RESEARCH ARTICLE

Antibiogram Pattern of Common Plant Leaves against *E. coli, Staphylococcus aureus* and Whole Milk Culture

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Abstract

The *in vitro* efficacy of ethanolic and aqueous extract of selected plants namely Neem (*Azadirachta indica*), Guava (*Psidium guajava*), Lemongrass (*Cymbopogon citratus)*, Mango (*Mangifera indica*), Tulsi (*Ocimum sanctum*) and Seasum (*Dalbergia sissoo*) leaves was studied against *E. coli, Staphylococcus aureus* and whole milk culture. The antibiogram revealed that *S. aureus* isolates were 100% susceptible to ethanolic extracts of only Neem and Guava in addition to standard drug Streptopenicillin followed by Lemongrass that showed 60 % sensitivity. The percent sensitivity of ethanolic extract of these plants against isolates of *E. coli* was 60, 40, 40, 0, 0 and 0, respectively. In whole milk culture the sensitivity percent was 100% for Neem, Guava, Lemon grass in comparison to 40% for Mango leaves and 0 % each for Tulsi and Seasum leaves. The aqueous extract of Neem, Lemongrass and Mango leaves did not show any activity against the isolates of *S. aureus* (0% sensitivity). All the leaf extracts were inactive against *E. coli* (0% sensitivity). The extract of Neem exhibited sensitivity against 40% isolates of whole milk culture

Keywords: Mastitis, Plants, Antibiogram, Extracts of leaves, *E. coli*, *S. aureus,* Whole milk culture.

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Introduction

Plants are the backbone of traditional Indian medicinal system since time immemorial. Herbal medicine remains one of the most common forms of therapy widely available throughout the world (Shukla *et al.,* 2000). Plants are mines of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Samie *et al*., 2010). However with time and advent of new generation antibiotics the use of herbal drugs took a back seat. Undoubtedly antibiotics have been of great value in controlling many infections, but their indiscriminate use has led to a serious threat of antibiotic resistance (Danso and Vlas, 2002). The worldwide problem of antibiotic resistance impacts negatively on antibiotic therapy thus making successful empiric therapy much more difficult to achieve. This menace of antibiotics resistance has prompted us to explore our traditional knowledge in finding new and innovative antimicrobial from plants and use medicines of plant origin to curb this nuisance of antibiotics. Phytochemical and biological studies have already been performed on a large number of plants by scientists all over the world. Therefore, the present study was carried out to screen locally available plants/trees for their antibiogram properties against *E. coli, S. aureus* and whole milk culture as an attempt to validate the ethno-veterinary practices.

Materials and Methods

The present study was conducted following approval of Institutional Animal Ethics Committee to screen the antibiogram potential of leaves of locally available plants against *E. coli*, *Staphylococcus aureus* and whole milk culture. The pure culture of *E. coli* and *S. aureus* was obtained from department of Veterinary Microbiology. The leaves of plants selected by ethno-botanical approach for pharmacological study were Neem (*Azadirachta indica*), Guava (*Psidium guajava*), Lemongrass (*Cymbopogon citratus)*, Mango (*Mangifera indica*), Tulsi (*Ocimum sanctum)* and Seasum (*Dalbergia sissoo*) .

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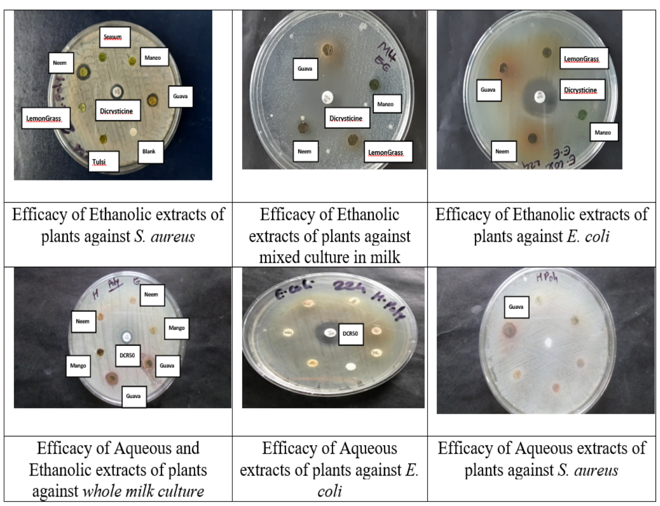
Disc Preparation

