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### PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATION OF GALLS OF *QUERCUS INFECTORIA*

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#### ABSTRACT

The galls of *Quercus infectoria* Olivier's was used in the treatment of skin infections. Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. The aim of the study was to find out the bioactive chemical constituents and to evaluate the antimicrobial activity of the aqueous, ethanolic and petroleum ether extracts of traditionally used medicinal plant, the galls of *Quercus infectoria*. A qualitative phytochemical analysis indicates the presence of alkaloids, glycosides, phenolic compounds, flavonoids, tannins and proteins and amino acids. The result of antimicrobial susceptibility assay showed promising evidence for the antimicrobial effects of Gall of *Quercus infectoria*. Ethanolic extract of Gall of *Quercus infectoria* showed maximum inhibition and the MIC values of ethanol extracts against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* were, respectively, 0.0391, 0.0781, 0.0391 and 0.0195 mg/mL. Whereas the MIC values of the extract (0.0391 mg/mL) against both the organisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, were the same. On the other hand, the MBC values of ethanol extract against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, were, respectively, 0.0195, 0.0391 and 0.0195 mg/mL. Whereas the MFC value of ethanol extract against *Candida albicans* was much lower, that is 0.0098 mg/mL. This scientific studies has revealed its potential to provide an alternative for modern medicinal products as well as cosmetics and skin care products.

#### KEY WORDS

Phytochemical screening, Plant extracts and Zone of inhibition.

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#### INTRODUCTION

Topical antimicrobial therapy is one of the most important methods of skin infections. The goal of topical antimicrobial therapy in skin infections is to control microbial colonization and subsequent proliferation thus promoting the healing of the wounds<sup>1</sup>.

*Quercus infectoria* is one of such plants employed by herbalists in the treatment of skin infections. In this research, *Quercus infectoria* was studied in order to investigate its antimicrobial properties. *Quercus infectoria* is a small tree or a shrub belonging to the Fagaceae (Quercaceae) family.

Gall of *Quercus infectoria* (QI), or better known as Manjakani, is originated from Western Asia and Southern Europe. Galls are irregular plant growth, which is stimulated by the reaction between plant hormones and powerful growth regulating chemicals produced by insects or mites<sup>2</sup>. The QI galls are produced by the insect, *Cynips quercufolii*, for depositing its eggs<sup>3</sup>.

The bioactivity and medicinal properties of herbal extracts are dependent on the presence of the chemical compounds. The chemical constituents of the galls have been reported to comprise a large amount of tannins and small amounts of free gallic acids, ellagic acid and synergic acid<sup>4,5</sup>.

<sup>6</sup>Rohana et al. (2004) reported that the QI galls aqueous extract showed high potential in skin whitening and antioxidant properties as the extract inhibited the superoxide and DPPH radical scavenging activities, and tyrosinase activities. The hydrolysable tannins including tannic acid and gallic acid are powerful astringent that are prescribed in diarrhoea.

The purpose of this study was to evaluate the *in vitro* antimicrobial activity of aqueous, ethanol and petroleum ether extracts and do the preliminary phytochemical evaluation to find out the responsible phytoconstituents in the above mentioned extracts.

## MATERIAL AND METHODS

### Preparation of extracts

The galls of *Q. infectoria* used in this study were collected from local drug store. The galls were washed with distilled water, and dried in air. The galls were crushed in mechanical mortar. Aqueous, acetone and ethanol extractions were performed by the following method. 50 gm of gall powders were used with 300 ml of solvents with an extraction period 24-72 hours. The extracts were filtered using filter paper and the solvents were evaporated using

rotary distillation apparatus. In order to obtain a completely dry extract, the resultant extracts were transferred to glass dishes, and were left in 50°C ovens for 24 hours. Then, they were left at 4°C until assessment of their antimicrobial activities<sup>7</sup>.

### Phytochemical Screening

The dried methanolic extract was analyzed for various phytoconstituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin by the following procedure:

#### Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added<sup>8</sup>. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent<sup>9</sup>. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

#### Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer<sup>8</sup>.

#### Terpenoid and steroid

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids<sup>8</sup>.

