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ANALYSIS OF PHYTOCHEMICALS AND ANTIBACTERIAL POTENTIAL OF *LONICERA JAPONICA* THUNB.

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ABSTRACT

Lonicera japonica (Honeysuckle) is one of the oldest medicinal herbs in known history. *Lonicera japonica* is an ingredient of herbal tea and has been known thousands of years for its cooling and detoxification effects. According to the Natural Medicines Comprehensive Database, *Lonicera japonica* is taken orally for digestive disorders, enteritis, dysentery, urinary disorders, diabetes, rheumatoid arthritis, malignant tumours etc. In the present study ethanolic extract was obtained from leaves and flowers of *Lonicera japonica* and phytochemical analysis was done to screen alkaloids, phenols terpenoids and flavonoids.Antibacterial activity was carried out by using bacterial strains *Staphylococcus aureus* MTCC 3160*, Bacillus subtilis* MTCC 736*, Vibrio cholerae* MTCC 3906*, and Salmonella typhi* MTCC 3220.Extracts of leaf and flower in various dilutions were found to have antibacterial activity against tested organisms.Chloroform and Ethanol leaf extracts of *Lonicera japonica* has shown high degree of inhibition (DIZ- 22mm and 22 mm) against test organisms. It is found that there was no antibacterial activity exhibited by some concentration. Ethanol and Methanol flower extracts of *Lonicera japonica* has shown high degree of inhibition (DIZ 21mm-23mm) against the test organisms and our results supported the usage of *Lonicera japonica* in traditional medicine.

KEYWORDS: ***Lonicera japonica* , Phytochemicals, Antibacterial activity.**

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INTRODUCTION

Herbal medicine is the world's most ancient form of medicine as it is evident from the fact that every ancient civilization used plants for healing and in many cultures, herbal knowledge was said to have been handed down from the God. Plants have always been the principle form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in count with the body's own defence [1.] There is a wide spread belief that green medicines are healthier and more harmless and safer than synthetic one. Antibacterial properties of various plant parts have been well documented for some of the medicinal plants for the past two decads [2] . Medicinal and aromatic plants are rich in bioactive compounds which could be an alternate way to combat various diseases even against some bacteria which are becoming resistant to certain synthetic medicines [3.] Nature has been a source of medicinal agents, and an impressive number of modern drugs have been isolated from natural sources, many of which based on their use in traditional medicine[4.] The world health organization (WHO) reported that about 80% of the world population depend mainly on traditional medicine and the traditional treatment involve mainly the use of the plant extract[5,] for primary health care, because of their safety, efficacy, compatibility and lesser side effects [6]. Traditional medicine in developing countries uses a wide variety of natural products in the treatment of common infections[7],[8]. Herbal medicines are an important part of the culture and traditions of African people. In India, a large number of medicinal plants occur in the wild state.Plants may offer a new source of antmicrobial agents for use as they produce secondary metabolites [9.]Antimicrobial activities of plant extracts form the basis for many applications, including raw and processed food preservation,pharmaceuticals, alternative medicines and natural therapies [10.] Since ancient times, plants have been model source of medicine as they are reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many human disorders. Today, most pathogenic organisms are becoming resistant to antibiotics. There has been increasing interest in the development of new types of effective natural and non toxic plant based antimicrobial compounds. The extracts of many plants have become popular in recent years and attempt to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications. Many studies have reported that phenolic compounds contribute to their antioxidant and pharmaceutical properties. There has been increasing interest in discovering new natural antimicrobials. The main objective of this study was to determine the effect of phytochemicals of *Lonicera japonica* under *in- vitro* conditions against the test microorganisms. These results were compared with the potent antibiotics. A number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants [11.] Essential oils (EOS) obtained from plant material have been used for centuries as antimicrobial agents. *Lonicera japonica* Thunb.(Honeysuckle) belongs to family caprifoliaceae is one of the oldest medicinal herbs in known history. Sometimes referred to woodbine was once used widely to treat urinary complaints, asthma and during child birth. However, in traditional Chinese medicine, it has been used medicinally for thousands of years. In China, South East Asia and Japan, many herbal medicine for colds or flu usually includes this botanical.It is also used as laxative, for colds and other respiratory tract infections, pneumonia, encephalitis, fever, inflammation, swelling, viral and bacterial infections.*Lonicera japonica* is traditionally used as a medicinal plant[12] in folk medicine in Northern Russia, China and Japan. The plant *Lonicera japonica* is a species of honeysuckle native to Eastern Asia including Japan, Korea China and Tiwan, which is a major invasive species in North America. Fruits of *Lonicera japonica* constitute one of the most important sources of potential health supporting phytochemicals in human diet[13.] Fruits and other herbals are a rich source of ascorbic acid and phenolic

