

Comparative studies on Phytochemical and Antibacterial Analysis of *C. sinensis* and *C. assamica*

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ABSTRACT

Medicinal plants are the prominent source of therapeutic agents used to prevent the human pathogenic bacteria. In the present investigation, comparative analysis on antibacterial activity of *Camellia sinensis* and *Camellia assamica* was done against human pathogenic bacteria viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Klebsiella pneumoniae* and *Salmonella typhi*. The extraction of plant leaves in different solvents like petroleum ether, chloroform and acetone were done by using Soxhlet apparatus and their antibacterial activity were analyzed by well and disc diffusion method against both Gram-positive and Gram-negative bacteria. The acetone soluble extract resulted in the highest zone of inhibition against the test pathogens. The chloroform and acetone soluble extracts of *C. assamica* possessed the potential antibacterial activity as compared with *C. sinensis*. The acetone soluble extract posed the highest inhibitory effect on the growth of six bacterial species viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Klebsiella pneumoniae* and *Salmonella typhi*. Tannins, saponins, terpenoids, alkaloids, steroids, reducing sugars, flavonoids and cardiac glycoside were present in different solvent-soluble extracts in two different species. Leaf based herbal preparations are cheaper and has no side-effects on human health. Therefore, herbal preparation should be used as medicine to cure the diseases caused by pathogenic and multi-drug resistance bacteria.

Keywords: *C. sinensis*, *C. assamica*, Petroleum ether, Chloroform, Acetone.

INTRODUCTION

Diseases are the most common cause of mortality. Foodborne diseases are increasing globally day-by-day. In constant, the microorganisms are capable of developing resistance against many antimicrobial agents. The medicinal plants play a significant role as potential antimicrobial agent.

Tea contains about 4000 bioactive compounds but one-third part is contributed by polyphenols. Polyphenols are the benzene ring compound with hydroxyl groups. More amount of flavonoids (20-30% of total dry weight than non-flavonoids are found in green tea (Sumpio *et al.*, 2006). The major flavonols found in tea are myricetin, quercetin and kaempferol. Another bioactive constituents present in tea leaves are flavonoids, alkaloids, tannins and phenolic compounds. Most of the plants are known to have many biological activities due to presence of phytochemical compound (Singh and Singh, 2017). Some study revealed that the polyphenolic compounds present in tea involves in prevention of cancer and cardiovascular diseases (Arts and Hollman., 2005; Lambert *et al.*, 2005; Joseph *et al.*, 2005). Polyphenols present in tea play a significant role to inhibit the growth of bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Clostridium perfringens*, *Vibrio parahaemolyticus*, *Bacillus cereus*, etc. (Toda *et al.*,

1989). These polyphenols inhibit the growth of food-borne pathogenic bacteria but it is ineffective against lactic acid bacteria. Polyphenols of tea shows effective result against both Gram-positive and Gram-negative bacteria but Gram-positive bacteria are more sensitive than Gram-negative bacteria. (Ray *et al.*, 2004).

Green tea bears 30 – 40% polyphenols in water extract but black tea about 3 - 10 % polyphenols. Epicatechin gallate (ECG), epigallocatechin gallate (EGCG), epigallocatechin (EGC), but Epicatechin (EC) is more significant antioxidant compound rather than other chemical compounds (Diane *et al.*, 2007). EGCG is the most abundant chemical component in tea extract and the actively powerful in biological activity. The concentration of polyphenols in fresh tea leaves remains approximately 30% of the dry weight (Archana and Abraham, 2011). A large number of pharmacological studies and epidemiological studies show that green tea has potent antioxidant effects (Chan *et al.*, 2007).

Catechins present in tea extract are very effective against different bacteria viz., *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Bacillus subtilis*, and *Staphylococcus aureus*. It shows effect on bacterial membrane by producing hydrogen peroxide and by alters the permeability of bacterial cell wall (Ferrazzano *et al.*, 2011).

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Table 1: Antibacterial activity of *Camellia* species against bacterial strains based on agar well diffusion method.