#### Flavonoid

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric

acid was added and red color was observed for flavonoids and orange color for flavones<sup>8</sup>.

#### **Tannins**

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins<sup>10</sup>.

#### **Determination of Antimicrobial Action**

##### **Microorganisms used**

The test organisms used were *Staphylococcus aureus* (ATCC29737), *Escherichia coli* (ATCC2068), *Pseudomonas aeruginosa* (ATCC9027), *Group A. Streptococcus* and *Candida albicans* (ATCC10231).

##### **Inoculum**

The microorganisms were inoculated into soybean casein broth (SBCB) and incubated at  $35 \pm 2^\circ\text{C}$  for 4 h. The resultant turbid suspension was diluted with SBCB to match with 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately  $3.0 \times 10^8$  CFU/ml.

##### **Agar diffusion assay**

Modified agar well diffusion method was employed. Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately  $60^\circ$  each time to ensure even distribution of the inoculum. As a final step, the rim of the agar was also swabbed. After allowing the inoculum to dry at room temperature, 6-mm-diameter wells were bored in the agar. Each extract was checked for antimicrobial activity by introducing 100  $\mu\text{L}$  of 4000 $\mu\text{g}/\text{ml}$  concentration into triplicate wells. Simultaneously, gentamicin sulfate (*S. aureus*, *P. aeruginosa*, and *E. coli*), and nystatin (*C. albicans*) were used as positive controls at a concentration of 1.0  $\mu\text{g}/\text{ml}$  and the dilution medium for the positive controls was sterile distilled water, ethanol and petroleum ether. The plates were allowed to stand at room temperature for 1hr for the extract to diffuse into the agar and then they were incubated at  $35 \pm 2^\circ\text{C}$  for 24 h, except *C. Albicans* which was incubated at  $29 \pm 2^\circ\text{C}$ .

#### **Determination of Minimal Inhibitory Concentration (MIC), Minimal Bactericidal Concentration (MBC) and Minimal Fungicidal**

The MIC was determined by micro-broth dilution methods. The reconstituted drug was serially diluted two fold in Mueller-Hinton broth (Oxoid) medium. Duplicate tubes of dilution ranging from 0.025 mg/ml to 25.6 mg/ml were inoculated with  $5 \times 10^5$  cells (CFU) of the test bacterial strain and cultures incubated at  $37^\circ\text{C}$  for 18 h. MIC was taken as the highest dilution (least concentration) of the drug showing no detectable growth.

MBC and MFC were determined by sub-culturing the test dilution in a fresh drug-free solid medium and incubating further for 18-24 h. The highest dilution that yielded no single bacterial and fungal colony on a solid medium was taken as MBC and MFC respectively.

#### **RESULTS AND DISCUSSION**

The results of preliminary phytochemical screening from Table No.1 show that extracts of Gall of *Quercus infectoria* revealed the presence of alkaloids, tannins, phenolic compounds, Fat and Lipids, Resins, proteins, glycosides and flavanoids. It will be noted that the ethanol extracts give more positive tests for different chemical constituents. The traditional healers make use of water primarily as a solvent but this study has shown that the ethanol extract of Gall of *Quercus infectoria* contains more of the pharmacologically active secondary metabolites. This is because most of these secondary metabolites being organic in nature are soluble in ethanol.

The result of antimicrobial susceptibility assay from Table No.2 showed promising evidence for the antimicrobial effects of Gall of *Quercus infectoria* against bacterial (*S. aureus*, *P. aeruginosa*, *E. coli*) and fungal (*C. albicans*) pathogens. Methanolic extract of Gall of *Quercus infectoria* showed maximum inhibition 28 mm against *C. albicans* than other *Quercus infectoria* extracts. Ethanolic

extract of Gall of *Quercus infectoria* showed maximum inhibition 24mm against *Staphylococcus aureus* than other Gall of *Quercus infectoria* extracts. Ethanolic extract of Gall of *Quercus infectoria* showed maximum inhibition 19mm against *P. aeruginosa* than other Gall of *Quercus infectoria* extracts. Extract of Gall of *Quercus infectoria* showed maximum inhibition 28mm against *C. albicans*, by this finding it may be suggested that Gall of *Quercus infectoria* have highest antimicrobial properties.