compounds like phenolic acids, anthocyanins, proanthocyanidins and other flavonoids. These compounds prove beneficial to human health[14, 15]. Their biological activities include:protection against the incidence and mortality rates of cancer[16], and as well as they have antitumorigenic[17,]antimicrobial[18,] anti-allergic[19,] and antimutagenic properties. *Lonicera japonica* possesses many other biological functions including hepatoprotective, cryoprotective,
antimicrobial,antioxidative,antiviral and anti-inflammatory[20].The major parts of this plant have medicinal properties,flower buds have anticancer and anti-inflammatory properties[21], leaf has antioxidant and tyrosinase inhibition properties [22.] A few species are used in idegenous medicine as antipyretic, stomachic,diuretic and antidysentric in India [23,24.]. This plant is also favoured because of their extreme hardiness to the cold. *Lonicera* plants have strong tolerance to severe low-temperature conditions.They can survive at temperature of -46^{0C} without damage[25,26].The freezing tolerance of perennial plants increases in winter to prevent injury under cold conditions. It is known as cold acclimation and seems to be connected with the content and accumulation of specific type of carbohydrates and proteins.The raffinose family of oligosaccharides have been shown to be potential cryoprotectants because of their capacity to modify the freezing behaviour of aqueous solutions[26,27]. Also the presence of galactose-containing oligosaccharides strongly correlates with increase in freezing, as well as dessication tolerance [26.]

MATERIALS AND METHODS

Plant material

Matured leaves and flowers of *Lonicera japonica* were collected in morning hours from the plants maintained in the Botanical garden. The plant was identified on the basis of morphological features with the help of flora and the database present in the library, Karnatak University Dharwad.

Preparation of extract

Fresh mature leaf samples(100g) were cleaned and naturally dried in shade at room temperature for 10 days, and ground into a fine powder. Dried plant samples were further air dried in a ventilated oven at 40^{0C} for 24hrs., then fine powder is passed through a sieve. Powdered sample (10g) was extracted with 95% ethanol at room temperature in a soxhlet apparatus with 300ml of solvent for 24hrs. The extract was filtered through a Millipore filter with a 0.45mm nylon membrane. The extract was concentrated under reduced pressure by a vaccum rotary evaporator to yield an ethanol extract. The sample extracted successively with chloroform,acetone and methonol respectively. Solvents (Analytical grade) for extraction were obtained from commercial sources.The samples were stored at 4^{0C} until use.

Isolation of the essential oil

The plant materials (flower) were dried in the shade at room temperature for 10 days. The air-dried flower parts (300 g) of *Lonicera japonica* were subjected to hydrodistillation for 3hrs. using a Clevenger type apparatus.The oil was dried over anhydrous Na_2SO_4 preserved in a sealed vial at 4^{oC} until further analysis.

