



## A Comparative study on the Antimicrobial Activity of normal and galled Leaves of five Medicinal Plants

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Published: 15, August, 2012; Vol. No.3(2):33-36; www.gbtrp.com; All Right Reserved, ©Gayathri Technological Publication, 2012.

### Abstract

Samples of normal and galled leaves of 5 selected medicinal plant species such as *Acacia ferruginea*, *Achyranthus aspera*, *Cinnamomum* sp, *Cinnamomum zeylanicum* and *Terminalia arjuna* were collected from the forest area in Kolli Hills, Namakkal District, Tamilnadu. Ethanol extracts of normal and galled leaves of the above five medicinal plant species were prepared separately. The antimicrobial activity of the extracts of normal and galled leaves against two bacterial strains namely *Escherichia coli* and *Bacillus subtilis* were tested by disc diffusion method. Based on the observed results on the inhibition zone against *E. coli* and *B. subtilis* for the extracts of normal and galled leaves, it is concluded that the antimicrobial activity of the medicinal plant appears to be enhanced by the gall formation and the effects of the active principles in the gall forming organisms could be the possible reason for the enhanced antimicrobial activity observed in the galled leaf extracts.

**Keywords:** Five medicinal plants, galled leaves, antimicrobial activity, *Escherichia coli*, *Bacillus subtilis*

### Introduction

India has a long history of empirical knowledge of the use of medicinal plant for the various diseases. Over the last 40 years, intensive efforts have been made to discover clinically useful antibacterial/antifungal drugs from different medicinal plants (Sardari *et al.*, 1998; Werner *et al.*, 1999; Kudi *et al.*, 1999; Perumalsamy *et al.*, 1999; Perumalsamy and Ignacimuthu, 2000). Natural product of higher plants may offer a new source of antibacterial agents for external use such as compresses, cataplasm goggles and ointments (Branther and Edith Grein, 1994). Synthetic antibiotic drugs are known to disturb the natural intestinal micro flora thereby depriving the benefits of those microbes to human body. On the other hand, antimicrobial agents of the plant origin are reported to be not associated with many side effects and have an enormous therapeutic potentials to heal many infectious diseases (Iwu *et al.*, 1999). In addition to the antimicrobial effects of the extracts obtained from normal leaves/stem/root of medicinal plants, the extract of galled leaves is reported to increase the medicinal property of plants (Fatima *et al.*, 2001), antifungal (Digraki *et al.*, 1993), larvicidal (Redwane *et al.*, 2002), anti diabetic (Hwany *et al.*, 2000), local anesthetic (Dar *et al.*, 1976), antiviral (Hussein *et al.*, 2000)

and anti-inflammatory (Kaur *et al.*, 2004) activities.

In the present study, an attempted made to comparatively assess the antibacterial activity (against a gram negative bacterial strain namely, *Escherichia coli* and gram positive bacterial strain namely *Bacillus subtilis*) of the extracts of normal and galled leaves of five species of medicinal plants such as *Acacia ferruginea*, *Achyranthus aspera*, *Cinnamomum* sp, *Cinnamomum zeylanicum* and *Terminalia arjuna*.

### Materials and Methods

#### Test micro organisms

The two bacterial strains such as *Escherichia coli* (gram negative) and *Bacillus subtilis* (gram positive) are selected for the present study. The cultured bacterial strain of *Escherichia coli* (ATCC 25922) and *Bacillus subtilis* (ATCC 25923) were obtained from IMTECH microbial type culture collection, Chandigarh, India. The bacteria were preserved at 4° C prior to use.

#### Preparation of nutrient agar culture medium

The nutrient agar culture medium was prepared by dissolving 10g peptone, 1.5 g beef extract, 3g yeast extract, 10g sodium chloride and 20g agar



in 2000ml of glass distilled water. The pH of the mixture was adjusted to 6.4 to 6.8 and sterilized by autoclaving at 120°C (15psi) for 20min. Selected Petri plates were sterilized, added with 10ml of broth (to get equal thickness) nutrient agar solution and were allowed to solidify. The bacteria were grown overnight in separate (agar plate) at 37°C and agitation (at 120rpm). For each plant extract (of normal and galled leaves), five agar plates were used separately in the disc diffusion method.

### Preparation of leaf extract

The extract of normal and galled leaves was prepared separately by using 80% ethanol as solvent. Samples of normal and galled leaves of each species of the selected medicinal plants (such as *A. ferruginea*, *A. aspera*, *Cinnamomum* sp., *C. zeylanicum* and *T. arjuna*) were collected, surface sterilized with 0.1% HgCl solution and washed 3 times with distilled water. 20g of sterilized and washed leaf material of normal and galled leaves of each plant species was ground (using a glass mortar and pestle) by adding 25ml of 8% ethanol. The homogenate was filtered using glass wool and then centrifuged at 5000 rpm for 10 min. The plant extract was concentrated into paste consistency using Soxhlet apparatus. The pasty leaf extract was stored at -20°C and was dissolved freshly in 10ml of 10% dimethyl sulfoxide (DMSO, EMERCK, Germany) before used in the assay.

