



Study on antibacterial activity of some medicinally important plants

L. Senthamil, P. M. Vetriselvi, C. Yamini and R. Kumaresan*

Department of Biotechnology, Periyar Maniammai University, Vallam, Thanjavur, Tamil Nadu, India

ABSTRACT

The conventional antimicrobial technique was based either on poisons or heavy metals, which may not killed the microbes completely, allowing the microbe to survive, change, and become resistant to the poisons and/or heavy metals. Hence, the identification and evaluation of new antimicrobial agents are inevitable. The medicinally important plants, *Camellia sinensis* (green tea), *cuminum cyminum* (cumin), *Cinnamomum cassia* (cinnamon) were taken for the present study. The aqueous extracts of these plants were used in different concentrations (0.5g, 0.25g, 0.12g / 2 ml) against four pathogenic strains namely *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Antibacterial activities of different extracts were evaluated by well diffusion method. Ampicillin, Ciprofloxacin were used as standard drugs. The aqueous extracts of all three plants showed significant antimicrobial activity against the selected microorganisms when compared to the standard drugs. The green tea showed the maximum zone of inhibition against the organisms used in the present study. *Camellia sinensis* (Green tea) could be used as an antimicrobial agent against the *E.coli*, *S.aures*, *B.subtilis* and *P.aeruginosa*.

Key words: Cumin (*cuminum cyminum*); Cinnamon(*Cinnamomum cassia*); Green tea (*Camellia sinensis*), Well Diffusion Method, Antimicrobial activity.

INTRODUCTION

India has a wide variety of medicinal herbs and spices. It has a rich tradition in use of medicinal plants to develop drugs. Natural products and its wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones [1,2,3]from plants offer new agents for defense against the attack of microorganisms such as bacteria, fungi etc. use of herbal antimicrobials has a main advantage of having less side effects and also biodegradable. Currently; drug companies are developing new antibiotics to replace those that are no longer effective [4]. With the increasing incidence of microorganism showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Since antiquity, plants have been used to treat common infectious diseases. Being nontoxic and easily affordable, there has been resurgence in the consumption and demand for medicinal plants [5].Therefore, this study was conducted to compare the antimicrobial effect of some medicinal plant extracts that are used in our daily life such as cinnamon (*Cinnamomum verum*), cumin (*Cuminum cyminum*), green tea (*Camellia sinensis*) are compared against gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) microorganisms since, the antimicrobial potential of these three plants have not been established well.

EXPERIMENTAL SECTION

Plant Material

Three medicinally important plants were used in the present study namely cinnamon (*Cinnamomum verum*), cumin (*Cuminum cyminum*), green tea (*Camellia sinensis*) and the parts of the plants were taken for extraction given in table.1. They were purchased from a local market in Tamil Nadu, India. The plants were brought to the laboratory and thoroughly washed in distilled water and dried in shade then stored in a plastic zip bag in 4°C until use.

Table 1. Characteristics of the Plants Extracts

Scientific name	Common Name	Part of the Plant used	Dry Weight
<i>Cinnamomum verum</i>	Cinnamon	Bark	5g, 2.5g & 1.2g in 20ml
<i>Cuminum cyminum</i>	Cumin	Seed	5g, 2.5g & 1.2g in 20ml
<i>Camellia sinensis</i>	Green tea	Leaf	5g, 2.5g & 1.2g in 20ml

Extract preparation:**Cumin (*Cuminum cyminum*)**

5g, 2.5g and 1.25g of cumin powder was taken in to the different beakers with 20 ml of sterile distilled water and boiled for 15 min at low flame. Then it was allowed to cool. Filter the extract using Whatman No.1 filter paper.

Cinnamon (*Cinnamomum cassia*)

5g, 2.5g and 1.25g of cinnamon powder was taken in to the different beakers and soaked in 20 ml of sterile distilled water for 24 hrs, then filter the filtrate which was heated at 40-50° C for 20 min. Then allowed to cool.

Green tea (*Camellia sinensis*)

5g, 2.5g and 1.25g of green tea was taken in to the different beakers with 20ml of sterile distilled water. Boil for 10 min at 50°C. Then it was allowed to cool. Filter the extract using Whatman No.1 filter paper.

