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Phytochemical analysis and antibacterial activity of galls of *Quercus infectoria* (Majuphala)

Archana Sawane[1*], Tejaswini Raut[2]
[1, 2] Department of Botany, S.M. Mohota College of Science, R. T. M. Nagpur University, Nagpur, Maharashtra, India

Abstract
The study was undertaken to screen phytochemical activity and to study antibacterial activity of aqueous and ethanolic extracts of *Quercus infectoria* against bacteria namely *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Phytochemical screening was done for different phytochemicals like tannins, alkaloids, phenolic compounds, glycosides, flavonoids, proteins and reducing sugar. Antibacterial activity was tested by Disc Diffusion technique. Tannins, alkaloids, phenolic compounds, glycosides and flavonoids were found to be present in both the extracts of *Q. infectoria.* Antibacterial activity of *Q. infectoria* was found to be comparable with the control antibiotics and even better for ethanolic extract against *P. aeruginosa* and *S. aureus*. The current study suggests potential of *Q. infectoria* as anti-bacterial agent with need of further studies on bioactive compounds in galls of *Q. infectoria* responsible for antibacterial activity.

Keywords: antibacterial activity, *Majuphala,* phytochemicals, *Quercus infectoria*

Introduction
Plant based drugs have been in use against various diseases since the time immemorial. The primitive man used plant as therapeutic agent and medicament, which they were able to procure easily. The nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues [1].
Plants produce a diverse range of bioactive molecules, called phytochemicals. They are considered secondary metabolites because the plants that produce them may not need them. These secondary metabolites such as flavonoids, steroids, alkaloids, resins, fatty acids, tannins and phenolic compounds, etc are synthesized naturally in all parts of the body of the plant; bark, leaves, stem, root, flower, fruit, seeds, etc., that is, any part of the body of the plant may contain active components, making them rich sources of different types of phytochemicals. Today the herbal or natural products have become more popular due to their high antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties. As the incidence of increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics agents is more, the researchers are developing interest towards alternative treatment options and products for diseases. Hence, the natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals. [2].
The *Quercus infectoria* (Family-Fagaceae) is a small tree or shrub found in Greece, Asia, and Iran. The galls arise on branches of this tree, resulting from the deposition of eggs by gall wasp [3]. In Indian traditional medicine, the galls have been used to treat diarrhoea, dysentery, internal haemorrhages, gonorrhoea, impetigo, tonsillitis, and menorrhagia. The drug Mazu (Gall of Quercus infectoria) is described in detail in ethnobotanical and classical Unani literature and various actions of the drug have been reported such as analgesic, antidote, anti-inflammatory, antipyretic, antiseptic, deodorant, desiccant, expectorant, germicidal, hypnotic, hypoglycaemic, powerful astringent, sedative, styptic, tonic, tonic to teeth and gum, and wound healing. [4]
Galls have also been shown to have high antibacterial activity, particularly against resistant bacteria. [5]
Incidence, and the emergence of multidrug resistant and disinfectant resistant bacteria—such as *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)—has increased rapidly, causing the increase of morbidity and mortality. [6]
The present study was, therefore, undertaken to screen phytochemical activity and to study antibacterial activity of aqueous and ethanolic extracts of *Q. infectoria* against bacteria namely *S. aureus, E. coli*, and *P. aeruginosa.*

Material and Methods
Seed collection and extraction process
The galls of *Q. infectoria* were purchased from traditional vendors of medicinal plants in local market of Nagpur. The galls were washed thoroughly with tap water followed with sterilized distilled water and shade dried for few days and then were grinded separately in mechanical grinder to get fine powder.
Each of 0.5 gm of the fine powder of seeds were dissolved in 5 ml of sterile distilled water to make aqueous extract and in 80 % ethanol for ethanolic extract, centrifuged and then were filtered by Whatman filter paper no. 1 till clear filtrates were obtained. The extracts were then stored in screw capped bottles in refrigerator for further use. Extraction procedure adapted was as described by Gowdhami et al. [7]

Phytochemical tests
Phytochemical analysis was carried out for identification of different phytochemicals like tannins, Alkaloids, Phenolic compounds, Glycosides, flavonoids, proteins and reducing sugar according to standard methods [8, 9, 10].

Antibacterial Activity
The antibacterial activity was investigated against pure cultures of pathogenic strains of *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram- positive), and

Pseudomonas aeruginosa (Gram- negative) by disc diffusion technique. [11] These bacterial strains were selected on the basis of their clinical importance in causing diseases in humans.

Bacterial cultures [*E. coli* (ATCC No. 25922), *P. aeruginosa* (ATCC No. 27853), *S. aureus* (ATCC No. 25923)] were obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune. Bacteria were sub-cultured on nutrient broth at 37°C overnight. 1 ml of the broth culture of each bacterium was spread over the nutrient agar taken in Petri dishes aseptically with the help of spreader. The filter paper discs (5 mm diameter) saturated with 10 microlitre of extract were placed over it and incubated at 37°C for 24 h. Distilled water and ethanol were used as the negative control. For positive control, chloramphenicol disc was used for Gram-positive *Staphylococcus aureus*, whereas tetracycline disc was used for Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. The Petri-dishes were observed for the presence of zone of inhibition around the discs and were measured by scale in millimetres. The tests were repeated thrice to confirm the findings, and the average of the readings was taken into consideration.