A stock solution of different extracts (aqueous and ethanolic) of different plants were prepared by soaking 5 gm of leaves with 25 ml of their respective solvents (sterile distilled water and Ethanol) for 3 days followed by maceration (Kumee *et al.*, 2015). Maceration extraction is crude extraction procedure ; solvents diffuse into solid plant material and solubilize compounds with similar polarity (Green, 2004). After the maceration, the liquid extract was obtained and clarified by gross filtration and centrifugation. Subsequently, ethanolic extract was evaporated out at 45-50 °C till the extract was reduced by 1/3rd of its volume. The aqueous extract was also reduced to 1/3rd of its volume by slow evaporation. The prepared extracts were impregnated in 5 mm sterilized blank discs which were cut with the help of commercial punch machine (Bauer *et al*., 1996). Dimethyl sulfoxide-loaded discs were used as negative controls for aqueous and ethanolic extracts. All impregnated discs were ensured to be fully dried at 45°C in an incubator for 18 to 24 hour prior to the application on bacterial lawn (Zaidan *et al.,* 2005). The standard antibiotic disc was used as positive controls. Ethanol was purposefully used in place of methanol for preparation because of its lesser biotoxicity (Kummee *et al*., 2015).



Efficacy of Ethanolic extracts of plants against *S. aureus*

Efficacy of Ethanolic extracts of plants against mixed culture in milk

Efficacy of Ethanolic extracts of plants against *E. coli*

Efficacy of Aqueous and Ethanolic extracts of plants against *whole milk culture*

Efficacy of Aqueous extracts of plants against *E. coli*

Efficacy of Aqueous extracts of plants against *S. aureus*

Fig. 1: Antibiogram pattern of ethanolic and aqueous extracts of common plants

Antimicrobial Susceptibility Procedure

The bacterial inoculums were uniformly spread using sterile cotton swab on a sterile Petri dish. The prepared plant discs were placed on top of the previously inoculated nutrient agar medium surface with the help of sterile forceps. Each disc was pressed down to ensure complete contact with the agar surface. The plates were incubated for 18–24 hrs at 37°C temperature in bacteriological incubator before interpretation of the result. A zone of inhibition was measured in millimeters (Table 1).

Determination of Relative Percentage Inhibition (RPI)

The Relative Percentage Inhibition (RPI) of the test extract with respect to positive control was calculated by using the following formula (Paluri *et. al.,* 2012).

$$RPI = 100 (X-Y)/(Z-Y)$$

Where, X= Total area of inhibition of the test extract; Y=Total area of inhibition of the solvent and Z= Total area of inhibition of the standard drug.

Results and Discussion

The antibiogram revealed that *S. aureus* isolates were 100% susceptible to ethanolic extracts of only Neem and Guava leaves in addition to standard drug Streptopenicillin followed by Lemongrass that showed 60 % sensitivity (Table 1, Fig 1). No inhibitory activity was noticed for leaves of Mango, Tulsi and Seasum. The zone of inhibition obtained against *S. aureus* isolates were 12.8±2.17 mm, 10.8±0.84 mm, 11.33±1.15 mm and 17.6±5.17 mm, respectively, for Neem, Guava, Lemongrass and Streptopenicillin. The percent sensitivity against isolates of *E. coli* for ethanolic extract of Neem, Guava, Lemongrass, Mango, Tulsi and Seasum leaves was 60, 40, 40, 0, 0 and 0, respectively. Guava showed the maximum zone of inhibition of 11.2±0.84 mm. In whole milk culture the sensitivity percent was 100% for ethanolic extract of Neem, Guava, Lemon grass in comparison to 40% for Mango leaves and 0 % each for Tulsi and Seasum leaves. Maximum zone of inhibition against whole milk culture was exhibited by Neem (14.8±2.28 mm) followed by Guava (13.6±1.14 mm) (Table 1).