### Antibacterial activity assay

The antibacterial activity of the extract of normal and galled leaves of the selected plant species is studied by disc diffusion method. 5 µl of the extract (containing 5mg of the extract) was impregnated on small disc of filter paper (of 6 mm diameter). The leaf extract impregnated-disc was placed on top of the agar medium cultured with bacterial strain (either *Escherichia coli* or *Bacillus subtilis*). The antibacterial assay plates were incubated at 37°C for 24 hours. After 24 hours, the diameter of the zone of inhibition around each of the disc (of leaf extract) was measured and recorded. For each of the normal and galled leaf extract for the two bacterial strains, five antibacterial assay plates were prepared and data were collected.

### Statistical analysis

The mean value of the zone of inhibition was calculated from the values obtained from five

plates of normal and galled leaf extracts against a particular bacterial strain. The difference between the zone of inhibition of normal leaf extract and of galled leaf extract was calculated as percentage. The significance of difference between the zone of inhibition of normal and galled leaves were analyzed by Student 't' test (Steel and Torrie, 1959).

### Results and Discussion

The data on the zone of inhibition (diameter in mm) of extracts of normal and galled leaves of the 5 species of medicinal plant against *Escherichia coli* and *Bacillus subtilis* are presented in Table 1. A perusal of Table 1 clearly indicated that the extracts of normal as well as galled leaves of 5 species of medicinal plants showed inhibitory effects (excepting that of the *Cinnomomum* sp. against *B. subtilis*) on the growth of *E. coli* and *B. subtilis*. However, the magnitudes of inhibition zone varied among the plant species. In all the plant species tested for the two bacterial strains, the inhibitory effect (based on the diameter of zone of inhibition) of galled leaves was found to be more than that of the extracts of normal leaves. The increased inhibitory activity of galled leaf extract was found to be significant /highly significant against *E. coli*. On the other hand, with reference to *B. subtilis*, the increased inhibitory effect of galled leaves extract was significant/highly significant in *Acacia ferruginea*, *Cinnamomum* sp., and *Cinnamomum zeylanicum*, whereas, it was not significant in *Achyranthus aspera* and *Terminalia arjuna*.

From the above results, it could be concluded that the extracts of normal (excepting that of the *Cinnomomum* sp. against *B. subtilis*) as well as galled leaves of all the 5 species of medicinal plants (*Acacia ferruginea*, *Achyranthus aspera*, *Cinnamomum* sp., *Cinnomomum zeylanicum* and *Terminalia arjuna*), in the present study, showed antimicrobial activity against *E.coli* and *B. subtilis*. Besides, the extracts of galled leaves showed significantly higher antimicrobial effects against the two bacterial strains compared to that of normal leaves. The increased antimicrobial effects of the galled leaf extracts were also reported by other researchers in the medicinal plant, *Quercus infectoria* (Dar *et al.*, 1976; Digraki *et al.*, 1993; Hwag *et al.*, 2000; Hussein *et al.*, 2000; Redwane *et al.*, 2002; Kaur *et al.*, 2004; Leela and Satirapipathkul, 2011 and Archa Vermani *et al.*, 2012). Sawsan *et al.* (2010) also reported that the extracts of *Quercus infectoria*



affects the bacteria as curing agent and also has an inhibitory effect on the resistance genes of the microorganisms thereby reducing the resistance to anti-microbial agents.

Table -1: Data on the zone of inhibition (diameter in mm) of extracts of normal and galled leaves of extracts of 5 species of medicinal plants against *Escherichia coli* and *Bacillus subtilis*

Plant species	Zone of inhibition (diameter in mm)			
	<i>Escherichia coli</i>		<i>Bacillus subtilis</i>	
	Normal leaf extract	Galled leaf extract	Normal leaf extract	Galled leaf extract
<i>Acacia feruginea</i>	17 ± 2	20 ± 1 (+18) (S)	11 ± 2	15 ± 1 (+36) (S)
<i>Achyranthus aspera</i>	11 ± 2	15 ± 3 (+36) (HS)	10 ± 2	12 ± 1 (+20) (NS)
<i>Cinnamomum sp.</i>	11 ± 2	14 ± 2 (+27) (HS)	0	9 ± 1 (+∞) (HS)
<i>Cinnomomum zylanicum</i>	11 ± 3	15 ± 2 (+36) (HS)	9 ± 2	12 ± 1 (+32) (HS)
<i>Terminalia arjuna</i>	11 ± 2	14 ± 1 (+27) (HS)	18 ± 3	19 ± 4 (+6) (NS)

Values are means of 5 observations ± S.E. Percent changes in the galled leaf extract over normal leaf extract are given in parenthesis.

