test	+	+
9	Diterpenes	+	+
10	Phenols		
	Ferric chloride test	-	+
11	Terpenoids	-	
12	Cardiac glycosides		
	12a. Legal's test	+	+
	12b. Keller kellani test	+	+
13	Carbohydrates		
	13a. Molisch's test	+	-
	13b. Bradford's test	+	-
	13c. Benedict's test	-	-
14	Phlobatannins	+	-

Footnote: (+) = present, (-) = absent

Phytochemical screening of Bougainvillea flower extracts: Phytochemical screening carried out on the ethanolic extract of Bougainvillea flower revealed the presence of 8 phytochemicals like alkaloids, saponin, steroids, tannins, flavonoids, diterpenes, cardiac glycosides and carbohydrates as shown in (Table 2) below.

Table 2: Results of phytochemical screening of ethanolic extract of Bougainvillea flower

Serial no.	Phytochemical constituents	Ethanolic extract
1	Alkaloids	
	a. Dragendoff's test	-
	b. Wagner's test	+
	c. Hager's test	+
2	Saponins	+
3	Steroids	+
4	Tannins	+
5	Anthocyanin	-
6	Coumarin	-
7	Amino acids	-
	Ninhydrin test	-
8	Flavonoids	
	8a. Alkaline reagent test	-
	8b. Zinc test	+
9	Diterpens	+
10	Phenols	
	Ferric chloride test	-
11	Terpenoids	-
12	Cardiac glycosides	
	12a. Legal's test	-
	12b. Keller kellani test	+
13	Carbohydrates	
	13a. Molisch's test	-
	13b. Barfoed's test	-
	13c. Benedict's test	+
14	Phlobatannins	-

Footnote: (+) = present, (-) = absent

As a research conducted by Rakam G. K. and Raja S., 2017, on standardization and phytochemical screening of *Bougainvillea glabra* revealed the presence of alkaloids, steroids, glycosides and flavonoids in chloroform extract; steroids, glycosides, and flavonoids in ethyl acetate extract and phytoconstituents like alkaloids, steroids, glycosides, flavonoids and tannins were shown in methanol extract. From this research one can understand that different solvents give different types of phytochemicals. As per the research conducted by Zahidul Islam *et al*., 2016, phytochemical screening of methanolic extracts of *B. glabra* flower was tested for the presence of some secondary metabolites and their result revealed the presence of alkaloid, flavonoid, reducing sugar, saponin, phenolic compound, tannin, protein and amino acid.

According to Nadia S. *et al.*, 2013, the qualitative analysis of phytochemicals in bougainvillea stem, leaves and flowers extract by using different solvents like distilled water, acetone, chloroform, ethanol and methanol, revealed the presence of alkaloids, flavonoids, glycosides, phlobatannins, saponins, steroids, tannins, and terpenoids. This result revealed the ethanolic extract of the flower part contains glycosides, phlobatannins, saponins, steroids, tannins, and terpenoids. But there were no alkaloids and flavonoids.

The other study conducted by Yolwin J. P. and Merlyn L., 2012, showed the presence of alkaloids, saponins, tannins, reducing sugars and cardiac glycosides and absence of anthraquinones, flavonoids and terpenoids in ethanolic extract of leaves of *Bougainvillea glabra.* There are reports showing that alkaloids, flavonoids, tannins and saponins are the compounds responsible for the antibacterial activities in higher plants (Adudu J. A. *et al.*, 2018). Tannins are used as antiseptic and this activity is due to presence of the phenolic group. Flavonoids contains anti-cancerous, anti-inflammatory, anti-microbial, anti-allergic activity and may, therefore be beneficial in therapeutic roles (Zahidul Islam *et al*., 2016).

ANTIBACTERIAL ACTIVITY TESTS OF *CURCUMA LONGA* EXTRACT AND *BOUGAINVILLEA GLABRA* EXTRACTS

The antibacterial activity test is an important method used in pharmacology to determine the efficacy of novel antimicrobial agents from biological extracts against micro-organisms. This was conducted by doing minimum inhibitory concentration of the extracts and by doing antibacterial susceptibility test. In this study, antibacterial effects of the extracts were conducted against isolated *S. aureus and K. pneumoniae.* The antibacterial activity test of the extracts of these plants were determined using agar disc diffusion method in which these bacterial isolates were dissolved in distilled water to compare with McFarland standards of 0.5×10^8.