The relative percent inhibition of ethanolic extracts of Neem, Guava and Lemongrass was 52.89, 37.66 and 41.44 for

Table 1: Antibiogram of ethanolic extracts of common plant leaves against *S. aureus, E. coli*, whole milk culture

	S. aureus		*E. coli*		*Whole milk culture*	
Plant	*% Samples showing sensitivity*	*Average zone (mm)*	*% Samples showing sensitivity*	*Average zone (mm)*	*% Samples showing sensitivity*	*Average zone (mm)*
Neem	100	12.8 ± 2.17	60	10.8 ± 0.84	100	14.8 ± 2.28
Guava	100	10.8 ± 0.84	40	11.2 ± 0.84	100	13.6 ± 1.14
Lemongrass	60	11.33 ± 1.15	40	10.4 ± 0.54	100	10.6 ± 0.89
Mango leaves	0	No zone	0	No zone	40	10.8 ± 1.09
Tulsi	0	No zone	0	No zone	0	No zone
Seasum	0	No zone	0	No zone	0	No zone
Dicrysticine 50	100	17.6 ± 5.17	100	16 ± 2	100	20.8 ± 2.68

Table 2: Antibiogram of aqueous extracts of common plant leaves against *S. aureus, E. coli*, whole milk culture

Plant	*S. aureus*		*E. coli*		*Whole milk culture*	
	% Samples showing sensitivity	*Average zone (mm)*	*% Samples showing sensitivity*	*Average zone (mm)*	*% Samples showing sensitivity*	*Average zone (mm)*
Neem	0	No zone	0	No zone	40	10.8±1.09
Guava	60	11.6±1.67	0	No zone	100	10.6±0.89
Lemongrass	0	No zone	0	No zone	0	No zone
Mango leaves	0	No zone	0	No zone	0	No zone
Tulsi	0	No zone	0	No zone	0	No zone
Seasum	0	No zone	0	No zone	0	No zone
Dicrysticine 50	100	17.2±4.6	100	16±2	100	20.4±2.62

Table 3: Relative percent inhibition of ethanolic extracts

Plants	*S. aureus*	*E. coli*	*Whole milk culture*
Neem	52.89%	45.56	50.62
Guava	37.66	49	42.75
Lemongrass	41.44	42.25	25.9
Mango leaves	-	-	26.96

Table 4: Relative percent inhibition of aqueous extracts

Plants	*S. aureus*	*E. coli*	*Whole milk culture*
Neem	-	-	28.03%
Guava	45.48 %	-	26.99%
Lemongrass	-	-	-
Mango leaves	-	-	-

S. aureus and 45.56, 49.00 and 42.25 for *E. coli*, respectively (Table 3). In whole milk culture the Neem, Guava, Lemongrass and Mango leaves exhibited 50.62%, 42.75%, 25.9% and 26.96% relative percent inhibition when compared to standard drug strepto penicillin.

The antibiogram efficacy of aqueous extracts of Neem, Guava, Lemongrass and Mango leaves studied against *S. aureus, E. coli* and whole milk culture (Table 2, Fig 1) revealed that the aqueous extract of Neem, Lemongrass and Mango leaves did not show any activity against the isolates of *S. aureus* (0% sensitivity). All the leaf extracts were inactive against *E. coli* (0% sensitivity). The extract of Neem exhibited sensitivity against 40% isolates of whole milk culture with average zone of inhibition of 10.8±1.09 mm. The aqueous extracts of Guava leaves exhibited sensitivity to *S. aureus* and whole milk culture to the tune of 60 and 100%, respectively. The relative percent inhibition of Guava leaves extract against *S. aureus* was 45.48% and Neem and Guava extracts against *E. coli* was 28.03% and 26.99%, respectively (Table 4).