Microorganisms

Four bacterial species namely *Staphylococcus aureus*, *Bacillus subtilis* (gram positive), *Pseudomonas aeruginosa* & *Escherichia coli* (gram negative) were obtained from MTCC, IMTECH, Chandigarh, India. All bacterial culture were maintained and subcultured regularly using nutrient agar media containing peptone 5g, beef extract 3g and agar 2% in a final volume of 11 ml.

Inoculation

The inoculum was spread onto the surface of the nutrient plates by using sterile cotton swabs. Each microorganism was inoculated in 3 plates. The plates were left for incubation for 24 hrs at 37°C.

Well diffusion assay

Using a sterile cork borer, 7mm diameter well were bored in the agar plate and a 100 µl volume of prepared extracts of different concentration was added into the well. All the plate were incubated at 37° C for 24 hrs. Antibacterial activity was determined by measuring the zone of growth inhibition around the well by diffusion assay technique. The antimicrobial activity of the extracts were compared against the standard drugs. The diameter of the zone of inhibition was measured using measuring scale.

RESULTS AND DISCUSSION

The result of the study showed that the three samples (*Cuminum cyminum*, *Cinnamomum cassia*, *Camellia sinensis*) with three concentrations (0.5g/2ml, 0.25g/2ml and 0.12g/2ml w/v) used in this study were shown in Table.2 and figures.1. The antimicrobial activity was classified as: if the diameter less than 8 mm considered as no sensitive, if it was from 8 mm to 15 mm considered as sensitive, the diameter from 16 mm to 20 mm considered as very sensitive and more than 20 mm considered as extremely sensitive. All plant extracts have been observed that they possess antimicrobial potential which was varied based on its effects. This difference was because of their activity depends upon the concentration and may also differ depending on the bacterial species. *E.coli* strains have shown the zone of inhibition ranges from 13-26mm for *Camellia sinensis* (green tea) (Fig.2), 9-11 mm for *Cinnamomum cassia* and 11mm for *Cuminum cyminum*. Similarly for *pseudomonas aeruginosa* (Fig.3), *staphylococcus aureus* (Fig.4), *Bacillus subtilis* (Fig.5) strains also have shown the zone of inhibition ranges from 13-28 mm for *Camellia sinensis* (green tea), 9-12 mm for *Cinnamomum cassia* and 11mm for *Cuminumcyminum*. Primarily, we have used disk diffusion method for the analysis of antimicrobial activity. There were no significant result observed in disk diffusion method against pathogen. Hence, we used the well diffusion method. *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtilis* were extremely sensitive to green tea extract indicates the presence of potential antimicrobial activity.