Minimum inhibitory effects of turmeric rhizome extracts: Minimum inhibitory concentration is the highest dilution or the least concentration of antimicrobial agent that will inhibit the growth or kill the micro-organisms (Oghenejobo *et al.*, 2017). The minimum inhibitory concentration of ethanolic extract of curcuma longa was conducted by using two-fold serial dilution technique, where different concentrations of the extract were prepared from 3.125 mg/ml to 200mg/ml (Table 3) and (Figure 3 and 4). The result of this study revealed that the MIC of the ethanolic extract of *Curcuma longa* extract against *S. aureus* and

K. pneumoniae were 50mg/ml and 25mg/ml respectively. This indicates the ethanolic extract of curcuma longa is more effective against *Klebsiella pneumonia* when compared to *S. aureus.*

The study conducted by Adudu J. A., 2018, showed that the Minimum inhibitory concentration of aqueous extract of *Curcuma longa* rhizome against *S. aureus* was 12.5 mg/ml.

Table 3: Minimum inhibition concentration (MIC) of ethanolic of Turmeric (*Curcuma longa)* Rhizomes

S. No	Test Organism	Concentration (mg/ml)						
		200	100	50	25	12.5	6.25	3.125
1	*S. aureus*	-	-	-	+	+	+	+
2	*K. Pneumoniae*	-	-	-	-	+	+	+

Foot note: (+) = bacteria have grown; (-) = no bacterial growth

Minimum inhibitory effects of Bougainvillea flower extracts: The minimum inhibitory concentration of ethanolic extract of *Bougainvillea glabra* flower was also performed by using two-fold serial dilution techniques, where different concentrations of the extract were prepared from 3.125 mg/ml to 200mg/ml (Table 4) and (Figure 3 and 4)). This test result revealed that the MIC of the ethanolic extract of *Bougainvillea glabra* extract against *S. aureus* and *K. pneumonia* was 25mg/ml.

Table 4: Minimum inhibition concentration (MIC) of ethanolic extract of *Bougainvillea glabra* flower

S. No	Test Organism	Concentration (mg/ml)						
		200	100	50	25	12.5	6.25	3.125
1	*K. pneumoniae*	-	-	-	-	+	+	+
2	*S. aureus*	-	-	-	-	+	+	+

Foot note: (+) = bacteria have grown; (-) = no bacterial growth

From this table and figure 6 below, we can see that the ethanolic extract of *C. longa* was less sensitive at lower concentrations against *K. Pneumoniae* when compared with ethanolic extract of *B. glabra* extract which shows MIC of 25 mg/ml against both micro-organisms.