The better results in ethanolic extracts can be attributed to the fact that the most active chemical compounds are only slightly hydrophilic in nature; however, they are freely lipophilic and more soluble in organic solvents, such as alcohol, ketones and esters (Pankaj *et al.*, 2011). Neem contains several active chemical compounds (Mondali *et al.*, 2009; Sudhir *et al.,* 2010), the most commonly active compounds in Neem are azadirachtin, nimbin and nimbidine (Mondali *et al.*, 2009). The result for Guava leaves corroborates with the results of Biswas *et al.* (2013) who also showed inhibitory activity of ethanolic extract against *S. aureus*. Many researchers too have reported that the Guava leaf extract had a potent antibacterial activity against various bacteria including *S. aureus* (Kummee *et al*., 2015), *Streptococcus* spp., and *E. coli* (Gutierrez *et al*., 2008; Kanbutra *et. al*., 2003). The *in vitro* antibacterial activities against bacteria by the crude extract could be due to presence of some active phyto chemicals in Guava leaves such as phenolic compounds, flavonoids, terpenoids, triterpene, tannins, essential oils, saponins, glycosides, carotenoid, and a number of other fixed substances (Gutierrez *et al*., 2008; El-Ahmadya *et al.,* 2013; Shruti *et al.,* 2013). These phyto chemicals are known to exhibit useful biological activities and a variety of medicinally important effects, including antibacterial activities and they may have acted alone or in combination to affect the bacterial organisms (Barbalho *et al.,* 2012; El-Ahmadya *et al.,* 2013). Nyamath and Karthikeyan (2018) opined that lemongrass leaf extracts is highly effective in controlling different types of pathogenic microorganisms with the maximum zone of inhibition in *S. aureus* followed by *Lysinibacillus macroides, Bacillus vallismortis, Vibrio cholera, E. coli*, and *Ps. aeruginosa*. Higher zone of inhibition of the ethanolic extracts of the leaves of Lemongrass against *S. aureus* was also reported by Danlami *et al.* (2011). Flavonoids and Tannins found in the extract are responsible for the activity (Gopinath *et al*., 2013). In the present study extracts (ethanolic and hydro) did not show any sensitivity towards the isolates of *E. coli* and *S. aureus*. Mango leaves however expressed sensitivity against whole milk culture. Manzur *et al.* (2020) concluded that the extracts of *M. indica* leaves represent natural alternatives against *Staphylococcus* spp. strains and the ethanolic extract shows potential as a natural sanitizer.

Our findings are in contrary to the findings of Shah *et al.* (2016), who studied antibacterial activity of the aqueous, alcoholic, chloroform extract and oil obtained from leaves of *Ocimum sanctum* against *E. coli*, *P. aeruginosa*, *S. typhimurium* and *S. aureus*. Contrary to our findings, Prasad *et al.* (2014) also recorded zone of inhibition against *E. coli, Ps. aeruginosa and S. aureus*. These variations can be due to variations in extraction methods, particularly the length of the extraction period, the solvent used, its pH, temperature, particle size, and the solvent-to-sample ratio (Ncube *et al.,* 2008).

Conclusion

The leaves of plants Neem (*Azadirachta indica*), Guava (*Psidium guajava*), Lemongrass (*Cymbopogon)*, Mango (*Mangifera indica*), can be used as an alternate to the antibiotics effectively in treatment and control of mastitis. The need is to standardize further the protocols in terms of extraction techniques and field applicability of the extracted product as pre or post dips or as a potent therapeutic agent.

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