Research Article

Phytochemical Screening, Formulation and Evaluation of Dried Galls of *Quercus Infectoria Oliv*

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ABSTRACT

Quercus infectoria oliv is an important medicinal plant of family Fagaceae comprises of galls called Oak galls. This is one of the traditionally used plant in Asian countries in the treatment of mouth ulcers, sores, and fungal infections. It is used as astringent, in anti-diarrhea preparations, ulcerative colitis and its dry extract is used as analgesic, hyperglycemic and has sedative hypnotic efficacy. The present study was aimed to carry out the phytochemical screening, antibacterial activity and formulation development of aqueous extract of the galls of *Quercus infectoria* oliv. The present study involved the collection, authentification, organoleptic, physicochemical, and gravimetric evaluation, soxhlet extraction of dried powdered galls of *Quercus infectoria* oliv using distilled water, preliminary phytochemical screening, determination of MIC and antibacterial activity of the aqueous extract by agar well diffusion assay followed by formulation of an antibacterial gel and its antibacterial evaluation. The major constituent of the galls are gallotannic acids and carbohydrates, proteins, amino acids, saponins, phenolic compounds and tannins were found in small amounts. Aqueous extract of *Quercus infectoria oliv* and the formulated gel showed significant antibacterial activity against *Psedomonas aeruginosa* spp. It was found that the galls are rich in tannins which mainly contribute the antibacterial property. The aqueous extract of *Quercus infectoria* oliv and the formulated gel have significant antibacterial activity. The gel was found to be non irritant to skin on application.

Keywords: Antibacterial activity, Phytochemical, *Pseudomonas aeruginosa,* Quercus *infectoria oliv.*

INTRODUCTION

Quercus infectoria oliv is an important medicinal plant of family Fagaceae comprises of galls called Oak galls is one of the traditionally used plants in Asian countries in the treatment of mouth ulcers, sores, fungal infections and other skin inflammations.[1] It is used as an astringent, in anti-diarrheal preparations, and in ulcerative colitis. Its dry extract is used as analgesic, hyperglycemic and has sedative hypnotic efficacy. The bark of the plant and acorns are astringent, they are used in the treatment of intertrigo, impetigo, and eczema.[2]

Quercus infectoria oliv is an evergreen shrub growing up to 1.8m. The galls are excrescences on the twigs, resulting from insect stack on the growing and rudimentary leaves. The galls are upto 4cm long. Oak galls are produced by female gall wasps (Andricus) of family Cynipidae who lays its eggs inside leaf buds.[3] Larva hatches and feed upon tissues of plant and secrets a peculiar fluid from its mouth that can stimulate the cells of the tissues to a rapid division and abnormal development resulting in the formation of galls.[4,5] The plant galls mostly develop directly after the female insect lays the eggs. The inducement for the gall formation is largely unknown; discussion speculates as to chemical, mechanical, and viral triggers. The hatching larvae nourish themselves with the nutritive tissue of the galls. Galls from which the wasps escape have lesser amounts of gallotannic acids. Gall wasps also called gallflies which reproduce partly by pure two-sex propagation and partly by parthenogenesis in which a male is completely unnecessary.[6]

MATERIALS AND METHODS

Reagents and materials

Mayer's reagent, Dragendroff's reagent, Wagner's reagent, Hager's reagent, Molisch's reagent, Barford's reagent, Fehlings A and B reagent, Liebermann Burchard reagent, Ninhydrin reagent, Million's reagent, Ferric chloride solution, Sodium hydroxide**.**

Collection and Cultivation

The dried galls were obtained from a local market in the month of August and authenticated by a botanist Dr.L.JOSE, Associate Professor and Head of Botany Division, St.Alberts College Ernakulam. The organoleptic characters like colour, odour, taste etc of the galls of the plant were studied.

Physicochemical evaluation

Fineness of the Powder

The fineness of the gall powder was classified according to norminal aperture size expressed in µm of the mesh of seize through which the powder will pass.

Foreign matter

Macroscopic examination was employed to determine the presence of foreign matter. Weighed air dried sample of *Quercus infectoria* oliv (50gm) was taken and spread into a thin layer. The foreign matter was sorted into groups by visual inspection, using a magnifying lens (10

X). Sift remainder of the sample through sieve no: 250. The dust was considered as mineral admixture.

Moisture Content

About 1.5 gm of the powdered drug was transferred into a weighed flat and thin porcelain dish. It was kept in an oven at 100-105°c for an hour. Then cooled in a desiccator and weighed again. The loss of weight was recorded as moisture content (WHO1988).

