

**INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY,  
BIOLOGY AND CHEMISTRY****Research Article****Preliminary Phytochemical Screening of *Quercus infectoria*****Olievier Fagaceae****Solanki Roshni<sup>1\*</sup> and Goyal Ramesh<sup>2</sup>**<sup>1</sup>Department of pharmacology & Toxicology, Faculty of pharmacy, Dharmasinh Desai University, Nadiad, Gujarat, India.<sup>2</sup>Institute of life sciences, Ahmedabad University, Ahmedabad, Gujarat, India.**ABSTRACT**

Herbs are growing as a medicine in wide spread diseases all over the world. Herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. *Quercus infectoria* is a well known plant of ayurveda belonging to the family Fagaceae. It consists abundant amount of hydrolysable tannins. It also contains gallic acid, ellagic acid and sitosterol in trace amount. It is well reported as astringent, anti-inflammatory, antiviral, antidiabetic, antibacterial, antiulcerogenic, gastroprotective and also shows its effectiveness in inflammatory bowel disease. In this paper primary phytochemical screening was carried out using acetone extract of galls of *Quercus infectoria*. Various parameters were screened like organoleptic and physical characterization, chemical testing, thin layer chromatography (TLC), qualitative and quantitative HPTLC.

**Keywords:** *Quercus infectoria*, TLC, HPTLC.**INTRODUCTION**

India is proud to be rich in biological diversity and tenth among the plant rich countries of Asia, sixth as far as centers of diversity especially agro diversity are concerned. *Quercus infectoria* Olivier (Fagaceae) is a small tree native of Greece, Asia Minor and Iran. The galls arise on young branches of this tree as a result of attack by the gall-wasp *Adleria gallae-tinctoria*<sup>1</sup>. The galls are locally known as anjakani in Malaysia, and are used in combination with other herbs as drinking remedy by women after childbirth to restore the elasticity of the uterine wall<sup>2</sup>. Majuphal, as it is widely known in Indian traditional medicine has been used as dental powder and in the treatment of toothache and gingivitis<sup>3,4</sup>. The galls of *Q. infectoria* have also been pharmacologically documented to possess astringent, antidiabetic<sup>5</sup>, antitremorine, local anaesthetic<sup>6</sup>, antiviral<sup>7</sup>, antibacterial<sup>8</sup>, antifungal<sup>9</sup>, larvicidal<sup>10</sup> and anti-inflammatory<sup>11</sup> activities. The main constituents found in the galls of *Quercus infectoria* are tannin (50-70%) and small amount of free gallic acid and

ellagic acid. Preliminary screening for the galls had been focused considering their widespread use.

**MATERIALS AND METHODS****Identification and Collection of Plant material**

Dried galls of *Quercus infectoria* was obtained from a commercial supplier of Ahmedabad, India. Drug was identified and authenticated by Dr. Hitesh A. Solanki, Reader Department of Botany, Gujarat University, Ahmedabad. Acetone extract of *Quercus infectoria* was prepared.

**Preparation of Acetone extract of *Quercus infectoria***

Dried and powdered galls of *Quercus infectoria* (20 g) was mixed with 500 ml of Acetone. The mixture was kept overnight. The extracted product was filtered and solvent was evaporated subsequently. A pale yellow powder was obtained with 46.6% yield.

**Organoleptic evaluation**

The organoleptic characters of the samples were evaluated such as color, odor, taste and texture.

**Determination of pH**

1% solution of *Quercus infectoria* was prepared in distilled water and pH was determined using pH meter.

**Determination of viscosity, surface tension and density**

Density, surface tension and viscosity of the 1% aqueous *Quercus infectoria* was estimated<sup>12</sup>.

**Chemical Test**

The separated compound was analyzed by chemical test with freshly prepared ferric chloride solution. 1 gm acetone extract powder was added in the 3 ml of ferric chloride solution. Change in the colour of the solution to the bluish black colour would be considered the presence of gallotannic acid.

**Thin Layer chromatography**

The technique used has been previously described<sup>13</sup>. About 10 $\mu$ l of the sample and 10 $\mu$ l of the standard were applied on silica gel plates. Thin layer chromatograms were developed by using Toluene: ethyl acetate: Formic acid (6:4:0.8 v/v/v) as mobile phase. The development was stopped when the solvent front had advanced about 7.5 cm. After drying plates in air, 10% solution of ferric was used as a spraying agent for the detection. Gallic acid in the sample was identified by comparison with the spot of the reference standard.

**HPTLC fingerprinting****[A] Preparation of stock solutions****Preparation of gallic acid standard solution**

A stock solution of standard gallic acid (1 mg/mL) was prepared by transferring 100 mg of gallic acid, accurately weighed, into a 100 mL volumetric flask, dissolving in 50 mL methanol. It was then sonicated for 10 minutes and the final volume of the solutions was made up to 100 mL with methanol to get stock solutions containing 1mg/mL. then take 0.1 ml of stock solution & dilute it upto 10ml with methanol to get 10  $\mu$ g/mL standard gallic acid solution for HPTLC.