Figure 1
Aerial parts of Lonicera japonica

Test microorganisms
The microbial strains i.e *Staphylococcus aureus* MTCC 3160*, Bacillus subtilis* MTCC 736*, Vibrio cholerae* MTCC 3906*,* and *Salmonella typhi* MTCC 3220 used in the present investigations were obtained from IMTECH, Chandigarh. The strains were cultured at 37^{0C} on plate count agar medium (PCA). Bacterial strains were maintained in sterile Glycerine stubbs at -20^{0C} **.**

Antibacterial assay
An agar-well diffusion method was employed for determination of antibacterial activities. The freeze-dried extract samples were sterilized by filtration. Bacteria were suspended in sterile water and diluted. The bacterial suspension (100µl) was spread on to the surface of PCA (plate count agar) medium. Wells (4.6mm in diameter) were made from the agar with a sterile borer and 60µl extract solution was delivered into them. Gentamicin is used as positive reference standard to determine the sensitivity of each microbial species tested. The inoculated plates were incubated at 35^{0C} for 24hrs. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters. All tests were performed in triplicates.

Determination of total phenolic content
Total phenolic content was estimated using the Folin-Ciocalteu Colorimetric method[28]. 0.2ml of appropriately diluted extract is oxidized for 4mins with 1ml of 0.5M Folin-Ciocalteu reagent and then the reaction was neutralized with saturated sodium carbonate (75g/l) 1ml. The absorbance of the resulting blue color was measured at 760nm with a spectrophotometer after incubation for 2hrs. at room temperature. Quantification was done based on a standard curve of gallic acid. Results were expressed as gram of gallic acid equivalent (GAE) per 100g of dry weight (DW). All tests were performed in triplicates.

Minimum inhibitory concentration (MIC)
Minimum inhibitory concentration (MIC) of essential oil and ethanolic extracts was tested by two –fold serial dilution method [29.] The test samples of oil and ethanolic extracts were first dissolved in ethanol, and incorporated into LB broth medium to obtain a concentration of 200µg/ml and serially diluted to achieve 1000,500,250,125,62.5 and 31.25 µg/ml. The final concentration of ethanol in the culture medium was maintained at 0.1% (v/v). A 10 µl standardised suspension of each tested organism (10^7 CFU/ml approximately) was transferred to each tube. The control tubes containing only bacterial suspension, were incubated at 37^0C for 24 hrs.The lowest concentration of the test samples, which did not show any growth of tested organism after microscopic evaluation, was determined as the MIC.

Phytochemical screening

Chemical tests were carried out with ethanol and methanol extracts of *Lonicera japonica* using standard procedures to identify the constituents as described by [11,30,31].

Test for Alkaloids

About 0.2 g of the extract of *Lonicera japonica* was warmed with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragendroffs reagent were added. Orange red precipitate indicates the presence of alkaloids (Table.1).

Wagner's test

A fraction of extract was treated with Wagner's reagent (1.27 gm of iodine and 2 gm of potassium iodide in 100 ml water) observe the formation of reddish brown colour precipitate indicates presence of alkaloids.

Liebermann's test

The extract was heated with sodium nitrite, added H_2SO_4 solution diluted with water and exess of dilute NaOH was added and observed for the formation of deep red or green or blue colour indicates presence of alkaloids.

Flavonoids

H_2SO_4 test

To 1 ml of sample 2 ml of H_2SO_4 was added, mixed well, formation of yellow colour was regarded as positive for the presence of flavonoids.

NaOH test

A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow-orange colour was regareded as positive for flavonoids.

Terpenoids

Salkowski test

0.2g of the extract of the leaf sample was mixed with 2 ml of chloroform [$CHCl_3$] and concentrated H_2SO_4 (3 ml) carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

Liebermann-Burchard test

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark green colour shows the presence of terpenoids.

Phenols

Ferric chloride test

1 ml of extract was treated with 0.5 ml of 1% $Fecl_3$ prepared in chloroform gives intense red, blue, purple or green colour indicates presence of phenol.

Steroids

10 ml of the ethanol extract was evaporated to dry mass and dissolved in 0.5 ml of solvent to this added 0.5 ml of aetic anhydride and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds [31,32].

Glycosides

1 gm of sample was added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5 ml of water was added to the other beaker. The two beakers were heated for 3-5 min and he contents are filtered into labelled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 minutes. The presence of reddish precipitate in the acid filtrate and absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides.