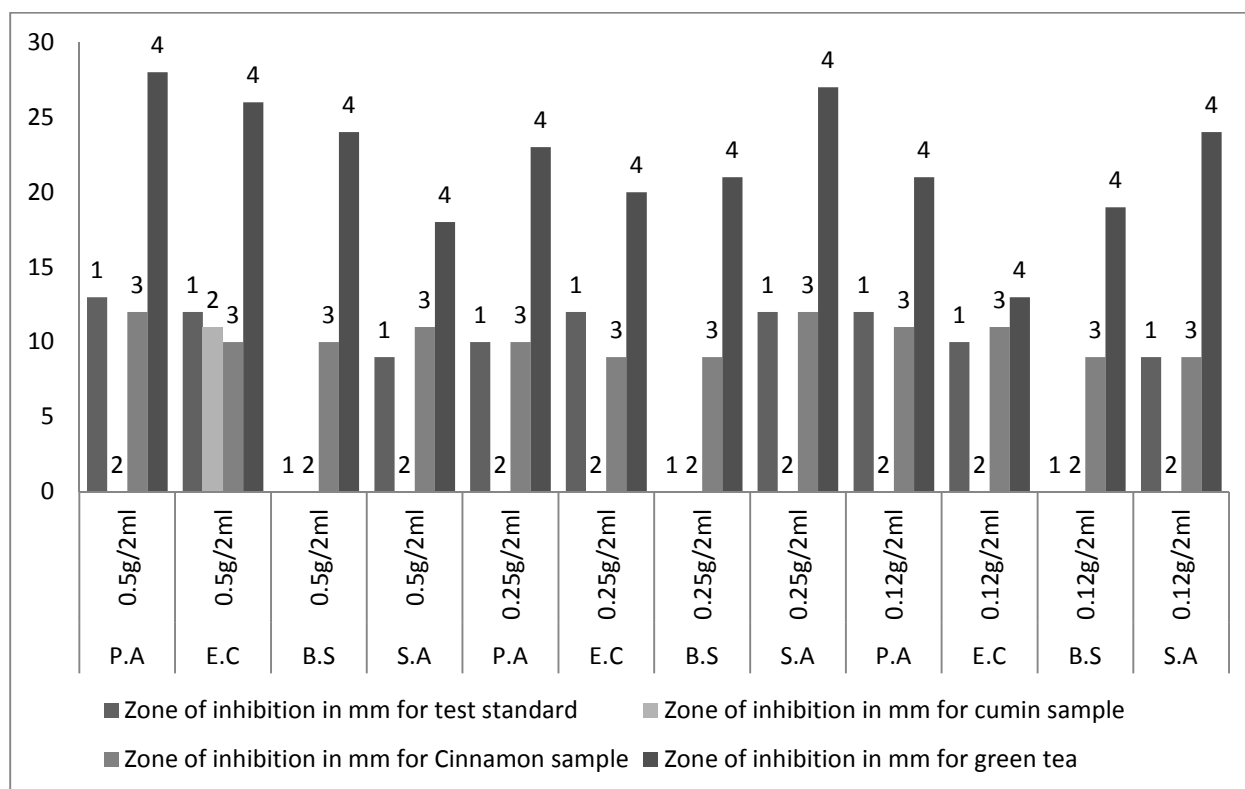
The assessment of the antimicrobial activity was based on the measurement of inhibition zone which was formed around the well. Toda et al., (1989) [6] reported that daily consumption of green tea can kill gram positive *S. aureus* and other harmful bacteria. Also it have been reported by previous studies [7,8,9,10,11] that the green tea contains catechin and polyphenols. These compounds have been found to possess antibacterial and antiviral action as well as anticarcinogenic and antimutagenic properties. These compounds could be responsible for the inhibition of pathogens.

Table 2. The Antimicrobial Activity of Different Plants Extract at Different Concentrations

Organism	Sample	Zone of inhibition at concentration 0.5g/2ml	Zone of inhibition at concentration 0.25g/2ml	Zone of inhibition at concentration 0.12g/2ml
P.A	T	13 mm	10 mm	12 mm
P.A	S1	Nil	Nil	Nil
P.A	S2	12 mm	10 mm	11 mm
P.A	S3	28 mm	23 mm	21 mm
E.C	T	12 mm	12 mm	10 mm
E.C	S1	11 mm	Nil	Nil
E.C	S2	10 mm	9 mm	11 mm
E.C	S3	26 mm	20 mm	13 mm
B.S	T	Nil	Nil	Nil
B.S	S1	Nil	Nil	Nil
B.S	S2	10 mm	9 mm	9 mm
B.S	S3	24 mm	21 mm	19 mm
S.A	T	9 mm	12 mm	9 mm
S.A	S1	Nil	Nil	Nil
S.A	S2	11 mm	12 mm	9 mm
S.A	S3	18 mm	27 mm	24 mm

P.A - *Pseudomonas aeruginosa*; E.C- *Escherichia coli*; B.S- *Bacillus subtilis*; S.A- *Staphylococcus aureus*
T - Antibiotic; S1- *Cinnamomum verum* extract; S2- *Cuminum cyminum* extract; S3- *Camellia sinensis* extract

Figure.1 Antibacterial Activity of different concentrations of standard, cumin, cinnamon and green tea against various strains



1-Antibiotic; 2- *Cinnamomum verum*; 3-*Cuminum cyminum*;4- *Camellia sinensis*

Figure.2 Zone of inhibition in *Escherichia coli* at different concentration of sample

Our present work was planned to carry out the study on in-vitro antibacterial activity of aqueous extract of *carmellia sinensis* against some gram positive and gram negative bacterial strain. The extract of *carmellia sinensis* exhibited greater extend of antibacterial activities.

The antimicrobial activities of medicinal plants are qualified due to the presence of alkaloids, and flavonoids [12,13]. These reports revealed that the presence of flavonoids and alkaloids in different extracts of *carmellia sinensis* confirms its prospective potential antimicrobial activity against all selected pathogenic bacterial strains. The plant active substances were soluble in organic solvents. Due to the solubility, plant extracts shown more activity than commercial antibiotic. The result of the present study showed that the potential usefulness of green tea in the treatment of various pathogenic diseases and it serves as selective agents for the protection of human health and may provide life tools for the study of bacterial diseases or infection.