Bacterial Species	Zone of inhibition (mm)*					
	<i>C. sinensis</i>			<i>C. assamica</i>		
	Petroleum ether	Chloroform	Acetone	Petroleum ether	Chloroform	Acetone
<i>E. coli</i>	-	-	21.6±0.33	-	17±0	22.6±1.20
<i>S. aureus</i>	-	-	26.3±0.88	-	23.3±0.33	31.3±1.33
<i>L. monocytogenes</i>	-	-	23.6±1.20	-	22±0	24.6±0.88
<i>P. aeruginosa</i>	-	-	24±0	-	27±0.57	31.3±2.33
<i>K. pneumoniae</i>	-	-	23.3±1.33	-	13±0	12.3±0.33
<i>S. typhi</i>	-	-	19±0.57	-	14±0	23.6±0.33

*Data are the means of three replicates ± standard error. (-) = No activity.

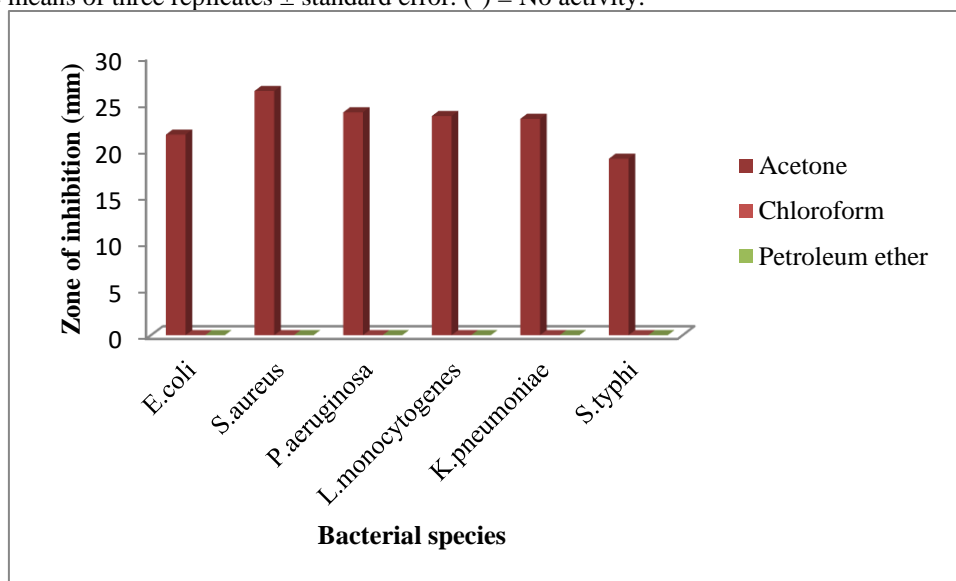


Figure 1: Antimicrobial activity of *C. sinensis* by agar-well diffusion method.

The present study was aimed to screen the phytochemicals and antibacterial activity of *C. sinensis* and *C. assamica* using different organic solvents were carried out.

MATERIAL AND METHODS

Sample collection

Freshly collected young and green leaves of *Camellia sinensis* L. and *C. assamica* L. from Tea Estate Dehradun, Uttarakhand, India were properly washed to remove the dust particles. It was shade-dried, cut into small pieces and powdered with the help of a grinder.

Collection of test organism

Six bacterial strains viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus* and *Listeria monocytogenes* were procured from Microbial Type Culture Collection (MTCC), Chandigarh (India). Luria Bertani (LB) broth was prepared and 3ml was dispensed in each culture tube and autoclaved at 120±1°C for 15 min. The culture tubes containing LB broth were separately inoculated with bacterial culture individually and incubated at 37°C for 24 hours to obtain turbidity (0.5 McFarland standard 1×10^8 CFU).

Extraction by soxhlet apparatus

The shade dried leaves 100 g of *C. sinensis* and *C. assamica* were weighted about 100 g and kept into soxhlet assembly. The tea leaves were extracted by using different

solvents such as petroleum ether, chloroform and acetone of different polarity for 6 hours. The solvents of the extracts were evaporated by using a rota-evaporator.

Agar well diffusion method

Different extraction with the different solvent systems of both tea plants was to evaluate antimicrobial activity against various pathogens. The microbial cultures (100 µl) of different bacteria were separately swabbed on Mueller Hinton agar plate (MHA). Wells (5 mm in diameter) were made with the help of a sterile borer each extract (80 µl) obtained as above and added to each well. All plates were incubated at 37 ±1°C in incubator for 24 h and zone of inhibition was measured after 24 h.

