WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 11, 1648-1655. Research Article ISSN 2277– 7105

INVESTIGATION OF *QUERCUS INFECTORIA* OLIVIER GALLS FOR ANTI-ARTHRITIC ACTIVITY

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Article Received on
11 July 2021,

Revised on 01 August 2021,
Accepted on 21 August 2021

DOI: 10.20959/wjpr202111-21408

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ABSTRACT

We investigated the ethanolic and aqueous extract of *Quercus infectoria* Olivier galls (local names: aleppo oak, majuphal, manjakani, masikai, maayaaphala) collected from the local market of Calicut for its anti-arthritic activity. The anti-arthritic activity was determined using various assays which include inhibition of protein denaturation, effect on membrane stabilization and proteinase inhibitory action. The ethanolic extract of *Quercus infectoria* gall showed a higher activity of inhibition of protein (bovine serum albumin) denaturation (58.1%), HRBC membrane stabilization (70.9%) and proteinase inhibitory action (52%) at a concentration of 100µl. Overall, the ethanolic extract had higher anti-arthritic activity than in the aqueous extract. We conclude that the *Quercus infectoria* galls investigated in this study are useful in the treatment of arthritis.

KEYWORDS: Arthritis, *Quercus infectoria*, Gall, Ethanolic extract.

INTRODUCTION

Traditional herbal medicines are naturally occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. Traditional herbal medicines are getting significant attention in global health debates.[1]

Natural products and their derivatives have continued to be the most significant sources of new leads into the development of new pharmaceutical agents. Approximately 25% of modern medications have been derived from previously used plant remedies.[2]

Quercus infectoria galls, locally known as "Manjakani" is one of the popular medicinal plants used to treat various ailments. It is a deciduous, small tree or shrubs that grow only up to the height of 2 metres and is mainly found in Asia, Greece, and Iran.

The galls of *Q. infectoria* are round-shaped abnormal growth found arising on young branches of the oak tree due to the attack by the gall-wasp Adleria gallae-tinctoria. Pharmacologically, the galls have been documented to possess astringent and antibacterial properties. The astringent properties are mainly derived from tannin, the main compound constituting 50-70% of *Q. infectoria* galls.[3]

"Arthritis" literally means joint inflammation. Although joint inflammation is a symptom or sign rather than a specific diagnosis, the term arthritis is often used to refer to any disorder that affects the joints. Joints are places in the body where bones come together, such as the knees, wrists, fingers, toes, and hips. These disorders fall within the broad category of rheumatic diseases.

These are diseases characterized by inflammation (signs include redness or heat, swelling, and symptoms such as pain) and loss of function of one or more connecting or supporting structures of the body. They especially affect joints, tendons, ligaments, bones, and muscles. Common signs and symptoms are pain, swelling, and stiffness. Arthritis affects 15% of Indian population (about 180 million people).[4]

Concerns regarding the safety and costs of conventional arthritis therapies have sparked interest in natural remedies. In addition, difficulty with chronic pain management in arthritis has led to the investigation of herbal therapies. Herbs may offer a complementary or

alternative method for effective and safe treatment. Thus, the present study is aimed to investigate the anti-arthritic potential of *Quercus infectoria* galls using different assays.

MATERIALS AND METHODS

Chemicals and Reagents: bovine serum albumin, phosphate buffered saline, human red blood cells (HRBC), alsever solution, isosaline, trypsin, tris-HCl buffer, casein, perchloric acid.

Gall Collection and Authentication: *Quercus infectoria* galls were collected locally from Calicut, Kerala on February 2021. Following collection, the galls were authenticated by Dr. Sreeja P, at Sir Syed College, Thaliparamba, Kannur, Kerala, and a voucher specimen of the gall was deposited for future reference.

Extract Preparation: *Quercus infectoria* galls were cleaned and shade dried for 1 day before being ground to a coarse-fine powder in an electric grinder. The powder was then used to prepare both ethanolic and aqueous extracts.

