EVALUATION OF IN-VITRO ANTHELMINTIC AND ANTIMITOTIC ACTIVITIES OF METHANOLIC EXTRACT OF QUERCUS INFECTORIA OAK GALLS

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ABSTRACT:

Quercus infectoria (oak galls) has been traditionally used for its medicinal properties. This study evaluates the in vitro anthelmintic and antimitotic activities of its methanolic extract. Anthelmintic activity was assessed using adult *Pheretima posthuma*, while antimitotic activity was evaluated through *Allium cepa* root tip assays. The extract demonstrated dose-dependent anthelmintic activity, causing significant paralysis and death of worms. Antimitotic evaluation revealed a marked decrease in mitotic index and increased chromosomal aberrations, indicating disruption of cell division. Phytochemical screening confirmed the presence of polyphenols, tannins, and flavonoid compounds likely contributing to the observed bioactivities. These findings support the traditional use of oak galls in treating parasitic infections and suggest potential for the development of natural anthelmintic and antimitotic agents.

Keywords: *Quercus infectoria*, anthelmintic, antimitotic, chromosomal aberrations

INTRODUCTION:

Helminthiasis and abnormal mitotic cell proliferation are two major health burdens, particularly in tropical and subtropical regions where sanitation and healthcare access are limited. Helminth infections caused by soil-transmitted and waterborne parasitic worms affect over 1.5 billion people globally, leading to a wide range of health issues such as anemia, gastrointestinal distress, malnutrition, and impaired cognitive development (World Health Organization [WHO], 2023). On the other hand, unregulated mitotic activity is a hallmark of cancer and other proliferative disorders, highlighting the need for novel antimitotic agents to modulate cell division mechanisms (Cragg & Newman, 2013).

The emergence of resistance to existing anthelmintic drugs, along with their associated toxicity and limited efficacy, has intensified the search for safer, cost-effective, and plant-derived therapeutics (Keiser & Utzinger, 2008). Likewise, identifying natural agents that interfere with the mitotic cycle holds significant promise for cancer chemoprevention and therapy (Rishton, 2008). Medicinal plants have historically provided leads for anthelmintic and anticancer agents, owing to their diverse phytoconstituents such as alkaloids, tannins, flavonoids, and phenolics, which exhibit a broad spectrum of bioactivities (Newman & Cragg, 2020).

Quercus infectoria Olivier (Family: Fagaceae), commonly known as oak gall or gallnut, is a small deciduous tree native to Asia Minor and the Middle East. The galls, formed as a result of parasitism by cynipid wasps, are rich in hydrolyzable tannins, mainly gallotannins and gallic acid, along with ellagic acid, flavonoids, and phenolic compounds (Chusri et al., 2011). Traditionally, oak galls have been used in various indigenous medical systems for the treatment of diarrhea, hemorrhage, wound infections, sore throat, and skin diseases (Kaur et al., 2004). Pharmacological studies have demonstrated that the extract of *Q. infectoria* possesses antibacterial, antifungal, antiviral, antioxidant, astringent, and anti-inflammatory properties (Al-Mustafa & Al-Thunibat, 2008; Dwivedi & Singh, 2011).

Although preliminary studies have hinted at its antiparasitic and cytotoxic potential, systematic evaluation of *Q. infectoria* for anthelmintic and antimitotic activities remains scarce. Anthelmintic screening using earthworms (*Pheretima posthuma*) serves as a widely accepted preliminary in vitro model due to their physiological similarity to parasitic nematodes (Vidyarthi et al., 2011). Similarly, the *Allium cepa* root tip assay is a well-established cytogenetic model for assessing antimitotic and genotoxic effects of test substances based on mitotic index and chromosomal abnormalities (Fiskesjö, 1985).

The present study was designed to evaluate the in vitro anthelmintic and antimitotic activities of the methanolic extract of *Quercus infectoria* oak galls. The study also aims to correlate these bioactivities with the phytochemical constituents present in the extract. Results from this investigation may validate the traditional uses of *Q. infectoria* and provide a scientific basis for its development as a plant-based therapeutic candidate for parasitic and proliferative diseases.