Disc diffusion method

The plant extracts were separately dissolved in different solvents were used to study the antimicrobial activity by disc diffusion method (Murray *et al.*, 1995). The bacterial suspension (100 µl) containing 1×10^8 CFU/ml was separately poured to prepare respective lawn on Mueller Hinton agar (MHA) plate. Whatmann filter paper discs (5 mm diameter) were impregnated with 10 µl of the plant extract and placed on the bacterial lawns at equidistance with positive and negative control discs. The inoculated plates were incubated at 37°C for 24 h.

Phytochemical analysis of *Camellia* species

Phytochemical analysis of tea leaves were carried out using the aqueous extracts of leaves using standard

Table 2: Antibacterial activity of *Camellia* species against bacterial strains based on Disc diffusion method.

Bacterial species	Zone of inhibition (mm)*					
	<i>C. sinensis</i>			<i>C. assamica</i>		
	Petroleum ether	Chloroform	Acetone	Petroleum ether	Chloroform	Acetone
<i>E. coli</i>	-	-	9.3±0.33	-	10.6±0.66	11.33±0.33
<i>S. aureus</i>	-	-	17.6±0.33	-	12.6±1.33	22.33±0.33
<i>L. monocytogenes</i>	-	-	16±0	-	12.3±1.45	15±0.57
<i>P. aeruginosa</i>	-	-	16±2.30	-	18±0	21.3±2.02
<i>K. pneumoniae</i>	-	-	11.6±0.66	-	8.6±0.33	11.6±0.33
<i>S. typhi</i>	-	-	7.3±0.33	-	9.3±0.33	11.3±0.33

*Data are the means of three replicates ± standard error. (-) = No activity.

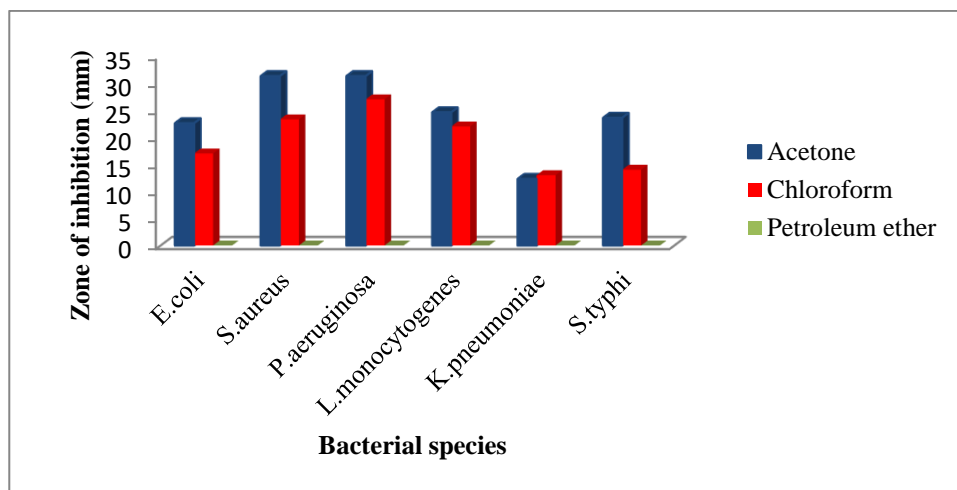


Figure 2: Antimicrobial activity of *C. assamica* by agar well diffusion method.