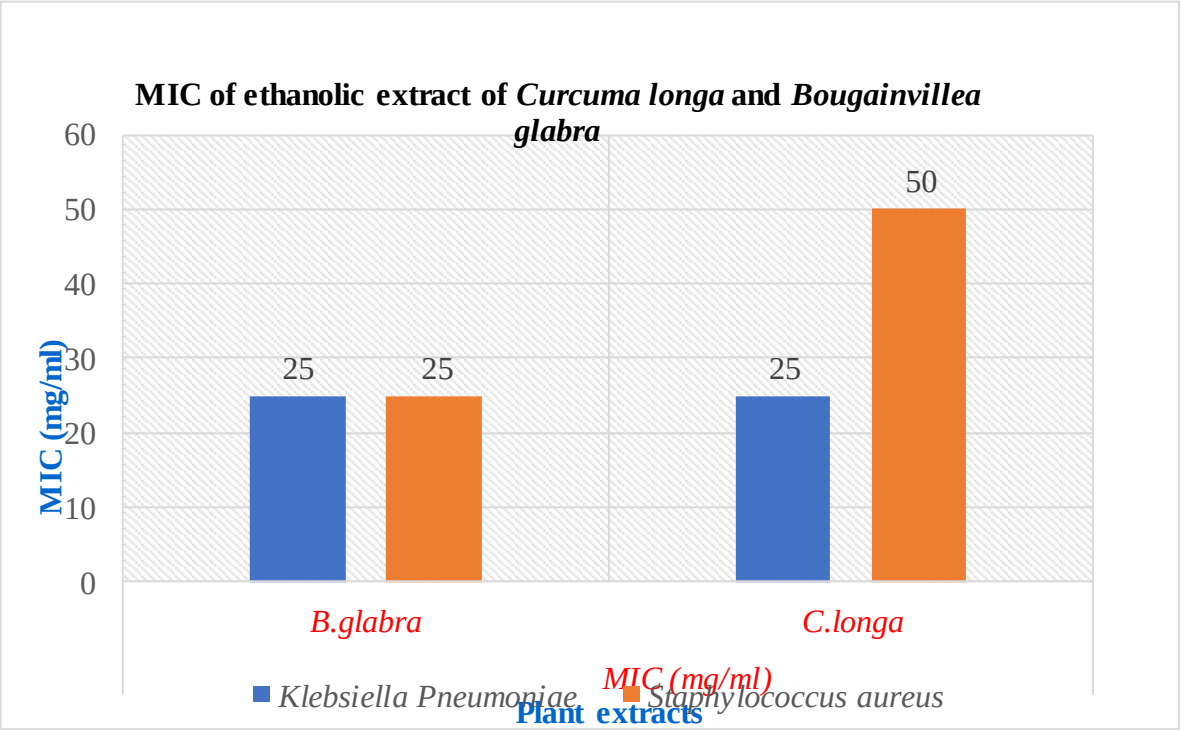


Figure 3: Graphical presentation of MIC of Ethanolic extracts of Curcuma longa rhizomes and Bougainvillea glabra flowers.

Bacterial sensitivity tests of turmeric extracts: The antimicrobial activity of ethanolic extracts of turmeric shown in (table 5) and (figure 5 and 7) indicates that this plant extract is very effective against both *K. pneumonia*e and S. aureus. This antimicrobial susceptibility test was performed by using different concentrations of the extracts (200mg/ml, 100mg/ml and 50 mg/ml against *K. pneumonia* and *S. aureus* and Chloramphenicol was used as positive control. The mean inhibition zone of the extract against *S. aureus* was 23mm,22mm and 11mm at 200mg/ml,100mg/ml and 50mg/ml respectively, while the mean inhibition zone of the extract against *K. pneumonia* was 27mm, 26.5mm and 11.5 mm at 200mg/ml, 100mg/ml and 50mg/ml respectively. The mean inhibition zone of chloramphenicol was 34mm and 35mm against *S. aureus* and *K. pneumonia* respectively.

This result indicates that the ethanolic extract of turmeric inhibited both micro-organisms with the highest average zone of inhibition (27 mm) against *K. pneumonia* at 200mg/ml and least average zone of inhibition (11 mm) against *S. aureus* at 50 mg/ml.

There was the study conducted by Oghenejobo *et al*., 2017, that revealed the ethanolic extract of *Curcuma longa* has the highest zone of inhibition (12 mm) at 200 mg/ml against *S. aureus*. There was a difference between the results of their study and the present study, which may be due to the method followed or the ecological origin of the plant. In their study, they had used well diffusion method for antibacterial susceptibility test, while in this study I have used disc diffusion method.

Table 5: Inhibition zone of ethanolic extract of *Curcuma longa* against *Klebsiella Pneumonia* and *Staphylococcus aureus*

S. No	Test organisms	Repetition	concentration (mg/ml) and inhibition zone (mm)			
1	*S. aureus*		200	100	50	CAF
		rep 1	22	23	10	
		rep 2	24	21	12	
		Mean	23	22	11	34
2	*Klebsiella Pneumonia*	rep 01	28	25	12	
		rep 02	26	28	11	
		Mean	27	26.5	11.5	35