Ethanolic extract was prepared by exhaustive extraction technique using soxhlet apparatus. About 50g of powdered drug was extracted in 400ml ethanol. A temperature of 70-78℃ was maintained throughout the extraction process. Extraction was continued for 3 days until it become colourless. The liquid extract was then distilled and evaporated to remove solvent and the residue were preserved in a dessicator.

Aqueous extract was prepared by hot maceration method. About 113g coarsely powdered drug was placed in a stoppered container with 1000ml water. Gentle heat was used during the process of extraction with frequent agitation. Extraction was continued for 7 days. The mixture was then strained and marc were pressed. The liquid extract was then evaporated to remove solvent by double boiler method and the residue were preserved in a dessicator.

Anti-arthritic Assays

Inhibition of protein denaturation

The reaction mixture consists of 0.2 ml of 5% aqueous solution bovine serum albumin, 2.8 ml of phosphate buffered saline (PBS, PH 6.4) and varying concentrations of extract. Then the mixtures were incubated at 37± 2ºC in a BOD incubator for 15 minutes and then heated at 70ºC for 5 minutes. The reaction mixture without test sample represents control. The absorbance produced by test sample is eliminated by including appropriate sample control

without albumin. After cooling, their absorbance was measured at 660nm. The Percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Test}} \times 100$$

Membrane stabilization assay (HRBC method)

The anti-inflammatory activity of gall extracts was assessed by in-vitro HRBC membrane stabilization method. Fresh whole human blood (10ml) was collected and transferred to the heparin zed centrifuged tubes. The collected blood was mixed with equal volume of Alsever solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL) and centrifuged with isosaline (0.85 %, Dissolve 8.5 g NaCl in water. Autoclave 15 minutes at 121°C. Cool to room temperature). To 1mL of HRBC suspension, equal volume of plant extracts in four different concentrations (10, 20, 50, 100 µg/mL) was added. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage of haemolysis was calculated then by the formula as given below:[5]

$$\% \text{ Membrane Stabilization} = \frac{(\text{OD of Control} - \text{OD of Test})}{\text{OD of Control}} \times 100$$

Proteinase inhibitory action

The reaction mixture (2.0 ml) contained 0.06 mg trypsin, 1.0 ml 25 mM tris-HCl buffer (pH 7.4) and 1.0 ml different fractions of extract. The mixtures were incubated at 37ºC for 5 minutes. Then 1.0 ml of 0.8% (w/v) casein was added. The mixtures were then incubated for additional 20 minutes. Then 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged and absorbance of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{OD of Control} - \text{OD of Test})}{\text{OD of Control}} \times 100$$

RESULTS AND DISCUSSION

This study of anti-arthritic activity on galls of *Quercus infectoria* depends on the type of solvent used during the extraction. Ethanol is an organic polar solvent suitable for the extraction of phenolic compounds and is safe for human consumption whereas polar inorganic solvent water is usually used for the extraction of various bioactive phytochemicals in different experiments. Our experiments indicate that the highest anti-arthritic activity can

be obtained from the ethanolic extract of *Quercus infectoria* galls when compared to the aqueous extract of the same.

Inhibition of protein denaturation

Denaturation of the protein involves the disruption of secondary, tertiary and quaternary structure of the molecules and finally leads to cell death, it occurs due to stress like a high level of salt, high temperature and high level of acidity. Denaturation of proteins is well documented as contributing to inflammatory conditions like RA. Most of the investigators have reported that denaturation of protein is one of the causes of RA due to the production of autoantigens in certain rheumatic diseases.[5]

Investigation of anti-arthritic activity of both ethanolic and aqueous extract of *Quercus infectoria* galls using this model was done in 5 different concentrations (20, 40, 60, 80, 100 µl). The detailed results are tabulated below:

Table 1: Effect of ethanolic and aqueous extracts of *Quercus infectoria* galls on heat induced protein denaturation.