Extraction

The dried galls were crushed into small pieces by using a mortar and pestle. Further size reduction was carried out by using an electric grinder. 250gms of the powdered drug was accurately weighed. The extraction was carried by soxhelation of the drug for seventy two hours using 600 ml water as solvent till the drug was exhausted. The extract was evaporated periodically to check the exhaustion of the drug.

Preliminary phytochemical screening of crude extract

The aqueous extract of *Quercus infectoria* was taken and then subjected to qualitative test for the identification of the plant constituent. Phytochemical analysis was done to find out the constituents such as carbohydrates, reducing sugars, amino acids, proteins, alkaloids, glycosides, flavonoids, phenolic compounds and tannins.

Test for Alkaloids

The extracts were treated with few drops of concentrated hydrochloric acid and filtered. The filtrate was tested with the following reagents:

Mayer's Test

A few drops of the solution were poured into the centre of a watch glass. Mayer's reagent is added in drops to the sides of the watch glass with the help of a glass rod and observed for the gelatinous white precipitate.

Dragendroff's Reagent

A few drops of Dragendroff's reagent were added to the extract. The orange brown precipitate indicates the presence of alkaloids.

Hager's Test

To the extract added a few drops of Hager's reagent (saturated solution of picric acid). A yellow precipitate indicates the presence of alkaloid.

Wagner's Test

To the extract added few drops of Wagner's reagent (dissolved 2g of iodine, 6g of KI in 100 ml water) and observed for the reddish brown precipitate**.**

Test for glycosides

The extracts were hydrolyzed with hydrochloric acid for few hrs in a water bath and the hydrolysate was subjected to various tests.

Legal's Test

To the hydrolysate, 1 ml of pyridine and few ml of sodium nitroprusside solution were added and was made alkaline with sodium hydroxide solution. A pink to red colour indicates the presence of glycosides.

Borntrager's Test

The hydrolysate was treated with chloroform and the chloroform layer was separated .To this, equal quantity of dilute ammonia solution was added. Note the presence of pink or red ammonical layer.

Test for Carbohydrates

Molisch's Test

To 2-3 ml of the extract added Molisch reagent (α-naphthol in alcohol). Shaken well and added conc. sulphuric acid from the sides of the test tube. Noted the presence of violet ring.

Test for Reducing Sugar

Fehling's Test

Mixed 1 ml of Fehling's A and Fehling's B reagent and boiled for 1 min. Add equal volume of test solution. Heated in a boiling water bath for 5-10 min. The solution was noted for a color reaction.

Benedict's Test

Mixed equal volume of Benedict's reagent and test solution in a test tube.Heated in a boiling water bath for 5-10 min. The green, yellow or red precipitate indicates the presence of reducing sugar.

Barfoed's Test

Equal volume of barfoed's reagent was mixed with test solution. Heated for 1-2 min in a boiling water bath and cooled. The presence of red precipitate was noted.

Test for Phenolic compounds

100 mg of the extract was boiled with 1 ml of distilled water and filtered. The filtrate was used for the following tests.

Ferric chloride test

To 2 ml of filtrate, 2 ml of 1% ferric chloride solution was added in a test tube. Bluish green color indicates the presence of phenolic compounds

Test for tannins

- To the extract 1 ml of 5% ferric chloride solution was added, formation of bluish black or greenish black precipitate indicates the presence of tannins. To the extract added few drops of 1% lead acetate. A yellowish precipitate indicates the presence of tannins.

Test for Flavonoids

- Extract was treated with few drops of aqueous sodium hydroxide solution and noted for the yellow color which becomes colorless on the addition of dilute acids.
- Extract was treated with concentrated sulphuric acid. A yellowish to orange colour indicates the presence of flavonoids.

Shinoda Test

The extract was dissolved in alcohol, pieces of magnesium were added followed by concentrated hydrochloric acid drop wise and heated. The presence of pink colour indicated the presence of flavonoids.

Determination of Proteins and Amino acids

Xanthoprotein Test

To 1ml of the extract, 1ml of concentrated sulphuric acid was added. A white precipitate which on boiling turns yellow. On addition of ammonium hydroxide yellow precipitate turns orange which indicates the presence of proteins.

Ninhydrin Test

To the extract, 0.25% w/v of Ninhydrin reagent was added and boiled for few minutes. A purple colour indicated the presence of proteins.