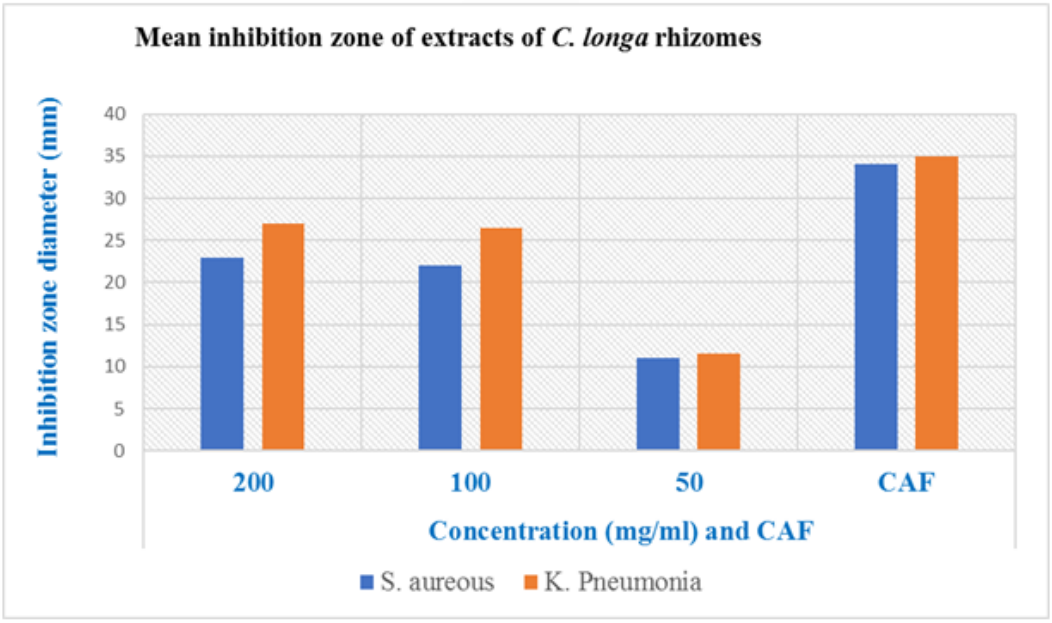


Figure 5: Graphical presentation of Mean inhibition zone of different concentrations of C. longa rhizome extracts against K. pneumoniae and S. aureus and positive control (Chloramphenicol).

According to Kruti K. *et al*., 2017, the methanolic extract obtained from the rhizomes of fresh *Curcuma longa* at 50 mg/ml concentration was studied for its antibacterial activity against different bacterial and fungal species. The study showed as this extract was effective against *S. aureus* with 27 mm zone of inhibition and there was no zone of inhibition when tested against K. pneumonia. They have also indicated as the *In-vitro* testing of turmeric showed that the activity of Turmeric powder against most bacteria was higher than for the crudes drugs (Streptomycin).

Bacterial sensitivity test of bougainvillea flower: The result of antibacterial activity of this study was illustrated in (table 6) and (figure 6 and 7) below. This antimicrobial activity test was also performed by using different concentrations of the extracts (200mg/ml, 100mg/ml and 50 mg/ml against *K. pneumonia* and *S. aureus* and Chloramphenicol was used as positive control. The mean inhibition zone of the extract against *S. aureus* was 22 mm, 24 mm and 21 mm at 200mg/ml,100mg/ml and 50mg/ml respectively, while the mean inhibition zone of the extract against *K. pneumonia* was 28 mm, 28 mm and 25.5 mm at 200mg/ml, 100 mg/ml and 50 mg/ml respectively. The mean inhibition zone of chloramphenicol was 34 mm and 35mm against *S. aureus* and *K. pneumonia* respectively. This antimicrobial activity of ethanolic extract of *Bougainvillea flower* indicates that this plant extract is very effective against *S. aureus* and *K. pneumonia*. The ethanolic extract of bougainvillea flower inhibited both micro-organisms with the highest average zone of inhibition (28 mm) against *K. pneumonia* at 200mg/ml and 100 mg/ml and least average zone of inhibition (21mm) against *S. aureus* at 50 mg/ml.

The antibacterial activity of *B. glabra* flower extract was varied on different types of bacteria. In this study, *K. pneumoniae* showed highest sensitivity with zone of inhibition range from 25.5-28 mm whereas *S. aureus* showed lowest sensitivity with zone of inhibition range from 21-24 mm compared.