**[B] Sample preparation**

100 mg extract of *Quercus infectoria* was taken and dissolved in methanol (10 ml) and passed through Whatman No. 1 paper (Merck, Mumbai, India). The final volume of the solution was made up to 100 mL with methanol to get stock solution containing 1

mg/mL. Successive dilution was carried out to get 10  $\mu$ g/mL of extract solution for HPTLC.

**[C] Instrumentation and chromatographic conditions**

HPTLC was performed on 20 cm  $\times$  10 cm aluminum backed plates coated with silica gel 60F254 (Merck, Mumbai, India). Standard solution of gallic acid and sample solution were applied to the plates as bands 8.0 mm wide, 30.0 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate by use of a Linomat V sample applicator equipped with a 100- $\mu$ L syringe. Ascending development to a distance of 80 mm was performed at room temperature ( $28 \pm 2^\circ\text{C}$ ), with chloroform: ethyl acetate: formic acid, 7.5 : 6: 0.5 (v/v/v), as mobile phase, in a glass chamber previously saturated with mobile phase vapor for 20 min. After development, the plates were dried with a hair dryer and then scanned at 254 nm with a TLC Scanner with WINCAT software, using the deuterium lamp.

**RESULT AND DISCUSSION**

Galls of *Quercus infectoria* were obtained from the local market as crude drug. It was crushed and subjected for extraction with acetone. The extracted product on evaporation gave pale yellow color extract. The yield obtained was 46.6%. (Figure 1).

The extract was further analyzed for various botanical parameters like colour, odor, taste & texture. The observation revealed that extract colour was Pale yellow with a pungent odor, bitter taste and fine texture. (Table 1)

Further physical characterization was done using 1% solution of the acetone extract in distilled water which revealed its density (0.99), Viscosity (1.01 cp) & surface tension (58.75) is nearer to water and pH (7-7.2 $\pm$ 0.2) was found to be neutral. Thus oral intake of drug can quietly be tolerated by the patient and good bioavailability can be obtained. (Table 2)

As galls contain rich amount of hydrolysable tannins which was confirmed by the chemical test with ferric chloride solution. It gave dark blue colour which reflects the presence of tannins in the acetone extract (Figure 2). Various chemical tests were performed for estimation of steroids, carbohydrate, alkaloids, glycosides, flavonoids, protein and saponins in acetone extract of *Quercus infectoria*. The result of chemical test showed that there is absence of steroids and triterpenoids. It reflects the absence of monosaccharides and presence of reducing sugar. Alkaloids are absent in the AEQI. There was also absence of glycosides and flavonoids. Extract showed presence of protein and absence of aminoacids. (Table 3)

As the gallotannic acid is hydrolysable tannin which, on dissolution in water hydrolysed to gallic acid. So, gallic acid was used as a standard for Thin layer chromatography. Retention factor ( $R_f$ ) value obtained for standard gallic acid was 0.36 & 0.38 with acetone extract of galls. The retention factor was same for the standard & test compound reflecting the presence of gallic acid which was formed from gallotannic acid.

The HPTLC finger printing was carried at 254 nm wavelength (Figure 3). The  $R_f$  value for standard gallic acid was 0.62 & for acetone extract of *Quercus infectoria* was 0.63. Here the identity of gallic acid in the plant extract was confirmed by overlaying the gallic acid in plant with that of the standard gallic acid. Gallic acid was found to be 0.68% w/w by HPTLC in the acetone extract. (Figure 5)

#### ACKNOWLEDGEMENT

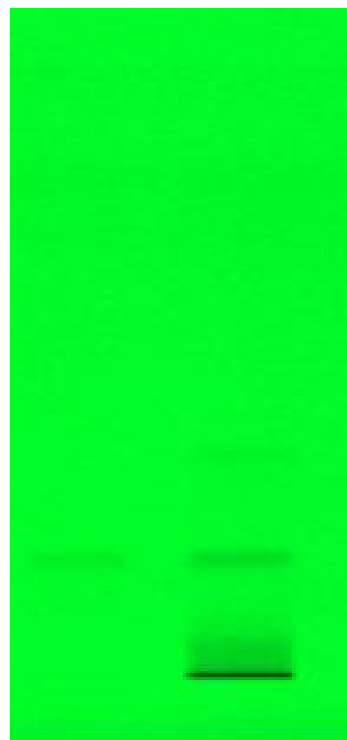
The authors are thankful to identified and authenticated by Dr. Hitesh A. Solanki, Reader, Department of Botany, Gujarat University, Ahmedabad, India for carrying out the identification and authentication of the plant.