RESULTS

Phytochemical screening

The phytochemical screening showed the presence of alkaloids, terpenoids, flavonoids,steroids and phenols in the extracts. The phytochemicals are known to have antimicrobial activity as well as other physiological activities Table 1.

In – vitro antibacterial assay

According to the results given in Table 2. and 3., the leaf and flower extracts exhibited a potent inhibitory effect against *S.aureus, V.cholerae, B.subtilis,* and *S.typhi,* chloroform flower extract did not inhibit the growth of any bacteria tested at used concentration.

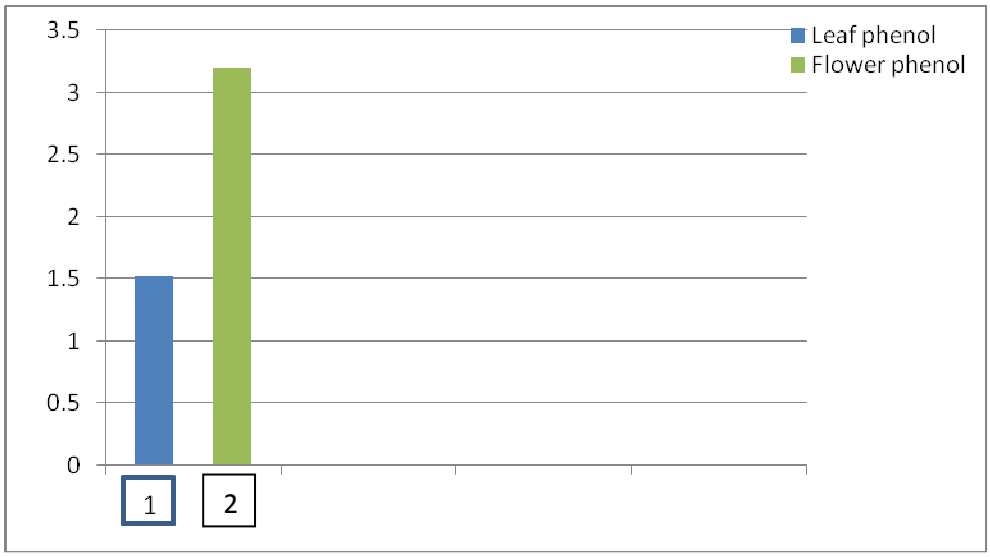

Figure 2
Concentration of phenolic compounds in leaf and flower of Lonicera Japonica

Table 1
Phytochemical constituents present in ethanolic leaf and flower extract of Lonicera Japonica

Sl.NO.	Phytochemicals	Ethanolic Extract
1	Alkaloids	+
2	Terpenoids	+
3	Phenols	+
4	Flavonoids	+
5	Steroids	+
6	Glycosides	–

Phytochemical analysis
Table.1 + Presence of phyochemicals - Absence of phytochemicals

Leaf extracts

Antibacterial sensitivity of leaf extracts was observed using agar well diffusion method by measuring the diameter of zone of growth inhibition. The results were shown in the Table 2.Gram's Positive and Gram's negative bacteria at a concentration of 50, 100µg/ml exhibited resistance to all extracts. These pathogens are sensitive at a concentration of 400 and 800µg/ml. Chloroform and Ethanol leaf extracts of *Lonicera japonica* has shown high degree of inhibition (DIZ- 22mm and 22 mm) against test organisms. It is found that there was no antibacterial activity exhibited by some concentration of leaf extracts (Table 2), where as there were some good antibacterial activities found from 400 to 800µg/ml against *S.aureus, V.cholerae, B.subtilis, and S.typhi*, Fig.3 and 5. Acetone extract even at 100µg/ml concentration show growth inhibition. The size of the zone increases as the concentration increased indicating concentration dependent effect. The Chloroform extract at low concentration had exhibited no significant antimicrobial activity. Some extracts at lower concentration had no significant antimicrobial activity