The MIC values of the ethanol extract from the galls of *Q. infectoria* are shown in Table No.3. Thus MICs, MBC and MFC assay are capable of verifying that the compound has antimicrobial activity and that it gives reliable indication of the concentration of drug required to inhibit the growth of microorganisms. The MIC values of ethanol extracts against *Staphylococcus aureus*

(ATCC29737), *Escherichia coli* (ATCC2068), *Pseudomonas aeruginosa* (ATCC9027), *Candida albicans* (ATCC10231) were, respectively, 0.0391, 0.0781, 0.0391 and 0.0195 mg/mL. Whereas the MIC values of the extract (0.0391 mg/mL) against both the organisms *Staphylococcus aureus* (ATCC29737), *Pseudomonas aeruginosa* (ATCC9027), were the same. On the other hand, the MBC values of ethanol extract against *Staphylococcus aureus* (ATCC29737), *Escherichia coli* (ATCC2068), *Pseudomonas aeruginosa* (ATCC9027), were, respectively, 0.0195, 0.0391 and 0.0195 mg/mL. Whereas the MFC value of ethanol extract against *Candida albicans* (ATCC10231) was much lower, that is 0.0098 mg/mL. This scientific studies has revealed its potential to provide an alternative for modern medicinal products as well as cosmetics and skin care products.

**Table No.1: Phytochemical analysis of Gall of *Quercus infectoria* extracts**

S.No	Phytochemical composition	Aqueous Extract	Ethanol Extract	Pet ether Extract
1	Alkaoids	+	+	-
2	Flavonoids	+	+	-
3	Phenolic	+	+	+
4	Tannins	+	+	+
5	Glycosides	+	+	-
6	Protens and Aminiacid	+	-	-
7	Resins	-	+	-
8	Carbohydrtes	-	-	-
9	Fat and Lipids	-	+	+

**Key:** + = detected, - = Not detected

**Table No.2: Antimicrobial activity of Gall of *Quercus infectoria* extracts**

Plant Extract	ZONE OF INHIBITION in mm(Mean± SD, n=3)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas</i>	<i>Candida albicans</i>
Aqueous extract	23±0	13±0	17±0	20±0
Ethanol extract	24±0	18±0	19±0	28±0
Pet. Ether extract	16±0	12±0	12±0	17±0
Gentamycin(1.0/ml)	22±0.0	21±0.0	23±0.0	NT
Nystatin (1.0-/ml)	NT	NT	NT	16±0.0
AQ, ET, PT	-	-	-	-

**Table No.3: MIC, MBC and MFC Values of ethanol extract of Gall of *Quercus infectoria***

S.No	Concentration (mg/mL)	<i>Staphylococcus aureus</i> (ATCC29737)		<i>Escherichia coli</i> (ATCC206)		<i>Pseudomonas aeruginosa</i> (ATCC9027)		<i>Candida albicans</i> (ATCC10231)		Control	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC		
		1	5.0000	-	-	-	-	-	-	-	-
2	2.5000	-	-	-	-	-	-	-	-	+	-
3	1.2500	-	-	-	-	-	-	-	-	+	-
4	0.6250	-	-	-	-	-	-	-	-	+	-
5	0.3125	-	-	-	-	-	-	-	-	+	-
6	0.1563	-	-	-	-	-	-	-	-	+	-
7	0.0781	-	-	+	-	-	-	-	-	+	-
8	0.0391	+	-	+	+	+	-	-	-	+	-
9	0.0195	+	+	+	+	+	+	+	-	+	-
10	0.0098	+	+	+	+	+	+	+	+	+	-
11	0.0049	+	+	+	+	+	+	+	+	+	-

## CONCLUSION

From the study it was found out that various bio-active constituents like Alkaloids, Flavanoids, Glycosides etc were present. The MIC, MBC and MFC values shows that, the compound has marked Anti-fungal and Anti-bacterial activity in terms of inhibition of fungal and bacterial growth *In-vitro*. It can be concluded that the potential of *Quercus infectoria* can be used for the preparation of pharmaceutical formulations.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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