MATERIALS AND METHODS

Collection and authentication of plant material: Quercus infectoria (Fagaceae) oak galls were collected from various areas of Kakinada and were identified and authenticated by Sri.K.S.N.KRISHNA, Former head of the department of Botany, P.R.Government Degree College, Kakinada**.**

Preparation of extract: The collected oak gall nuts were subjected to shade drying, and they were made into a fine powder. The powder is extracted with methanol in the ratio of 1:2 ratio for a time period of 2 weeks. After extraction, the extract was filtered, and the solvent was distilled off by using a simple distillation technique.

Identification of phytoconstituents by preliminary phytochemical tests:

1) Test for alkaloids: a) Mayer's test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids. B) Wagner's test: To the methanolic extract, add a few drops of Wagner's reagent and observe for the formation of any precipitate. The formation of reddish brown precipitate is an indication of the presence of alkaloids. c) Dragendroff's test: To the methanolic extract add a few drops of acetic acid, followed by Dragendroff's reagent. The mixture was observed for the formation of any precipitate. The formation of red precipitate is an indication of the presence of alkaloids.

2) Test for carbohydrates: A) Molish's test: To a small amount of methanolic extract, add 1ml of α-napthol and concentrated sulphuric acid was added slowly into a side test tube. The mixture was observed for the formation of any violet ring at the junction of the two liquids indicates the presence of carbohydrates. B) Fehling's test: To a small amount of methanolic extract, add an equal amount of Fehling's solution A and B. The mixture was observed for the formation of any precipitate. Formation of red precipitate indicates the presence of carbohydrates. C) Benedict's test: To a small amount of methanolic extract, add an equal amount of Benedict's reagent and heat gently. The mixture was observed for the formation of any precipitate. Formation of red precipitate indicates the presence of carbohydrates.

3) Test for glycosides: A) Brontrager's test: - (for anthraquinone glycosides) The extract was macerated with ether, and the ether layer was separated and added to aqueous ammonia. The

mixture was observed for any colour change. Appearance of pink (or violet colour in the aqueous layer is an indication of the presence of anthraquinone glycosides. B) Killer-killani test: The methanolic extract was dissolved in acetic acid containing ferric chloride, and it was transferred to a test tube containing sulphuric acid. The mixture was observed for any colour change at the junction between the two liquid layers. At the junction formation of a reddish brown colour, which gradually became is indication of the presence of cardiac glycosides. C) Legal's test: The extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides

4) Test for saponins: A) Froth test: To the methanolic extract, add 20ml of distilled water and shake in a graduated cylinder for 15 minutes. The mixture was observed for the formation of foam and the thickness foam. The formation of a two-centimeter layer of foam is an indication of the presence of saponins. B) Foam test: 0.5g of extract was shaken with 2ml of water. If foam persists for ten it indicates the presence of saponins.

5) Test for phenols: A) Ferric chloride test: To a small amount of methanolic extract, add a few drops of ferric chloride solution. The mixture was observed for the formation of any colour change. Formation of bluish green (or) bluish black colour is an indication of the presence of phenols.

6) Test for tannins: A) Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7) Test for flavonoids:- A) Alkaline reagent test: To methanolic extract was treated with a few drops of sodium hydroxide (or) ammonia. The mixture was observed for the appearance of any colour change. The formation of dark yellow is an indication of the presence of flavonoids. B) Lead acetate test: To the methanolic extract, add basic lead acetate solution, and the mixture is observed for the formation of any precipitate. The formation of yellow precipitate is an indication of the presence of tannins.

8) Test for proteins and amino acids: A) Ninhydrin test: To the methanolic extract, add the ninhydrin reagent and boil. The mixture was observed for the appearance of any colour change. The formation of purple colour is an indication of the presence of an amino acid. B) Xanthoprotein test: To methanolic extract was treated with a few drops of concentrated. Nitric acid. The formation of yellow colour indicates the presence of proteins.