Macroscopy

Galls are spherical or pear shaped, hard and brittle having 1.2 to 2.5cm in diameter They have a short basal stalk and numerous rounded projections on the upper part of the gall; they usually sink in water; surface is smooth rather shining, bluish green, olive green or white brown, a few galls show the escape route of the insect, in the form of a small rounded hole leading to cylindrical canal which passes to the centre of the gall. The taste of the drug is astringent, followed by sweetness. The average weight of ten galls picked at random should not be less than 2.5gms.

Microscopy

Transverse section of the gall shows an outer zone of small thin walled, irregularly shaped parenchymatous cells. Oval shaped sclerenchymatous cells are arranged as a ring in the center. Also small thick walled parenchymatous cells were present in the center zone. The outer zone of parenchyma has 3 types of cells arranged as layers. The uppermost cells are small, irregular and thin walled. Middle cells are large and oval in shape. Innermost cells are long parenchymatous cells, all having intercellular spaces. Vascular bundles consisting of xylem and phloem are irregularly distributed. Around the central cavity, sclerenchymatous cells are arranged as a ring. They vary in size and shape. Rectangular, ovoid, elongated and thick walled scleroses having pits and large lumen usually filled with dense brown material. Rosette crystals of calcium oxalate are present in the outer and middle region and prismatic crystals in the inner parenchymatous cells. Starch grains are either simple or compound with central hilum. Simple grains are present abundantly in the innermost zone of the parenchyma.[3]

Gravimetric evaluation

Ash value

The total ash, water soluble ash and acid insoluble ash was used to measure the total amount of the material remain a preening after the ignition. This includes both physiological ash which is derived from the plant tissue itself, which is the extraneous matter adhering to the plant surface.

Total ash

Place about 2 to 4gm of the ground air dried material accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). The material was spreaded as an even layer and was ignited by gradually increasing the heat to 500 to 600°C until it is white, indicating the absence of carbon. Collected in desiccators and weighed if carbon free ash cannot be obtained in this manner, cooled the crucible and moistened the residue with about 2 ml of water or a saturated solution ammonium nitrate or the residue is dried on a water bath on a hot plate and ignited to constant weight. Allow the residue to cool in suitable desiccators for 30min then weighed without delay. The content of total ash in milligram or gram of air dried material is calculated.

Acid insoluble ash

It is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present especially sand. To the crucible containing the total ash, added 25ml of hydrochloric acid (70 gram per liter) and covered with a watch glass and boiled for 5 min. Rinsed watch glass with 5 ml of hot water and pour this liquid to the crucible. Collected the insoluble i.e.; matter using filter paper and washed with hot water until filtrate is neutral. Transfer the filter paper containing insoluble matter to the crucible dried on a hot plate and it is ignited to constant weight. Allow the residue to cool in a suitable desiccators for 30 min .Then weighed without delay. The content of acid insoluble ash in milligram or gram of air dried material is calculated.

Water soluble ash

It is the difference in weight between the total ash and the residue after treatment. To the crucible containing the total ash added 25 ml of water and boiled for 5 minutes. Collect the insoluble matter in a sintered glass crucible or a filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not increasing 45°C. Subtract the weight of this residue in milligram from the weight of total ash. The water soluble ash value was calculated.

In-Vitro Screening of Aqueous Extract for Anti Bacterial Activity of *Quercus Infectoria Oliv*

Agar well diffusion assay

The antibacterial activity of the extract was determined by agar well diffusion method. Briefly, overnight bacterial culture were diluted in the Mueller-Hinton broth to obtain a bacterial suspension of 10^8 CFU/ ml. Petri plates containing 20ml of Muller-Hinton broth Agar media were inoculated with 100µl of diluted cultures by spread plate technique and were allowed to dry in a sterile chamber. 5mm well was cut using a cork borer on the surface of the inoculated agar. The gall extract were filtered, sterilized using 25ml syringe filter loaded into wells and were allowed to dry completely. The antibacterial activity was assessed by measuring the inhibition zone.

Determination of MIC and Zone of Inhibition

A minimum inhibitory concentration is the lowest concentration of an antimicrobial that inhibit the growth of micro-organism after 18-24hrs. The samples were tested at different concentration. Sterile NA plates were prepared and 0.1 ml of the inoculums of test organism was spread uniformly. Wells were prepared by using a sterile borer of diameter 6 mm and the samples at different concentration (1µl, 2µl, 3µl, 4µl and 5µl) were added in each well separately. The plates were incubated at 35-37^{o}C for 18-48 hours, a period of time sufficient for the growth. The zone of inhibition of microbial growth around the well was measured in cm. MIC was calculated from the fully grown plates.