S- Significant statistically, P< 0.05; HS- Highly significant statistically, P< 0.01.; NS – Not significant statistically, P>0.05

In galled leaves, chemicals such as alkaloids, glycosides, flavanoids, tannins, saponins and phenols are reported to be present in higher levels (Sawsan *et al.*, 2010). Among these chemicals, Leela and Satirapipathkul (2011) reported that the higher levels of tannin found in galled leaves of *Quercus infectoria* is responsible for the higher antibacterial activity of the extract. The observed higher antibacterial activity in the galled leaves of 5 species of the medicinal plants, in the present study, together with the report of Sawsan *et al.*, (2010) and Leela and Satirapipathkul (2011), could be probably due to the presence of higher levels of alkaloids, glycosides, flavanoids, tannins, saponins and phenols present in the gall-forming micro organisms in the galled leaves. However, a final conclusion on the increased antimicrobial activity of galled leaves could be arrived at only after a detailed biochemical analysis of the galled leaf tissues.

### Acknowledgements

The authors are thankful to the Principal and HOD of Microbiology, Kandaswami Koundar's College, Velur for providing laboratory facilities and also our special thanks to the Management of Karpagam University, Coimbatore for permit literature collection.

### References

Archa Vermani, Navneet and Praphat, 2009. Screening of *Quercus infectoria* gall extracts as

anti-bacterial agents against dental pathogens. *Indian Journal of Dental Research*, 20 (3): 337-339.

Branther, A. and Edith Grein, 1994. Antibacterial activity of extracts used externally in traditional medicine. *Journal of Ethnopharmacology*, 44: 35-40.

Dar, M.R.S., Ikram, M. and Famouhi, T. 1976. Pharmacology of *Quercus infectoria*. *Journal of Pharmaceutical Sciences*, 65: 1791-1974.

Digraki, M., Alma, M.H., Alma, A., Ilcim and Sen, S. 1993. Antibacterial and antifungal effects of various commercial plant extracts. *Pharmaceutical Biology*, 37: 216-20.

Fatima, S., Farooqi, A.H.A., Kumar, R., Kumar, T.R.S. and Khanuja, S.P.S. 2001. Antibacterial activity possessed by medicinal plants used in tooth powders. *Journal of Medical Aromatic Plant Science*, 22: 187-9.

Hussein, G., Miyashiro, H., Nakamura, N., Hattori, M., Kakiuchi, N. and Shimothno, K, 2000. Inhibitory effects of medicinal plant extracts on hepatitis C virus protease. *Phytotherapy Research*, 14: 510-516.

Hwang, J.K., Kong, T.W., Back, T.W.N.I. and Pyun, Y.R. 2000.  $\alpha$ -Glycosidase inhibitory activity of hexagalloylglucose from the galls of *Quercus infectoria*. *Planta Medica*, 66: 73-74.

Iwu, M.W., Duncan, A.R. and Oknnji, 1999. New antimicrobials of plant origin. *Perspectives on new crops and new uses*, 457-462.

Kaur, G., Hamid, H., Ali, Alam, M.S. and Athar, M. 2004. Anti-inflammatory evolution of alcoholic extracts of galls of *Quercus infectoria*. *Journal of Ethnopharmacology*, 90: 285-92.



Kudi, A.C., Umoh, J.V., Eduvie, L.O. and Getu, J. 1999. Screening of some Nigerian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*, 67: 225-228.

Leela, T. and Satirapipathkul, C. 2011. Studies on the antibacterial activity of *Quercus infectoria* galls. *International Proceedings of Chemical, Biological and Environmental Engineering*, 5: 410-414.

Perumalsamy, R., Ignachimuthu, S. and Patric Raja, D. 1999. Preliminary screening of ethno medical plants from India. *Journal of Ethnopharmacology*, 66: 235-240.

Perumalsamy, R. and Ignachimuthu, S. 2000. Antibacterial activity of some folklore medicinal plants used by tribal in Western Ghats of India. *Journal of Ethnopharmacology*, 69 : 63-71.

Redwane, A., Lazrek, H.B., Bouallam, S., Markouk, M., Amarouch, H. and Jana, M. 2002. Larvicidal activity of extracts from *Quercus iusitanica* var. *Infectoria* galls (Oliv.) *Journal of Ethnopharmacology*, 79:261-263.

Sardari, S., Gtolamreza Amin, Ronald, G. M. and Daneshtalab, M.1998. Phytopharmaceuticals. Part I. Antifungal activity of selected Iranian and Canadian plants. *Pharmaceutical Biology*, 36:180-188.

Sawsan, M.A.A., Abbas, A.K.R. and Juma, I.M. 2010. The effect of extract of plants on the causative agents of diarrhoea in Erbil Children. *World Family Medicine Journal*, 8(7): 3-9.

Steel, R.G.D. and Torrie, J.H. 1960. *Principles and procedures of statistics with special reference to the biological sciences*. Mc Graw Hill, Inc, New York, 451.

Wernar, F., Paul Olcemo and Rainer Ansorg. 1999. Antibacterial activity of East African medicinal plants. *Journal of Ethnopharmacology*, 60:79-84.

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**Manuscript Progress Date**

Received : 07.05.2012

Revised : 30.07.2012

Accepted : 14.08.2012

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