9) Test for steroids and terpenoids: A) Salkowski test: To the methanolic extract, add an equal volume of methanol and concentrated sulphuric acid. The mixture was observed for the appearance of colour change. Formation of bluish red to cherry red colour in the methanolic layer and green fluorescence of the acid layer indicates the presence of terpenoids

PHARMACOLOGICAL EVALUATION: EVALUATION OF IN-VITRO ANTHELMINTIC ACTIVITY: Collection of earthworms: Indian adult earthworms (Pheretima posthuma) were used to evaluate the anthelmintic activity of methanolic extract of Quercus infectoria. The earthworms were collected from the moist soil of Korangi, Andhra Pradesh. Worms were washed with saline water to remove the fecal matter and stored in Tyrode solution. Worms about 9 cm long and 0.2 - 0.3 cm wide were selected for the experiment. Preparation of Tyrode's solution: Weigh an accurate quantity of all the ingredients mentioned in the table below. Mix all the ingredients in a small amount of water until they dissolve and make up the volume to 1000ml

Table 1: Composition of Tyrode's solution

INGREDIENTS	QUANTITY
Sodium chloride	8grams
Potassium chloride	0.2grams
Calcium chloride	0.2grams
Sodium di hydrogen phosphate	0.1grams
Magnesium chloride	0.1grams
Glucose	1grams
Sodium bicarbonate	1grams
Distilled water	1000ml

Experimental Procedure: Evaluation of anthelmintic activity against Pheretima posthuma: The anthelmintic activity was performed according to the standard methods on the adult Indian earthworm Pheretima posthuma. The standard drug Albendazole was diluted with normal saline solution, and three concentrations of the standard drug, viz, 25, 50, and 100 mg/ml, were prepared and poured into the petri dishes. The methanolic extract of Quercus Infectoria was diluted with normal saline solution to achieve 25, 50, and 100 mg/ml concentrations. Normal saline solution (0.9% NaCl) alone was used as the negative control. All these dilutions were poured into the petri dishes. The equal size of seven petri dishes was taken and numbered. After that, similar-sized (about 8 cm) six earthworms (n=6) were placed in every petri dish at room temperature. Then the paralysis and death (lethal) time were observed and noted down from all petri dishes. The paralysis time and lethal time were recorded in terms of minutes. The experiments were performed in triplicate.

Analysis: The anthelmintic screening was followed by the investigation of the time of paralysis and death occurring in the earthworm. The time taken for paralysis was noted when no movement or loss of movement (Not retrieve even in normal saline) of earthworms, and the death time was recorded if the earthworms did not have any movement after shaking forcefully and also dipped in 50oC warm water and also fading away the colour of the worm.

Evaluation of In-Vitro antimitotic activity: Anti-mitotic activity using seed germination assays: An agent that prevents or disrupts mitosis is called an antimitotic agent. Antimitotic constituents can stop the mitosis in anywhere of the cell cycle. Methanolic extract of Quercus Infectoria was tested for antimitotic activity using green grams, using tap water as a control, and Methotrexate as a standard anticancer drug. Stock preparation of *Quercus Infectoria* methanolic extract: The stock solution of *Quercus Infectoria* methanolic extract was prepared by dissolving 1g of extract in 10 ml of distilled water, from which the dilutions of 25,50,100 µg/ml solutions and 25,50,100 mg/ml were prepared.

Stock preparation of standard drug: The stock solution of standard drug was prepared by dissolving 1 ml of Methotrexate(15mg) in 10ml of water from which serial dilutions of 25,50,100µg/ml are prepared. Seeds preparation: Seeds of Vigna radiata L used in this study were obtained from a local store. Dry seeds of equal weight were sterilized with 5% NaOCl for 2 min, followed by rinsing with sterile distilled water (4 -5times).

Assay procedure: The prepared dilutions were taken in petri plates, and sterilized dry seeds were added to each petri plate, incubating at room temperature for 72 hr. Seeds were soaked in tap water in the control group for 72 hr. For morphological studies, photographs were taken. The percentage of seed germination was evaluated in the control, plant extract, and anticancer drug (Methotrexate). Water was used for dilution. Water as a control and an anticancer drug as a standard were used for the study. Each experiment was performed in triplicate, and the mean value was computed by using the formula.

$$\%\ inhibition = Lc - \frac{Lt}{Lc} \times 100$$

RESULTS & DISCUSSION:

Phytochemical Evaluation: The methanolic extract of *Quercus infectoria* contains some phytochemical constituents, and all the phytochemical constituents are present, like alkaloids, glucosides, tannins, saponins, phenols, flavonoids, amino acids, and phytosterols.