Figure.3 Zone of inhibition in *Pseudomonas aeruginosa* at different concentration of sample



Figure.4 Zone of inhibition in *Staphylococcus aureus* at different concentration of sample



Figure.5 Zone of inhibition in *Bacillus subtilis* at different concentration of sample



S. Archana and Jayanthi Abraham (2011) [14] have been concluded, 10 g of the powdered leaves extract has shown better activity from their comparative analysis of antimicrobial activity of leaf extract from fresh green tea, commercial green tea and black tea on pathogens like *Escherichia coli*, *Enterococcus faecalis*, *Salmonella typhi*, *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio cholera* and fungal strains *Fusarium*, *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans*. In the present study, better results were observed with 250 mg/ml of aqueous extract. Sana Mukhtar and Ifra Ghorri (2012) [15] confirmed from their study of antibacterial activity of aqueous and ethanolic extract of garlic cinnamon and turmeric against *E.coli* and *Bacillus subtilis*. They have used only 100 gm of powdered cinnamon in 200 ml distilled water for the extraction. But in the present study, we have used three different concentrations against four different organisms and also we have observed the better activity at

250 mg/ml against *E.Coli*. Anita Dua et.al in their Antimicrobial properties of methanolic extract of Cumin (*cuminum cyminum*) seeds concentration of 1g/10 ml against *E.coli*, *P.aeruginosa*, *S.aureus*, *B.pumilus* in their studies. But in our study, 0.5 g / 2 ml of aqueous extract showed maximum activity against all the studied organism. This present study results significantly varied may be due to its extraction solvent.

CONCLUSION

Since the aqueous extract of *Camellia sinensis* (Green Tea) has been observed extremely sensitive in the present study. *Camellia sinensis* may be considered as a potential antimicrobial source for the treatment of gram positive organisms *S.aureus* and *B. subtilis* and gram negative organisms *P.aeruginosa* and *E.coli*. Further studies will be beneficial in providing data on the possible effects of these plant extracts if it is to be used as a relevant therapeutics.

REFERENCES

- [1] SR Al-Zubaydi, MA Al-Hmdany and SJ Raesan. *Journal of Duhok University*, **2009**;12(1): 244-249.
- [2] J Leon, E Rojo and J Sanchezerrano. *Journal of Experimental Botany* **2001**; 52: 1-9.
- [3] MM Cowan, *Clinical Microbiology Reviews*. **1999**; 12(4): 564-582.
- [4] EK Silbergeld, L Price, and J Graham. Antimicrobial Resistance and Human Health. Retrieved from Pew Commission on Industrial Farm Animal Production. **2008**.
- [5] A Jayashree, and S Maneemegalai. *Biomedicine*. **2008**; 28:190-194.
- [6] M Toda, S Okubo, R Hiyoshi and T Shimamura. *Nippon Saikingaku Zasshi* **1989b**; 44(4): 669-672.
- [7] S Sakanaka, M Aizawa, M Kim and T Yamamoto. *Biosci Biotechnol Biochem* **1996**; 60: 745 - 749.
- [8] YJ Ahn, T Kawamura, M Kim, T Yamamoto and T Mitsuoka. *Agric Biol Chem*. **1991**; 55: 1425 - 1426.
- [9] H Makhtar, S Hatiyar and P Agarwal. *J. Invest Dermatol*. **1994**; 102:3-7.
- [10] M Wakayama, K Suzuki, M Toda, S Okubo, Y Hara and T Shimamura. *Antiviral Res*. **1993**; 1: 289 - 299.
- [11] Y Kuroda and Y Hara. *Mutat. Res* **1999**; 436 (1): 60 -67.
- [12] S Burapedjo, A Bunchoo. *Planta medica* 61. 365-366, **1995**.
- [13] AM Fewell, JG Roddick. *Phytochemistry* 33, 323-328, **1993**
- [14] S Archana, and Jayanthi Abreham, *Journal of Applied Pharmaceutical Science* 01 (08); **2011**: 149-152
- [15] Sana Mukhtar and Ifra Ghori, 9-15, Vol 3, Issue-2, *IJABPT-2012*.