Sample	Concentration (µl)	OD of Control	OD of Test	% Inhibition
Ethanolic extract	20	1.41	1.15	18.4
	40	1.41	0.95	32.6
	60	1.41	0.86	39
	80	1.41	0.71	49.6
	100	1.41	0.59	58.1
Aqueous extract	20	1.41	1.28	8.5
	40	1.41	1.19	15.6
	60	1.41	0.96	31.9
	80	1.41	0.83	41.1
	100	1.41	0.69	51

From the results of the present study it can be stated that ethanolic extract of *Q. infectoria* galls may prevent denaturation of proteins in rheumatic diseases to a larger extend than aqueous extract of the same.

Effect on membrane stabilization

Stabilizing effect on heat and saline induced erythrocyte lysis is very good index of anti-inflammatory activity and there by anti-arthritic activity. The membrane of RBC is similar to that of lysosomal membrane. In inflammatory condition stabilising the lysosomal membrane helps to prevent the release of lysosomal constituents which cause further inflammation and damage.[5]

Investigation of effect on membrane stabilization by in-vitro HRBC membrane stabilization method was carried out in 5 different concentrations (20, 40, 60, 80, 100 µl) of both ethanolic and aqueous extract of *Quercus infectoria* galls. The results are tabulated below:

Table 2: Effect of ethanolic and aqueous extracts of *Quercus infectoria* galls on HRBC membrane stabilization.

Sample	Concentration (µl)	OD of Control	OD of Test	% Stabilization
Ethanolic extract	20	0.62	0.55	11.2
	40	0.62	0.46	25.8
	60	0.62	0.35	43.5
	80	0.62	0.26	58
	100	0.62	0.18	70.9
	20	0.62	0.58	6.4
Aqueous extract	40	0.62	0.51	17.7
	60	0.62	0.41	33.8
	80	0.62	0.32	48.3
	100	0.62	0.25	59.6

From the results of above study it can be stated that ethanolic extract of *Q. infectoria* galls may stabilise the lysosomal membrane to a larger extend than aqueous extract of the same.

Proteinase inhibitory action

Proteinases have been implicated in arthritic reactions. Rich sources of proteinase are neutrophil lysosomal granules. The proteases act enzymatically to degrade the collagen and proteoglycan matrix of bone and cartilage. It was previously reported that leucocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors.[5]

In certain forms of rheumatoid arthritis trypsin is activated hence in present study trypsin was used. Proteinase inhibitory action was also carried out in 5 different concentrations (20, 40, 60, 80, 100 µl) of both ethanolic and aqueous extracts of *Quercus infectoria* galls. The results are tabulated below:

Table 3: Effect of ethanolic and aqueous extracts of *Quercus infectoria* galls on proteinase inhibitory action.

Sample	Concentration (µl)	OD of Control	OD of Test	% Inhibition
Ethanolic extract	20	1.5	1.24	17.3
	40	1.5	1.01	32.6
	60	1.5	0.91	39.3
	80	1.5	0.83	44.6
	100	1.5	0.72	52
	20	1.5	1.31	12.6
	40	1.5	1.2	20
	60	1.5	0.99	34
	80	1.5	0.91	39.3
	100	1.5	0.85	43.3

From the results above, ethanolic extract of *Q. infectoria* galls showed significant inhibition of proteinase activity than aqueous extract of the same.

Overall, our study indicates that the ethanolic extract of *Quercus infectoria* galls can produce higher activity in inhibition of protein denaturation (58.1% at 100 µl concentration), HRBC membrane stabilization (70.9% at 100 µl concentration) and proteinase inhibitory action (52% at 100 µl concentration).

We hope that the findings from this research will encourage the design of appropriate studies to identify potent anti-arthritic agents in herbs. Further study is warranted to identify the individual bioactive compounds in *Quercus infectoria* galls underlying this high anti-arthritic potential.

CONCLUSION

Our findings strongly suggest that the galls of *Quercus infectoria* Olivier are promising source of natural anti-arthritic agent as indicated by their high activity of inhibition of protein denaturation, *in-vitro* HRBC membrane stabilization and proteinase inhibitory action. On comparing the activity of both ethanolic and aqueous extracts, ethanolic extract is found to have higher anti-arthritic potential.

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