Evaluation of in-vitro anthelmintic activity: The methanolic extracts of Quercus infectoria were evaluated for in-vitro anthelmintic activity against Pheritima posthuma at three concentrations. Paralysis times ranged from 20 to 87 minutes for Quercus infectoria and 41 to 100 minutes for the standard drug Albendazole. Death times varied from 31 to 141 minutes for Quercus infectoria and 80 to 134 minutes for Albendazole. Both paralysis and death times showed a significant decrease with increasing concentrations of the extracts compared to Albendazole.

Table 2: In-vitro anthelmintic activity of methanolic extract of Quercus infectoria against *Pheritima posthuma.*

Treatment	Concentration(mg/ml)	Paralysis Time(min)	Death Time (min)
Albendazole (Standard)	25	100.83±4.57	134.5±5.08
	50	65.66±7.68	102.83±4.75
	100	41.83±3.86	80±3.57
Quercus infectoria (Extract)	25	87±5.79	138.16±2.78
	50	45±3.84	61.33±3.44
	100	20±3.34	35.16±2.48
Control (Saline solution)	0.9 % Nacl	No paralysis	No death

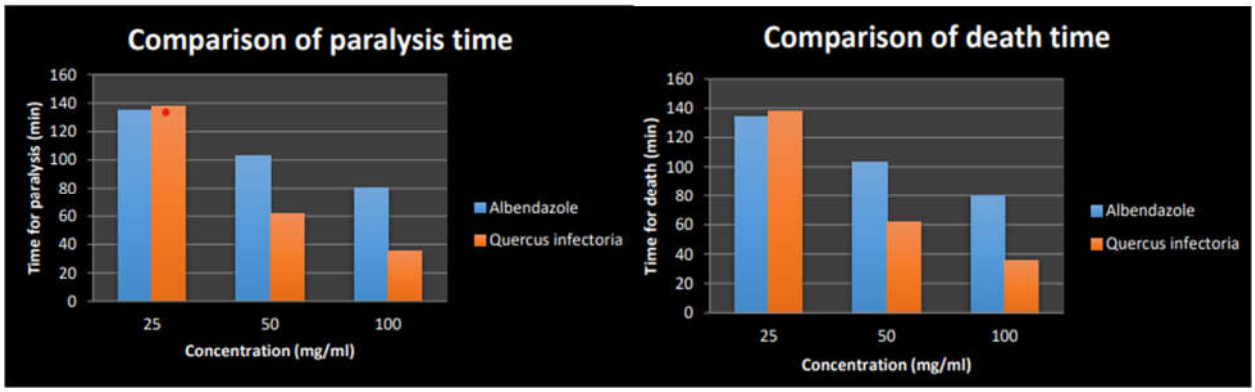

Figure: Data representing the comparison of paralysis time and death time between Albendazole and the extract

Evaluation of in-vitro antimitotic activity: The methanolic extracts of Quercus Infectoria nut galls at three different concentrations were tested against on Vigna radiata L for In-Vitro antimitotic activity with reference to standard drug Methotrexate. The results were tabulated below. The extract exhibited dose dependent inhibition i.e in microgram concentration it showed almost similar activity as that of the standard where as in milligram concentration it showed a drastic total inhibition

Table 3: Percent growth inhibition

Name	concentration	Length of the root(cm)	% inhibition
Quercus infectoria	25 µg/ml	3.92	68.58
	50 µg/ml	3.1	75.18
	100 µg/ml	1.94	84.77
	25 mg/ml	0	100
	50 mg/ml	0	100
	100mg/ml	0	100
STANDARD (Methotrexate)	25µg/ml	0.45	93.57
	50 µg/ml	0.35	95
	100 µg/ml	0.32	95.42

