Full Length Research Paper

Preliminary phytochemical screening of *Quercus infectoria* Oliv. for treatment of skin diseases

Vaibhav Vaidya^{1*}, C. B. Mahendrakumar² and Kiran Bhise³

¹Pad. Dr. D.Y. Patil College of Pharmacy, Akurdi, Pune Maharashtra- 411 044, India.

²St. Marry College of Pharmacy, Secunderabad Andhra Pradesh- 500 025, India.

³MCE Socy's Allana College of Pharmacy, Camp, Pune Maharashtra- 411 001, India.

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In present work, fruits of *Quercus infectoria* are selected on the basis of traditional claim. Fingerprinting analysis for the said extract along with the biomarker, gallic acid was performed using high performance thin layer chromatography (HPTLC). The extract and biomarker were standardized with various instrumental techniques like Newton magnetic resonance (NMR), Fourier transform infrared (FTIR) and ultraviolet (UV) spectroscopies. Preliminary phytochemical screening was performed for establishing the profile of extract for its nature of chemical composition. The extracts showed the presence of tannins, mucilage and saponins. HPTLC fingerprinting showed better separation of the components. Planer chromatogram generated was used to determine existence of present phytoconstituents. The R_f value was found to be 0.19. The extract exhibited good correlation with selected marker in different analytical techniques like NMR, FTIR and UV spectroscopies. UV-Spectroscopic analysis showed good correlation between plant extract and standard (gallic acid). The extract and biomarker exhibited 0.074, 0.0511 and 0.003, 0.0429 intercept and slope values, respectively.

Key words: *Quercus infectoria*, gallic acid, high performance thin layer chromatography (HPTLC), Newton magnetic resonance (NMR), Fourier transform infrared (FTIR), ultraviolet (UV) spectroscopy, tannins.

INTRODUCTION

The ancient literature of Ayurveda has served the wellbeing of mankind because of empirical knowledge of the observations and the experience of Ayurveda practitioner. Though traditional medicines offer a safe and inexpensive approach to treat many skin diseases, it has been neglected due to some reasons like shortcoming in treating chronic condition and unavailability of pathological data (Govindarajan, 2007; Al-Quran, 2008).

In this work, fruits of *Quercus infectoria* Oliv. (Fagaceae) which is also traditionally known as Majuphal was selected on the basis of traditional claim (Khouzami, 2009; Kaur, 2008). The reported constituents of plant are tannins, saponins and mucilage. This study has been focused on phytochemical investigation of *Q. infectoria* which has been reported as potent candidate for treating

skin diseases and inflammatory conditions (Umachigia, 2008; Leela, 2011). The existence of the aforementioned phytoconstituents is determined using proximate analysis, fingerprinting analysis of extract and biomarker using high performance thin layer chromatography (HPTLC) and other instrumental techniques like Newton magnetic resonance (NMR), Fourier transform infrared (FTIR) and ultraviolet (UV) spectroscopies.

MATERIALS AND METHODS

Plant collection and extraction

The fresh fruits of *Q. infectoria* were selected for this study. Selected plant was collected from Pune region and authenticated

*Corresponding author. E-mail: v.vaidya123@gmail.com. Tel: +91- 9423324249.

Figure 1. Fruits of *Q. infectoria*.

from Department of Botany, University of Pune, Maharashtra, India (Voucher No. Bot/34/2010) (Figure 1). Proximate analysis of powder was carried out for different physicochemical standards such as ash values, extractive values and loss on drying (Mukherjee, 2002).

The hydroalcoholic extracts in the proportion 60:40 for the selected herbal plant was prepared by simple maceration for about 72 h and concentrated and stored in air tight container (Chusri, 2009; Vermani, 2010).

Phytochemical analysis

The crude extract was subjected to preliminary phytochemical screening for the detection of various phytoconstituents (Ghafour, 2010; Wallis, 1985).

Histology

The plant specimen for the study was cut and fixed in formalin acetic acid (FAA) and was dehydrated (Sass, 1940). Infiltration was carried out by addition of paraffin wax and this paraffin embedded specimens were sectioned with the help of rotary microtome. The sections were stained with Toluidine blue and observed under microscope (O'Brien et al., 1964). Photographs of different magnifications were taken with Nikon Laboratory Photo 2 Microscopic Unit with the help of plane polarized light for the study of various organs (Esau, 1964).

Fingerprinting and spectroscopic analysis

The crude extract of selected plant was analyzed for HPTLC fingerprinting using Camag HPTLC system equipped with an automatic TLC sampler and TLC scanner with a UV cabinet. Planer chromatograms of samples prepared using general solvent system Toulene:Chloroform:Ethanol (8:8:2) and solution prepared as 100 mg in 5 ml of methanol. UV-Spectroscopic analysis of extract and biomarker was performed using Shimadzu 1700 UV spectrophotometer. NMR as well as FTIR investigation was performed on extract and biomarker (Jamil, 2012; Rodriguez, 2008; Carmen, 2008).