Determination of microbial growth inhibitory properties by Zone of inhibition

The antibacterial activity of the sample against *Pseudomonas aeruginosa* bacteria was determined as follows. The antibacterial activity was carried out at four different concentrations (0.01 mg, 0.02 mg, 0.03mg, 0. 04mg, 0. 05mg).

Table 1: Formulation

Composition	Formulae
Crude Extract	1gm
Carbopol 940	5gm
Triethanolamine	0.06ml
Water	q.s up to 10 gm

The appropriate amount of carbopol 940 powder was powdered well using a mortar and pestle. It was then dispersed into vigorously stirred (stirred by magnetic stirrer at 1200 rpm for 30min) distilled water (taking care to avoid the formation of indispersible lumps) and allowed to hydrate for 24 hours. The dispersion was neutralized with triethanolamine to adjust the pH. The pH was then checked. Each formulation contained 10 gm of gel[7] as shown in Table1.

Evaluation of gel

The prepared gels were evaluated for physicochemical parameters like colour and odour .The clarity was examined by visual examination under a black and white background. Direct measurements were made using a digital pH meter. Viscosities were determined in a cone and plate viscometer of the gels prepared. A spindle (no.7) was rotated at 100 rpm. Samples of the gels (0.5gm) were left to settle over 30min at the assay temperature (37°C) before measurements were taken. Spreadability was determined by applying weight above the slides in which the formulation was placed, and time in seconds required to separate the slides was noted. Spreadability of formulation was reported in seconds. Spreadability was then calculated by using the formula

S=M.L/T

Where,

S=spreadability

M=weight tied to upper slide

L=length of glass slide

T=time taken to separate the slide completely from each other.[8, 9]

Extrudability was measured using a closed collapsible tube. A collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5cm ribbon of the formulation in 10 seconds was determined. The average extrusion pressure in grams was reported.[10, 11]

$$\text{Extrudability} = \frac{\text{Applied weight to extrude gel from the tube (gm)}}{\text{Area in cm}^2}$$

The formulations were tested for their homogeneity by visual appearance after the gels have been set in the container. Also a small quantity of each gel is pressed between the thumb and the index finger and the consistency of the gel is noticed whether homogeneous or not.[12]

Anti bacterial evaluation of formulated gel

The gel was tested for antimicrobial activity using agar diffusion on solid media. The inoculum was spread onto Nutrient Agar (Peptone 10g/l; NaCl 5g/l; Yeast extract 1.5g/l; agar 2%; pH 7.0) plate using a sterile swab and then spotted with 10 mg gel[13] using the microbial test strain Pseudomonas *aeruginosa* and the control used was 20 µl of *Chloramphenicol (5 mg/ml)*

RESULTS AND DISCUSSION

The galls were collected from the local market and authenticated by the botanist Dr.L.JOSE, Associate professor and Head of Botany Division, St.Alberts College Ernakulam. The organoleptic characters of the galls *Quercus infectoria* oliv was identified. The phytochemical

evaluations of the aqueous extract of galls were carried as per stated procedure which revealed the presence of tannins, carbohydrates, saponins, amino acids and proteins. Gravimetric analysis for determination of ash value of crude powder *of Quercus infectoria* oliv were performed and the values for total ash acid insoluble ash, water soluble ash were obtained as 4.27%w/w, 0.87%w/w and 1.29%w/w respectively. The antibacterial activity and MIC of the aqueous extract of *Quercus infectoria* oliv were determined by agar well diffusion assay. The MIC of the aqueous extract of *Quercus infectoria* oliv was found to be 20µg.Gel was formulated using the aqueous extract of *Quercus infectoria* oliv and various physicochemical parameters like clarity, colour, pH, viscosity, spreadability, extrudability, homogeneity were determined. The pH of the gel complied with the skin pH which was found to be 6.8. The formulated gel was found to have optimum viscosity, spreadability, extrudability i.e., 2.78 poise, 1.5 g cm/sec, 0.35 g/cm^2 respectively. The gel was found to be clear and homogenous and free from skin irritation on application. The gel was evaluated and showed significant antibacterial activity. The organoleptic characters like color, odor and taste were studied and percentage yield was calculated for 250 gm of powder which yielded 15.132 %w/w of the crude extract.

Phytochemical Evaluation

The extract showed positive results for carbohydrates, saponins, proteins, amino acids, tannins and phenolic compounds.