Flower extracts

The results were shown in the Table 3. Gram's positive and Gram's negative bacteria at concentration of 50µg 100µg 400µg for Ethanol, Acetone and Methanol extracts exhibited resistance to all extracts of flowers. These pathogens are sensitive at concentration of 800 µg/ml for chloroform and 400µg and 800 µg for Ethanol, Acetone and Methanol flower extracts of. *Lonicera japonica* . Ethanol and Methanol flower extracts has show high degree of inhibition (DIZ 21mm-23mm) Fig.4 and 6., against the test organisms. Some extracts at lower concentration had no significant antimicrobial activity. Chloroform extract had exhibited no significant antimicrobial activity. It is found that there was no antibacterial activity exhibited by some concentration where as there were some good antibacterial activities

found from 400µg-800µg against *S.aureus, V.cholerae, Bacillus subtilis,* and *S.typhi* for Ethanol, Acetone and Methanol extracts (Table 3.). However these antibacterial activities against these bacteria were shown to be equal or less active when compared to the control Gentamicin. This differential action of antibacterial property of flower extracts of *Lonicera japonica* may be depending upon the active compounds specific for bacteria. The effectiveness of the plant was not due to one constituent, but to the combined action of other chemical compounds involved in it [33]. Bioactive compounds like alkaloids, flavonoids, triterpinoids, thymol and other phenolic compounds are classified as antimicrobial compounds[34]. The present study shows the effect of the extracts on pathogenic bacterial agents which really shows the presence of biological compounds.These observations suggest that the bioactive compounds responsible for the activity can be extracted through the organic solvent medium. The potential developing antimicrobial drugs from higher plants appears rewarding, as it will lead to the development of phytomedicine to act against microbes. Therefore such screening experiments form a primary platform for further phytochemical and pharmacological studies that may open the possibility of finding new clinically effective antibiotic compounds.

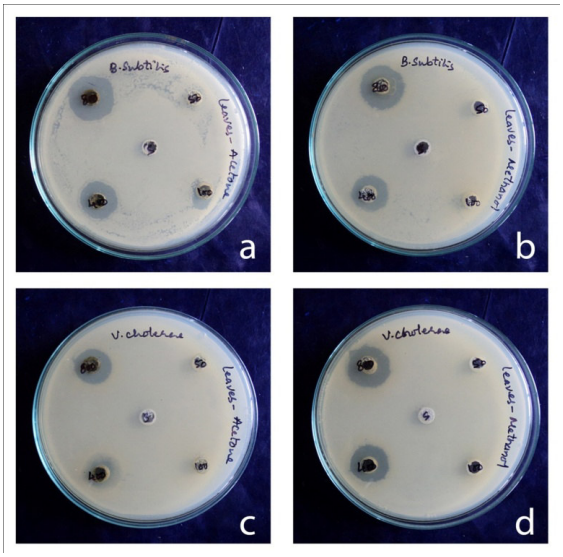

Figure 3
Antibacterial activity of different leaf extracts of L.japonica against test organisms

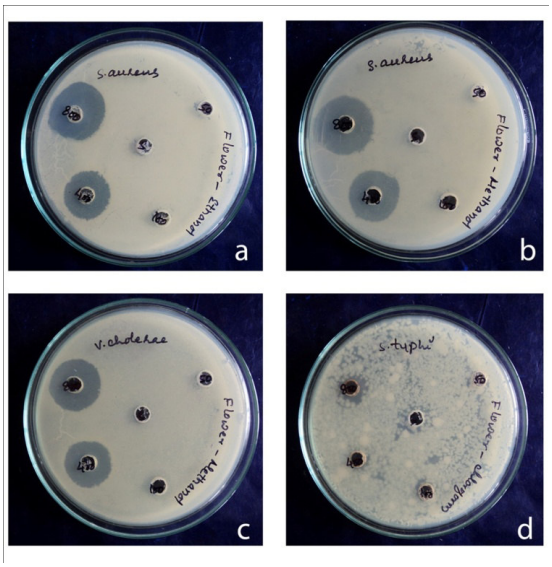

Figure 4
Antibacterial activity of different flower extracts of L.japonica against test organisms