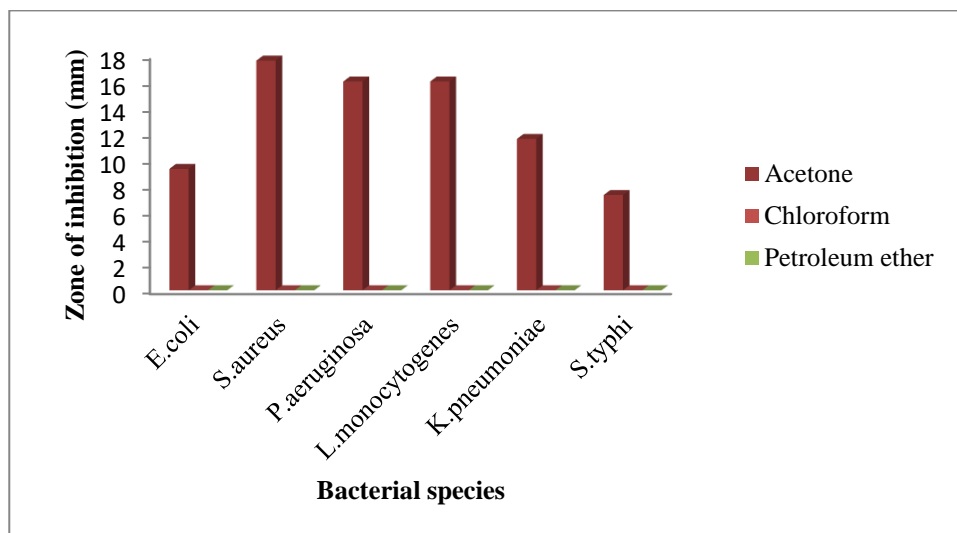


Figure 3: Antimicrobial activity of *C. sinensis* by disc diffusion method.

method to determine the essential components as defined by Sofowara (1993), Trease and Evans (1989) and Harborne (1973) with slight modification.

Test for tannins

Tea leaf extract (1 ml) was boiled with 20 ml water and filtered with Whatmann filter paper. After filtration, few drops of 0.1% of ferric chloride was added and appearance of blue-black and brownish green colour showed positive result.

Test for saponins

Leaf extract (1 ml) was boiled in 10 ml distilled water in a water bath and the extract was filtered. 10 ml of filtrate was mixed with 5 ml distilled water and shaken vigourously to see the stable persistent froth. 3 drops of olive oil was mixed with frothing, shaken vigourously and the formation of emulsion was examined.

Test for terpenoids (Salkowski test)

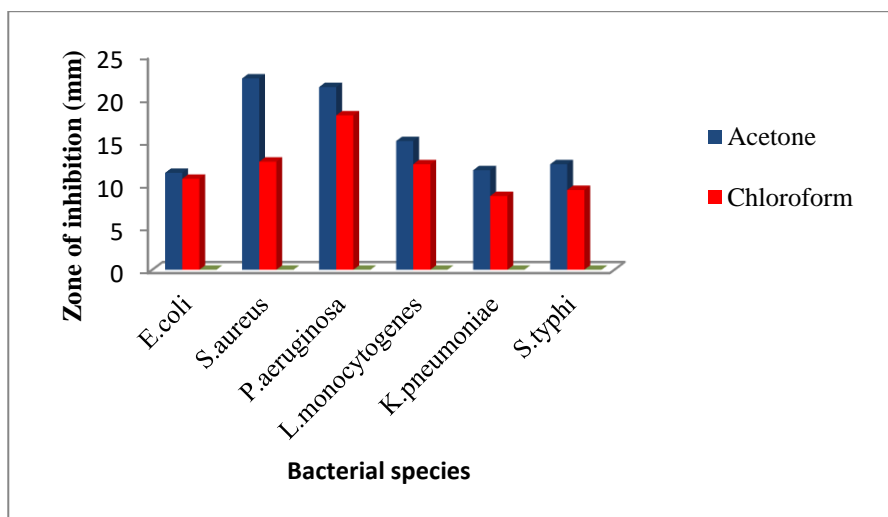


Figure 4: Antimicrobial activity of *C. assamica* by disc diffusion method.

Table 3: Phytochemical analysis of *C. sinensis*.

	Petroleum ether	Chloroform	Acetone
Alkaloids	+	+	+
Flavanoids	-	+	+
Carbohydrates	-	-	+
Saponins	-	-	-
Tannins	-	+	+
Steroids	-	-	+
Terpenoids	-	-	-
Cardiac glycoside	-	-	+

Plant extract (2ml) was mixed with 2 ml chloroform 3 ml concentrated sulfuric acid was added carefully and formation of the thin layer was examined. Development of reddish brown colouration of the inter face shows positive results.

Test for Flavonoids

The presence of flavonoids were carried out following Sofowara, (1993). Dilute ammonia solution (5 ml) was added in aqueous extract with the addition of sulfuric acid. Disappearance of yellow colour shows the positive result.

Test for steroids

Leaf extract (1 ml) and dissolving 10 ml of chloroform in a test tube followed by gradual mixing of equal amount of concentrated sulphuric acid in equal amount. Sulphuric acid layer showing yellow with green fluorescence and the upper layer that turning into red colour showed positive result.