Table 6: Inhibition zone of ethanolic extract of Bougainvillea glabra against klebsiella Pneumonia and Staphylococcus aureus

S. No	Test organisms	Repetition	Concentration (mg/ml) and inhibition zone (mm)			
1	*S. aureus*		200	100	50	CAF
		rep 01	20	23	22	
		rep 02	24	25	20	
		Mean	22	24	21	34
2	*K. Pneumoniae*	rep 01	27	27	26	
		Rep 02	29	29	25	
		Mean	28	28	25.5	35

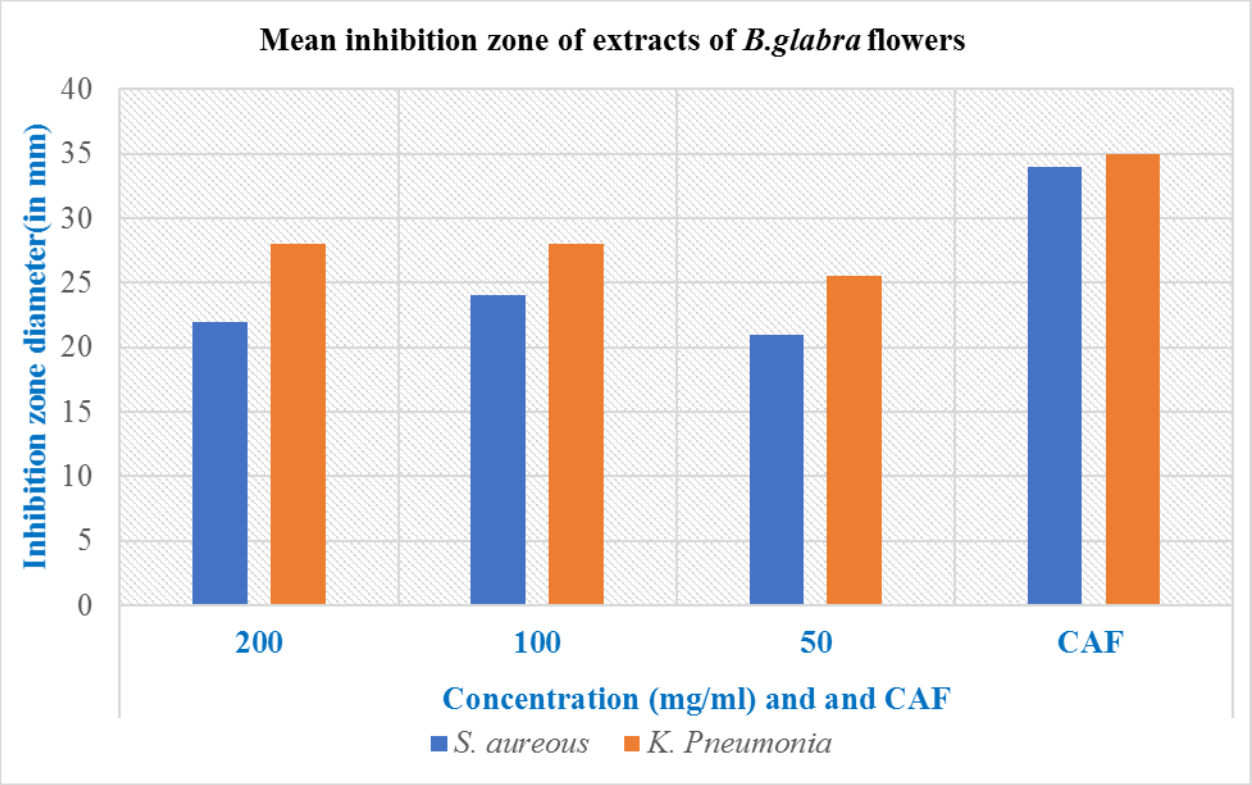
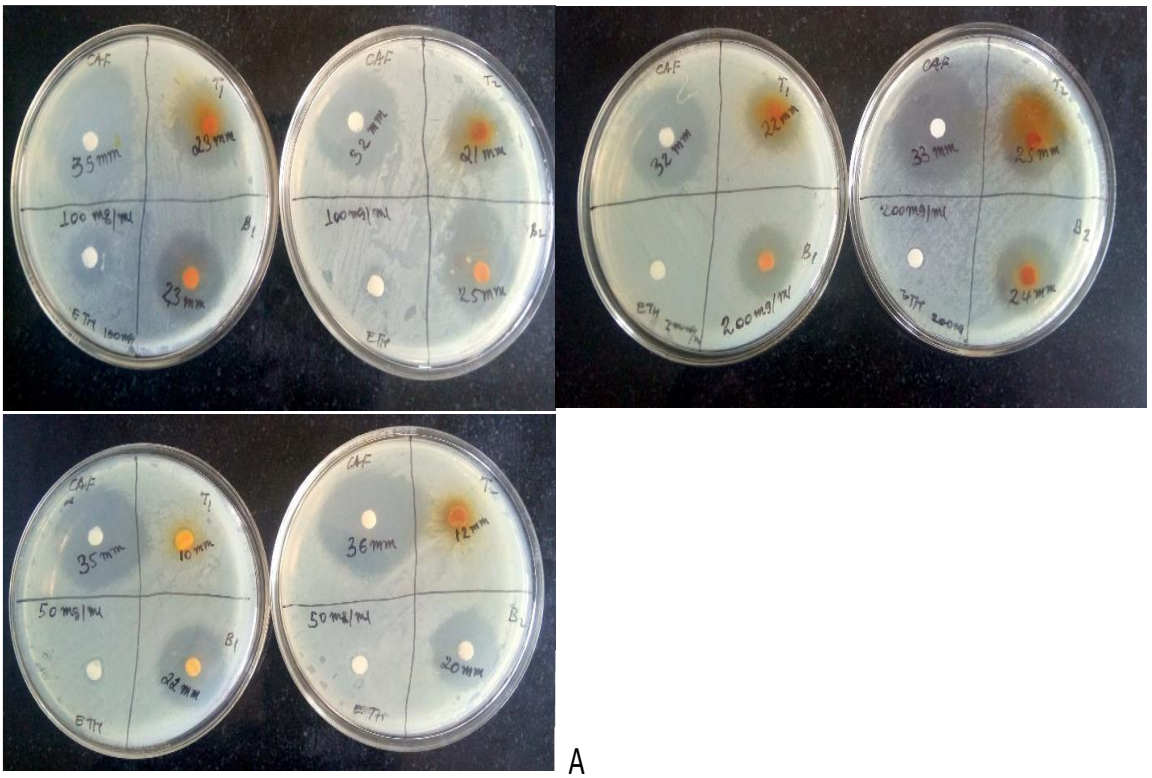
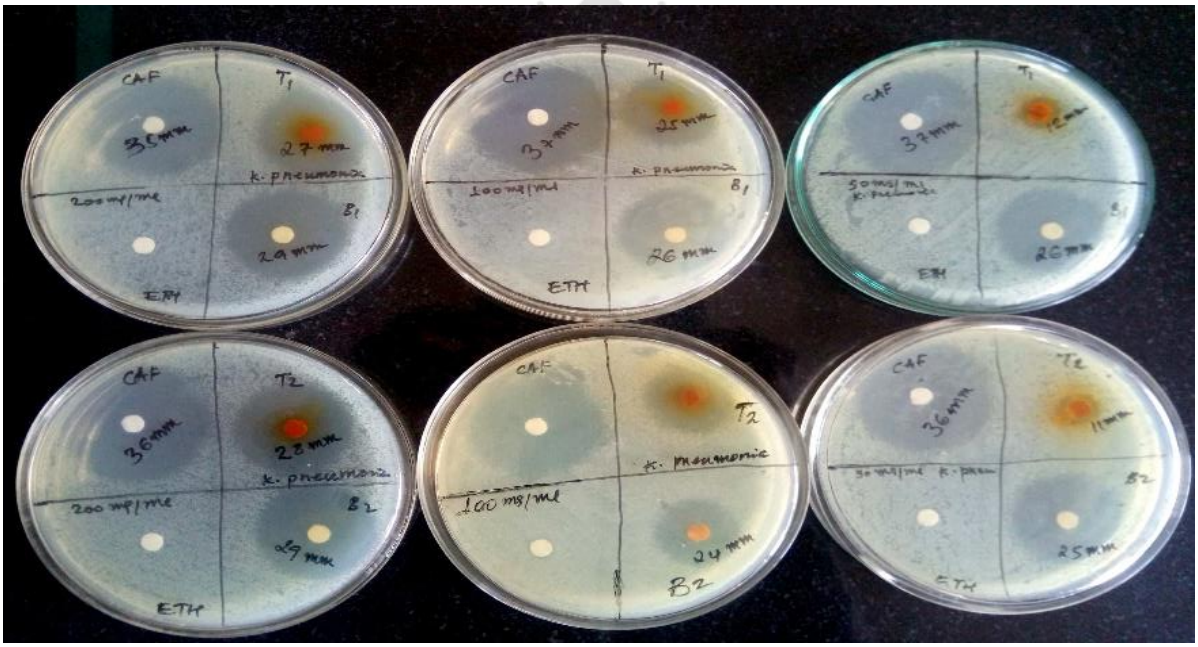