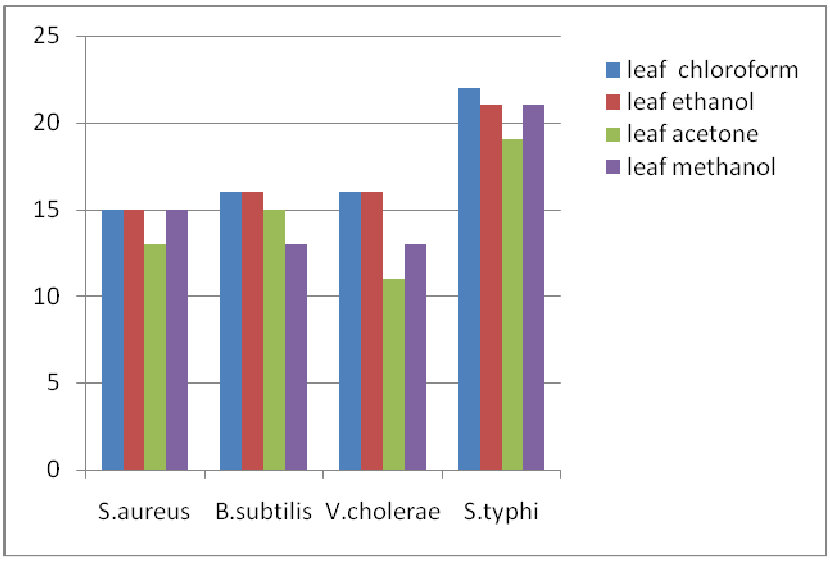

Figure 5
Antimicrobial activity of L. japonica leaf extracts

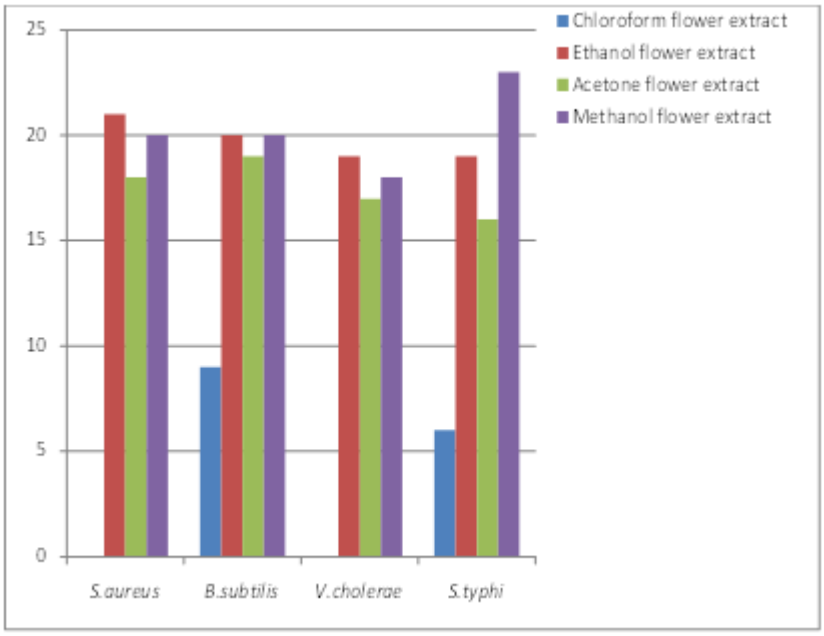

Figure 6
Antimicrobial activity of L. japonica flower extracts

Table 2
Antimicrobial activity of Lonicera japonica Leaf extracts

Test organism	Extraction mecium	Zone of inhibition (mm) in various cencentaions				
		50µg/ml	100µg/ml	400µg/ml	800µg/ml	MICµg
S-aureus +ve	Chloroform	-	-	12	15	400
	Ethanol	-	-	12	15	400
	Acetone	-	-	11	13	400
	Methanol	-	-	12	15	400
		50 µg	100 µg	400 µg	800 µg	MIC µg
	Gentamicin	18	21	27	34	25
B- subtilis +ve	Chloroform	-	-	13	16	400
	Ethanol	-	-	13	16	400
	Acetone	-	3	11	15	100
	Methanol	-	-	10	13	400
		50 µg	100 µg	400 µg	800 µg	MIC µg
	Gentamicin	10	15	22	25	25
V- cholerae -ve	Chloroform	-	-	14	16	400
	Ethanol	-	-	14	16	400
	Acetone	-	-	4	11	400
	Methanol	-	-	11	13	400
		50 µg	100 µg	400 µg	800 µg	MIC µg
	Gentamicin	15	21	23	27	25
S-typhi -ve	Chloroform	-	-	18	22	400
	Ethanol	-	-	18	21	400
	Acetone	-	-	13	19	400
	Methanol	-	-	15	21	400
		50 µg	100 µg	400 µg	800 µg	MIC µg
	Gentamicin	13	21	25	27	25

Values are the average of triplicate, includes the cup diameter (5mm), - no zone of inhibition

Table 3
Antimicrobial activity of Lonicera japonica flower extracts

Test organism	Extraction medium	Zone of inhibition (mm) in various cencentaions				
		50µg/ml	100µg/ml	400µg/ml	800µg/ml	MICµg
S-aureus +ve	Chloroform	-	-	-	-	NF
	Ethanol	-	-	15	21	400
	Acetone	-	-	11	18	400
	Methanol	-	-	15	20	400
		50 µg	100 µg	400 µg	800 µg	MIC µg
	Gentamicin	18	21	27	34	25
B- subtilis +ve	Chloroform	-	-	-	9	800
	Ethanol	-	-	14	20	400
	Acetone	-	-	11	19	400