Table 1. Preliminary Phytochemical Screening of *Q. infectoria*.

S/N	Nature of constituent	Sample (<i>Q. infectoria</i>)
1	Alkaloids	-
2	Carbohydrates	+
3	Flavonoids	-
4	Glycosides	-
5	Lipids	+
6	Mucilage	+
7	Phytosterols	-
8	Proteins and amino acids	-
9	Saponins	+
10	Tannins	+
11	Volatile oils	-

RESULTS

Pharmacognostic study

The various physicochemical standards such as ash values, extractive values and loss on drying was performed. Preliminary phytochemical screening was performed for establishing the profile of extract for its nature of chemical composition (Soon et al., 2007; Aroonrerk, 2009).

Phytochemical analysis

Preliminary phytochemical screening was performed for establishing the profile of extract for its nature of chemical composition. The extracts showed the presence of tannins, mucilage and saponins. The results are listed in Table 1.

Histology

It shows the pericarp of around 250 μm thick and consist of thin walled parenchymatous cells somewhat radially oblong and possess dense accumulation of tannins. Mesocarp shows thin layers of parenchyma with the vascular strands including masses of fibers, xylem and phloem. The endocarp shows columnar sclerides. The cotyledon is seen in parallel longitudinal sections. It is bilobed and embryo is placed in wide longitudinal cylindrical chamber (Figures 2 to 4).

Fingerprinting and spectroscopic analysis

HPTLC fingerprinting showed better separation of the components. Planer chromatogram generated was used to determine existence of present phytoconstituents. The R_f value was found to be 0.19 (Graph 1 and Figure 5).

Figure 2. Histology showing the pericarp region.

Figure 3. Entire section of fruit.

UV calibration curve was plotted for plant extract as well as biomarker, gallic acid at 275 and 260 nm, respectively. UV-Spectroscopic analysis showed good correlation between plant extract and standard (gallic acid).

Table 2. Calibration of *Quercus infectoria* at 275 nm.

Concentrations (mcg/ml)	Absorbances
0	0
15	0.407
30	0.798
60	1.568
90	2.161
120	2.763

Table 3. Calibration curve of gallic acid at 260 nm.

Concentrations (mcg/ml)	Absorbances
0	0
2	0.101
4	0.215
6	0.313
8	0.399
10	0.51

The extract and biomarker exhibited 0.074, 0.0511 and 0.003, 0.0429 intercept and slope values, respectively (Tables 2 and 3; Graphs 2 and 3). UV-Spectroscopic analysis showed good correlation between plant extract and standard (gallic acid). FTIR and NMR spectra were run for extract and biomarker. The extract and marker showed good correlation in FTIR.

Extract showed prominent peak values at 2364.81, 2148.77 and 1915.38 cm^{-1} whereas gallic acid showed peak at 2048.47 cm^{-1} (Graphs 4 and 5). Similarly, NMR also exhibited good correlation for sample and biomarker (Graphs 6 to 9).

DISCUSSION

Thus in recent years there is spurt in the interest regarding survival of Ayurvedic forms of medication due to shortcomings of modern medicines. Tannins serves as natural defense mechanism against microbial infections can also be used in some inflammatory conditions (Abdul, 2005; Adel, 2010). Phytochemical analysis of hydroalcoholic extract of fruits of *Q. infectoria* showed the presence of tannins (Basri, 2004) which is confirmed by fingerprinting and spectroscopic analysis. The study can be further extended in formulation of this potent candidate for treatment of skin diseases as tannins act as free radical scavengers as well as antimicrobial agents.

Figure 4. Endocarp showing columnar sclerides.

Figure 5. HPTLC band of *Q. infectoria*

Track 2, ID: Q.infectoria

Graph 1. Planer Chromatogram of *Quercus infectoria*. Graph 2. Calibration Curve of *Quercus infectoria* at 275 nm.

Graph 3. Calibration curve of gallic acid at 260 nm.

Graph 4. FTIR Spectra of *Q. infectoria*.

Graph 5. FTIR Spectra of Biomarker (Gallic acid).

Graph 6. ^1H NMR of gallic acid.

Sample Name:
STD-GALIC-ACID
Data Collected on:
Varian-SMR-mercury300
Archive directory:
Sample directory:
Fidfile: CARBON
Pulse Sequence: CARBON (zgpg3)
Solvent: dms
Data collected on: Nov 8 2011

STD-Gallic
acid
DYPati

Graph 7. Carbon NMR of Gallic acid.

Sample Name:
EXT-S-D-2
Data Collected on:
Varian-SMR-mercury300
Archive directory:
Sample directory:
Fidfile: Proton
Pulse Sequence: Proton (zgpg3)
Solvent: dms
Data collected on: Nov 9 2011

Extract B
S-I
DYPati

Graph 8. NMR of *Q. infectoria*.

Graph 9. Carbon NMR of *Q. infectoria*.

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