Table 2: Gravimetric Evaluation

Test	Observation
Total ash	4.27% w/w
Water soluble ash	1.29% w/w
Acid insoluble ash	0.87% w/w
Moisture content	4.25%w/w

In-Vitro Screening Of Aqueous Crude Extract Of *Quercus Infectoria*

Table 3: Evaluation of In-vitro Antibacterial Activity by Zone of Inhibition of aqueous Extract of *Quercus infectoria*

Microorganism	Concentration (mg)	Zone of inhibition of Test sample (cm)	Zone of inhibition of Positive sample (Chloramphenicol) (cm) (Conc. 0.1mg)
Pseudomonas aeruginosa	0.01	0	1.4
	0.02	0.2	
	0.03	0.4	
	0.04	0.5	
	0.05	0.7	

Determination of Minimum Inhibitory Concentration of the samples

The minimum inhibitory concentration of the formulation with *Pseudomonas aeruginosa* was determined and the value was found to be 20µg.

Aqueous extract of *Quercus infectoria* showed significant antimicrobial activity against *Pseudomonas aeruginosa spp* as shown in Figure 1.

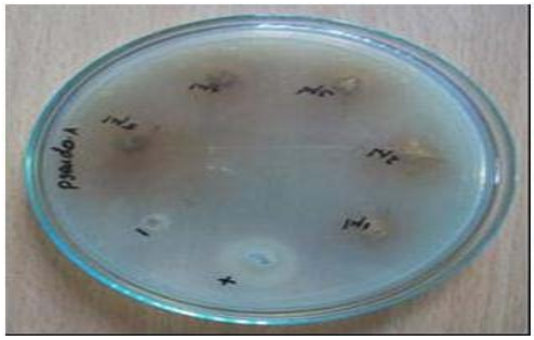

Figure 1: Photograph of antibacterial activity of different concentration of extract *Quercus infectoria* oliv using *Pseudomonas aeruginosa* and positive sample is chloramphenicol

Table 4: Evaluation of gel

Parameters	Observation
Clarity	Clear
Colour	Light brown shade
Odour	Aromatic
Ph	6.8
Extrudability	0.35g/cm^2
Spreadability	1.5 g cm/sec
Viscosity	2.78 poise
Homogeneity	Homogeneous

Figure 2: Picture of the formulated gel in an open jar

Table 5: Antibacterial evaluation of formulated gel

Test	Observation
Zone size (diameter) of test plate (sample 1), after 48 hours	2.8 cm
Zone size (diameter) of positive control plate after 48 hours	4.5 cm

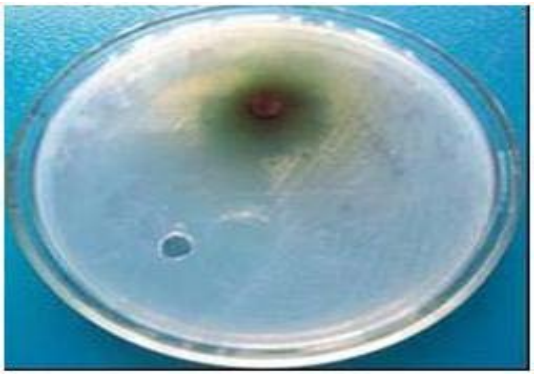

Figure 3: Photograph of antibacterial activity of gel of *Quercus infectoria* oliv using *Pseudomonas aeruginosa* and positive sample is chloramphenicol

CONCLUSION

The antibacterial activity of the aqueous crude extract of dried galls of *Quercus infectoria* was evaluated against the gram negative stain *Pseudomonas aeruginosa.* It was found that the aqueous extract possessed optimum antibacterial activity. They are used in the treatment of impetigo, eczema and intertrigo. The galls produced on the tree are strongly astringent and can be used in treatment of hemorrhages, chronic diarrhoea, dysentery etc. It is traditionally used in the treatment of mouth ulcers. Phytochemical screening was performed and was found out that it was rich in tannins. Tannins are commonly used in the leather industry for shoe preparations, ink etc. The other constituents found are alkaloids, proteins, amino acids, carbohydrates, phenolic compounds etc in moderate amounts. Tannins mainly contribute to the anti microbial activities of the drug. Gel (Figure 2) was prepared using carbopol 940 and triethanolamine as excipient which induced gelling property and adjustment of pH respectively. The gel had optimum clarity, spreadability, extrudability, viscosity thus making it an ideal formulation as shown in Table 4. The gel was found to be non irritant to the skin. The gel has significant antibacterial activity shown in Table 5 and Figure 3 posed a scope for its further research and development into suitable formulations.

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