	Methanol	-	-	19	20	400
		50 µg	100 µg	400 µg	800 µg	MIC µg
	Gentamicin	10	15	22	25	25
V- cholerae -ve	Chloroform	-	-	-	-	NF
	Ethanol	-	-	15	19	400
	Acetone	-	-	14	17	400
	Methanol	-	-	15	18	400
		50 µg	100 µg	400 µg	800 µg	MIC µg
	Gentamicin	15	21	23	27	25
S-typhi -ve	Chloroform	-	-	-	6	800
	Ethanol	-	-	15	19	400
	Acetone	-	-	11	16	400
	Methanol	-	-	21	23	400
		50 µg	100 µg	400 µg	800 µg	MIC µg
	Gentamicin	13	21	25	27	25

Values are the average of triplicate, includes the cup diameter (5mm), - no zone of inhibition

Minimum inhibition concentration (MIC)

Antimicrobial efficiency was quantified using the minimum inhibitory concentration (MIC), as an indication of non- detected group of microorganisms. The hot extracts were found to be more effective, when compared with

cold extracts of leaf and flower of *Lonicera japonica*. As shown in Table 2. and 3., the MIC values for the acetone extract were found more susceptibile to *B.subtilis* (100µg/ml) than those of *S.aureus, V.cholerae* and *S.typhi.* On the other hand MIC values of chloroform, ethanol and methanol leaf extracts against the tested bacteria were about 400µg/ml. For flower extracts MIC value for chloroform extract is 800 µg/ml and values of acetone, ethanol and methanol against the tested bacteria were about 400 µg/ml.

Total phenolic content of leaf and flower

Total phenolic content of leaf is 1.51mg/gm and flower is 3.20mg/gm respectively.The most active constituents (essential oils) of many spices having wide spectra of antimicrobial activity are aromatic phenolic compounds, such as thymol, carvacrol, eugenol and cinnamic aldehyde [35,36] Fig.2.