Figure 6: Graphical presentation of Mean inhibition zone of different concentrations of B. glabra flower extracts against K. pneumonia and S. aureus and positive control (Chloramphenicol).



A



B

Figure 7: Results of Zone of inhibition of different concentrations of extracts of B. glabra and C. longa on the test bacteria. A = S. aureus and B= K. pneumoniae. CAF (Chloramphenicol), T (turmeric extract), B (bougainvillea extract), ETH (ethanol).

CONCLUSION AND RECOMMENDATIONS

Scientists are currently focusing on the Phytochemicals to treat numerous ailments affecting mankind. These traditional medicinal plants are often cheaper, locally available and easily consumable and when used as a simple medicinal practice they can form an integral part of complementary or alternative medicines. Even though their effectiveness and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents.

This study was carried out for screening of phytochemical constituents and for studying antibacterial activity and minimum inhibitory concentration of fresh *Curcuma longa* rhizomes and *B. glabra* flowers. Phytochemical screening of ethanolic extract *C. longa* revealed the presence of alkaloids, saponins, anthocyanins, flavonoids, diterpenes, cardiac glycosides, carbohydrates and phlobatannins, while the aqueous extract of *Curcuma longa* revealed the presence of alkaloids, saponins, steroids, tannins, anthocyanins, flavonoids, diterpenes, phenols and cardiac glycosides. Alkaloids, saponins, diterpenes, flavonoids, anthocyanins and cardiac glycosides are present in both ethanolic and aqueous extract of *C. longa*. Phytochemical screening of ethanolic extract *B. glabra* flower revealed the presence alkaloids, saponins, steroids, tannins, flavonoids, diterpenes, cardiac glycosides and carbohydrates. phytochemicals like alkaloids, saponins, steroids, tannins, flavonoids, diterpenes, cardiac glycosides and carbohydrates are present in both ethanolic extracts of *C. longa* rhizomes and *Bougainvillea glabra* flower. Both the ethanolic extracts of *C. longa* rhizome and *B. glabra* flower found to be very effective against the bacterial isolates (*S. aureus and K. Pneumoniae*). From the whole study, it can be concluded that antimicrobial activity of the extracts of medicinal plants are dependent upon some parameters such plant material used, methods employed, growth media and most importantly micro-organisms tested. The solvent and extraction systems can also modify the final results.

The result of the present study shows that the extracts of *Curcuma longa* rhizomes and *Bougainvillea glabra* flower contains potential antimicrobial components that may be a great use for the development of medicines by pharmaceutical industries as therapy against various diseases. Some of the secondary metabolites detected in this plant extract may be responsible for the antibacterial activity observed and thus it justifies their traditional use as medicinal plants for the treatment of various bacterial and fungal diseases.

Generally, medicinal plants possess valuable medicinal properties and are promising choice over modern synthetic drugs. They are believed to show minimum or no side effects and are considered to be safe. But, most of their advantages are still confined to tribal areas because of raw knowledge and absence of proper scientific studies. Therefore, the following recommendations will be provided:

- The medicinal benefits of the medicinal plants should be used properly and scientifically and reach the larger populations to the world.

- For the useful application of the plant part in modern medicine, the Physico-chemical and detail phytochemicals is very important.

- Correct knowledge of crude drugs is very important aspect in preparations, safety and efficacy of the herbal products.

- The *in-vivo* studies of the extracts of these plants should be properly carried out to ensure the selection of effective microbial substances with low side effects and adverse effects.

- The combined effects of these plants should be also studied with each other and with other medicinal plants.

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