Fig. 1: Acetone extract of *Quercus infectoria*



Fig. 2: Silica gel thin layer chromatography for *Quercus infectoria* with standard Gallic acid



A B

Fig. 3: Photograph of chromatograms obtained, at 254 nm, from gallic acid standard (A) acetone extract of *Quercus infectoria* (B)

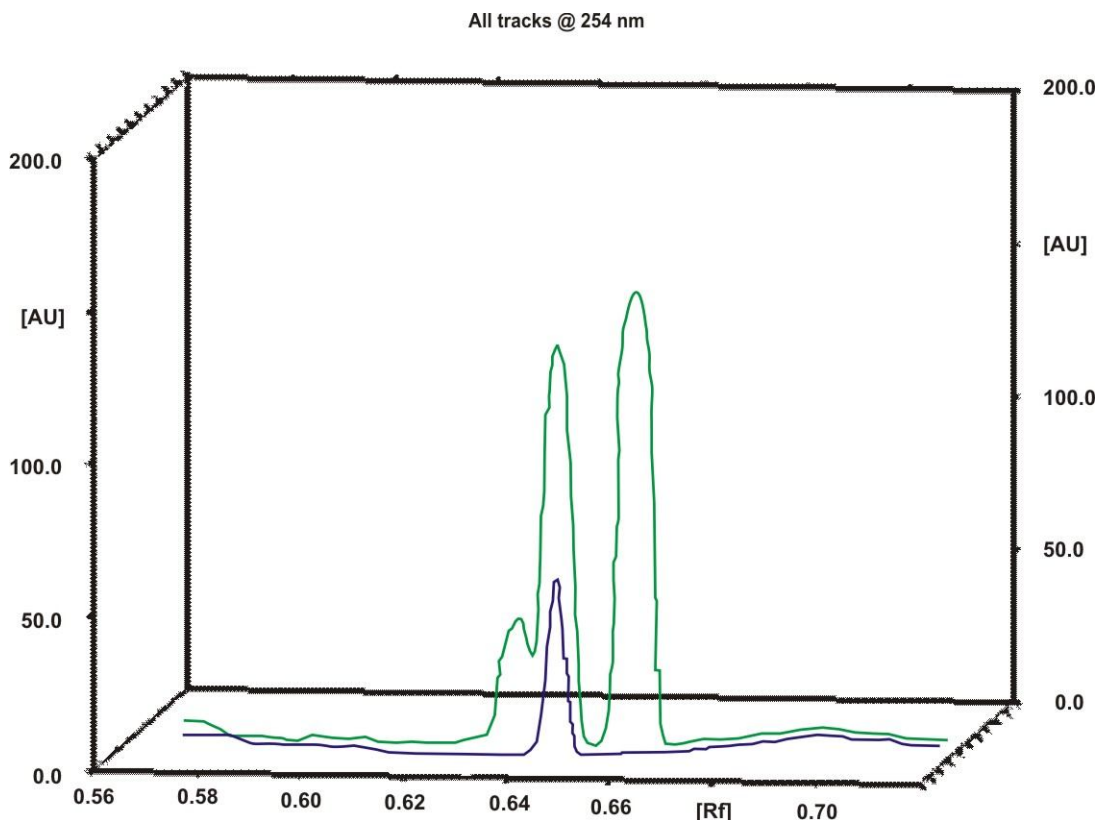


Fig. 4: Chromatogram obtained at 254 nm in Green color band for Acetone extract of *Quercus infectoria* and blue color band for gallic acid

Table 1: Organoleptic properties

Appearance	Colour	Odour	taste	Texture	Particle size
Powder	Light Brownish yellow	Pungent	Bitter	Fine	100#

Table 2: Density, Viscosity, surface tension & pH

Density	Viscosity	Surface tension	pH
0.99	1.01 cp	58.75	7-7.2±0.2

Table 3: Chemical test for acetone extract of *Quercus infectoria*

Sr. No.	Test	Result	Inference
1.	Salkowski test	Negative	Steroids absent
2.	Barfoed's Test	Negative	Mono sacharide absent
3.	Benedict's test	Positive	Reducing sugar present
4.	Fehling's Test	Positive	Reducing sugar present
5.	Mayer's test	Negative	Alkaloid absent
6.	Heger's test	Negative	Alkaloid absent
7.	Draggondroff's test	Negative	Alkaloid absent
8.	Balget's test	Negative	Glycoside absent
9.	Test with Ferric chloride	Positive (Blue colour)	Tannin's present and flavonoids absent
10.	Millon's test	Positive	Protein present
11.	Ninhydrine test	Negative	Amino acid absent
12.	Biuret test	Negative	Amino acid absent

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