DISCUSSION

Since ancient times aromatic plant extracts have been in use for many purposes, such as food,drugs and perfumery.Historically many plant oils and extracts have been reported to have antimicrobial properties.Also, the renewal of interest in food industry and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required.Essential oils, which are odorous and volatile products of plant secondary metabolism, have a wide application in food flavouring and preservation industries.In recent years, several researchers have reported that the oxygenated mono-or sesquiterpenes, and mono-sesquiterpene hydrocarbons are the major components of essential oils which exhibit potential antibacterial activities [37.] Also, the results of the antibacterial screening showed that leaf extracts and essential oil of flower have potential activity against tested micro-organisms. The antibacterial activity of different extracts could be attributed to the presence of some bioactive phenolic compounds in *Lonicera japonica* and other phytochemicals such as flavonoids, alkaloids, terpenoids and steroids, and these findings are in agreement with a previous report [20]. Individual essential oil components, many of them being approved food flavourings imparts certain flavour to foods. It has been shown that phenolic compounds were abundant in leaves, while oxygenated sequiterpenes, alcohols and phenolics were the main constituents in the flowers [38] .The use of essential oils may improve food safety and overall microbial quality. Phenolic compounds have been found in extracts of *Lonicera japonica* leaves [39.] And recent studies have suggested that they may possess multiple therapeutic functions for various human diseases including liver cancer[40.] In recent years phenolic compounds have gained increasing interest because they exhibit beneficial health effects due to their potential antioxidant and pharmaceutical properties[41.]The ethanolic leaf extracts and flower oil of *Lonicera japonica* with high antibacterial activity selected in this study could be a potential source for inhibitory substances against pathogens and may be candidate for using in food or food processing system and herbal medicine. The presence of the phytochemical constituents such as alkaloids, flavanoids, terpenoids and phenolic compounds have been reported to be important compounds in many other medicinal plants [42, 43.] In the present study, among the four bacteria tested Gram positive bacteria *B. Subtilis* was the most sensitive to the extracts. In general the plant antibiotic substances appear to be more inhibiting to Gram positive bacteria than Gram negative bacteria. Unlike Gram positive bacteria, lipopolysaccharide layer along with protein and phospholipids are the major components in the outer surface in Gram negative bacteria [44.]The highest sensitivity of the bacteria may be due to its cell wall structure and outer membrane[45.] A possible explaination for these observations may lie in the significant differences in the outer layers of Gram negative and Gram positive bacteria. Gram negative bacteria posses an outer membrane and a unique periplasmic space not found in Gram positive bacteria. It is often reported that Gram negative bacteria are more resistant to essential oils. The hydrophilic cell wall structure of Gram negative bacteria is constituted essentially of a lipopolysaccharide that blocks the penetration of hydrophobic oil and avoids the accumulation of essential oils

in target cell membrane [46]. The results emphasized the importance of phenolic compounds of *Lonicera japonica* extracts and also indicated that phenolic compounds significantly contributed to their antibacterial activity. The most active constituents (essential oil) of many aromatic plants having wide spectra of antimicrobial activity are aromatic compounds such as thyme, eugenol in clove, cinnamon and cinnamic aldehyde in cinnamon [36.] This suggested that phenolic compounds might significantly contribute to their antibacterial activity. The present study also demonstrated that extract of *Lonicera japonica* contain high levels of phenolics and possessed strong antibacterial activity.

CONCLUSION

The observed *in-vitro* biological activity of these extracts are to be confirmed by bioassay guided isolation and identification of the active phytochemicals in the extracts. It is important to validate the various extracts of the *Lonicera japonica* by *in-vitro* methods. Scientific validation of herbal medicine by the ethanopharmacological research is a primary concern in developing countries. Various crude extracts of the plant exhibited potential bactericidal properties therefore it can be used as antibacterial supplement in the development of new therapeutic agents. In conclusion, the results of our study suggest the possibility of using the oil or organic extracts of *L.japonica* as natural antimicrobials in the food industry because they possess strong antibacterial activities. The use of natural antibacterial agents will be suitable for applications in the food industry because this plant is not toxic, and it has been consumed by mankind for centuries without showing any signs of toxicity. A further research is needed to evaluate the bio-active compounds present in various organic extracts of *L. japonica* and also to obtain information regarding the practical effectiveness of essential oil or extracts to prevent the growth of food borne and spoilage microbes under specific application conditions.

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Conflict of Interest

Conflict of Interest declared none.

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