Test for cardiac glycoside (Keller-Killiani test)

Leaf extract (5 ml) was treated with glacial acetic acid and one drop of ferric chloride solution was added to it. After pouring of 1 ml sulphuric acid appearance of brown ring at the interface indicated a deoxysugar characteristic of cardenolides.

Test for alkaloids

Aqueous leaf extract (1 ml) was taken in a test tube and 3 ml of hexane was added. It was shaken and filtered through filter paper. 5 ml of 2 % HCl was poured into a

test tube, heated in a water bath, filtered and few drops of picric acid was added in this contents. Formation of yellow colour precipitate showed the positive result of alkaloids (Wadood et al., 2013).

RESULTS

Both the extracts showed significant ($p < 0.05$) antimicrobial activity against *E. coli*, *S. aureus*, *L. monocytogenes*, *P. aeruginosa*, *K. pneumoniae* and *S. typhi*. The acetone extract of *C. sinensis* and *C. assamica* was more potent in inhibiting the growth of pathogens than chloroform extract of *C. assamica*. Petroleum ether extract did not show any effect against any bacterial strain (Table 1 and Fig. 1 and 2 by well diffusion method, Table 2 and Fig. 3 and 4). Different types of chemicals have been found in the tea extracts dissolved in different solvents. Moreover, difference in bioagents lies according to different solvents (Table. 3 and 4). The effectiveness of the extracts of both species of *Camellia* supports to develop the new strategies against the adverse effects of antibiotics and drugs, providing protection for human health.

DISCUSSION

Analysis of plant extract showed the presence of glycosides, saponins, steroids, terpenoids, flavonoids, alkanoids and reducing sugar. Research work proved that the antioxidant property of medicinal plant are due to rich in phenolic compounds (Brown and Rice-Evans., 1998; Krings and Berger., 2001). The inhibitory property of medicinal plants are due to the presence of flavonoids and alkaloids (Saikia et al., 2006). The presence of alkaloids, flavonoids and other bioactive compounds showed antimicrobial activity against pathogenic bacterial strains (Archana and Abraham., 2011). Phenolic compounds are the natural antioxidant compounds (Ali et al., 1998). Tannins found in plant extract bind to proline rich protein and affect protein synthesis. Flavonoids are the phenolic substances that are synthesized by plants in response to microbial infection and show antimicrobial properties against wide range of microorganisms. Their activity may be effect the complex extracellular and soluble proteins

Table 4: Phytochemical analysis of *C. assamica*.

	Petroleum ether	Chloroform	Acetone
Alkaloids	+	+	+
Flavanoids	+	-	+
Carbohydrates	-	+	+
Saponins	-	-	+
Tannins	-	+	+
Steroids	-	+	+
Terpenoids	-	+	+
Cardiac glycoside	-	+	+

and also complex with bacterial cell wall (Marjorie., 1996). They possess potential antioxidant and anticancer properties (Salah *et al.*, 1995; Del-Rio *et al.*, 1997; Okwu and Okwu., 2004). Saponins are also found in plant extract that coagulate and precipitate the RBC. Some other features of saponins are formation of foams in aqueous medium, cholesterol binding property, bitterness, inhibitory inflammation (Just *et al.*, 1998). Steroids have been reported the property of antibacterial and it is a very significant compounds related to sex hormone (Sodipo *et al.*, 2000; Okwu and Okwu., 2004). Alkaloids have been uses for centuries due to presence of medicinal and cytotoxicity properties (Nobori *et al.*, 1994). Several researcher's reported that the presence of alkaloids shows antibacterial property (Stray., 1998; Okwu and Okwu., 2004). Alkaloids are also known to lower the blood pressure (Nyarko and Addy., 1990). The presence of phytochemicals is the primary way that proved for presence of bioactive components having potential and valuable properties for the human health.

CONCLUSION

Medicinal plants may replace the usages of antibiotics in future. Tea plays a significant role against the pathogenic and drug-resistant microorganism. Several studies have shown that the phytochemicals contribute the medicinal properties that have been no side-effects on human-health. The presence of these phytochemical revealed that further work should be carried out to purify the active components responsible for the antimicrobial, antioxidant properties.

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