Result and Discussion

In the present study, galls of *Quercus infectoria* were tested for presence of different phytochemicals like tannins, alkaloids, phenolic compounds, glycosides, flavonoids, proteins and reducing sugar. Tannins, alkaloids, phenolic compounds, glycosides and flavonoids were found to be present whereas proteins and reducing sugars were found to be absent with the results of the screening tests used (Table 1). Similar results have also been reported earlier [12].

Table 1: Screening of aqueous and ethanolic extracts of *Quercus infectoria* for different phytochemicals

Phytochemical test	Phytochemicals (Present/Absent)	
	Aqueous extract	Ethanol Extract
Tannins (Dilute Iodine Solution test)	++	++
Alkaloids (Wagner's Test)	+	+
Phenolic compounds (Lead acetate test)	+	+
Glycosides	+	+
Flavonoids	+	+
Protein (Nitric acid test)	-	-
Reducing sugar (Benedict's test)	-	-

Antimicrobial activity results are presented in Table 2. Antimicrobial activity of ethanolic and aqueous extract were observed. It was found that antimicrobial activity was statistically significantly more for ethanolic extract than for the aqueous extract for *P. aeruginosa* and *S. aureus.*

Table 2: Antibacterial activity of aqueous and ethanolic extract of *Q. infectoria*

Bacteria	Zone of inhibition (mm ± SEM)				*t test, p value (For difference in Mean between Aqueous and ethanol extract)*
	Aqueous extract	*Ethanol extract*	*Positive Control*	*Negative Control*	
E. coli	18.00 ± 0.58	18.33 ± 0.33	18.66 ±0.33	---	p>0.05
P. aeruginosa	12.67 ± 0.33	23.00 ± 0.58*	20.00 ± 0.00	---	P<0.001
S. aureus	18.33 ± 0.33	20.67 ± 0.33**	18.33 ± 0.33	---	P<0.01

* p = 0.0065 for *P. aeruginosa* as compared to Positive control

** p = 0.0077 for *S. aureus* as compared to positive control

Antibacterial activity of *Q. infectoria* was found to be comparable with the control antibiotics and even statistically better for ethanolic extract against *P. aeruginosa* (p = 0.0065) and *S. aureus* (p = 0.0077).

Similar results demonstrating good antimicrobial activity of galls have been reported by earlier researchers. [13] Diethyl ether extract of *Quercus infectoria* was found to record the maximum activity against *S. aureus*, *K. Pneumoniae* and *V. cholera* in a study by Shamila et al. [14] *Q. infectoria* extract has also been shown to be effective against Methicillin-resistant Staphylococcus aureus (MRSA). The appearance of pseudomulticellular bacteria in the treated cells and the synergistic effect of the plant extract with beta-lactamase-susceptible penicillins suggest that the extract may interfere with staphylococcal enzymes including autolysins and beta-lactamase [15]. Some fraction of *Q. infectoria* extracts have also been shown to be effective against verocytotoxin producing enterohemorrhagic *E. Coli* (VTEC) making them potential promising candidate for natural food additive for the control of food poisoning by *E. coli* as well as other VTEC srtains. [16] Wan Nor Amilah, et al. (2014) have also suggested the potential use of *Q. infectoria* gall extracts as one of the effective phytotherapeutic agents in the treatment of multi drug resistant (MDR) bacterial infections. [5]

Findings of the present study showed that ethanol extract from the galls of *Q. infectoria* was most effective as antimicrobial for *P. aeruginosa.* This is in accordance with a study by Nimri, et al. (1999) [17] that one of the most susceptible bacteria to the effect of the ethanol extract from the galls of *Q. infectoria* was *P. aeruginosa.*

The antimicrobial properties of galls are mainly due to the presence of tannin which is the major constituent present in *Q. infectoria* [18]. Tannin is a hydrophilic compound usually extracted from aqueous and hydrophilic organic solvent and inhibits microbial growth by forming complex molecules with microbial enzymes as well as membranes of microorganisms, altering the bacterial metabolism by inhibition of oxidative phosphorylation and reducing iron concentration through precipitation with various nitrogen containing groups of protein (Scalbert, 1991). [19]

Conclusion

In the present investigation, aqueous and ethanolic extract *Q. infectoria* were evaluated for phytochemical screening and anti-microbial activity against *E. Coli*, *P. aeruginosa* and *S. aureus* and it was found that both the extracts have high anti-bacterial activity against the studied bacteria with better anti-bacterial activity for ethanolic extract. The current study suggests potential of *Q. infectoria* as anti-bacterial agent with need of further studies on bioactive compounds in galls of *Q. infectoria* responsible for antibacterial activity.

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