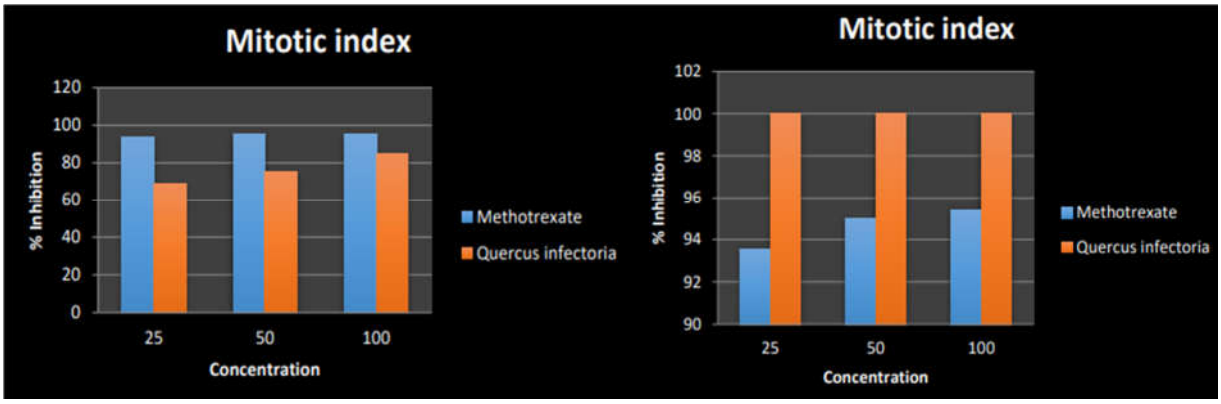

Figure: Data representing the comparison between Methotrexate and Extract (µg/ml)

The present study demonstrates that the methanolic extract of *Quercus infectoria* (oak galls) possesses significant in-vitro anthelmintic and antimitotic activities. The extract exhibited dose-dependent effects in both *Pheretima posthuma* and *Allium cepa* models, highlighting its broad-spectrum biological potential.

In the anthelmintic assay, the extract caused rapid paralysis and death of *Pheretima posthuma*, particularly at higher concentrations. The potency may be attributed to the high tannin content of oak galls—particularly gallotannins and gallic acid—which are known to interact with parasite cuticle proteins and cause their denaturation and disruption (Nawaz et al., 2014). Tannins can also interfere with energy generation in helminths by uncoupling oxidative phosphorylation, ultimately leading to parasite death (Athanasiadou et al., 2001).

The extract also demonstrated strong antimitotic activity in the *Allium cepa* root tip assay, as evidenced by a reduced mitotic index and the presence of chromosomal aberrations such as c-mitosis, chromosome bridges, and sticky chromosomes. These findings suggest that the extract contains compounds capable of inhibiting mitotic spindle formation or interfering with DNA synthesis. Polyphenolic constituents, especially gallic acid and ellagic acid, are known to inhibit topoisomerase activity, induce cell cycle arrest, and trigger apoptosis in rapidly dividing cells (Kaur et al., 2004; Cragg & Newman, 2013).

These results are consistent with earlier studies reporting antimicrobial and cytotoxic activities of *Q. infectoria* extracts. Chusri et al. (2011) reported its inhibitory activity against *Helicobacter pylori*, while Al-Mustafa and Al-Thunibat (2008) highlighted its antioxidant potential. The anthelmintic and antimitotic effects observed in this study further broaden the pharmacological scope of this traditionally used plant.

The use of *Pheretima posthuma* and *Allium cepa* models provides a reliable and ethical preliminary screening platform. While these models do not fully represent in vivo conditions, they offer insight into the potential mechanisms and guide further studies. The findings also suggest that the methanolic extract may be a valuable source of lead molecules for the development of plant-based anthelmintic and anticancer agents.

In conclusion, the study validates the traditional therapeutic applications of *Quercus infectoria* and underscores the need for further phytochemical isolation, molecular docking studies, and in vivo evaluation to fully explore its pharmacological relevance and therapeutic safety.

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