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Research Article

IN VITRO PHYTOCHEMICAL SCREENING, ANTIOXIDANT & ANTIMICROBIAL ACTIVITY OF THE METHANOLIC EXTRACT OF *QUERCUS INFECTORIA* L.

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ABSTRACT

Various herbal products are being tried to treat common ailments. *Quercus infectoria* has been found to possess antioxidant and antibacterial properties against some common oral pathogens. Also, the incidences of an assortment of diseases are becoming important with the augment in rate of population. The diseases chiefly comprise respiratory disorders, cardiovascular disorders, throat inflammation, skin infections etc. In the current study, broadly claimed crude drug *Quercus infectoria*, have been screened for their antiodxidant activity by radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and antibacterial activity against *Shigella flexneri, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Enterobacter aerogenes, Aspergillus niger, Candida albicans, Trichophyton rubrum*. Also, Phytochemical screening of methanolic extracts of *Quercus infectoria* have been screened. Phytochemical screening recorded positive results for terpenoids, tannin and flavonoids. Further,the antioxidant activity shows moderate to potent antioxidant activity, with the ED_{50} value i.e. 0.254 µg/ml. When screening for antimicrobial activity, the results were expressed in terms of the diameter of the inhibition zone: The maximum efficacy of methanolic extract was showed against *Proteus vulgaris* (20 mm) of MIC & *Aspergillus fumigates* (12 mm).

Keywords: Antioxidant activity, Antimicrobial activity, DPPH Scavenging, *Quercus infectoria*.

INTRODUCTION

At the present time, the fact of destructive effect of reactive oxygen species on human health is well-known. The ability of natural defense systems of living organisms alongside intemperance production of these species decreases when predisposed with negative environmental factors otherwise aging. As a consequence, diverse cellular as well as extracellular components, in addition to nucleic acids, are damaged, causing or enhancing a quantity of degenerative diseases. As a result, antioxidants that scavenge free radicals are of enormous value in preventing such "oxidative" pathologies. That is why natural products with antioxidant properties turn out to be more and more well-liked all above the world. Natural phenolic phytochemicals in fruits as well as vegetables have advantageous health effects on coronary heart diseases moreover cancers chiefly due to their antioxidant activity [1]. As plants fabricate a lot of antioxidants to organize the oxidative stress caused by sunbeams along with oxygen, they can symbolize a foundation of new compounds with antioxidant activity. In the midst of natural antioxidants, phenolic antioxidants are in the front position since all the phenolic classes i.e. simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives as well as flavonoids have the structural desires of free radical scavengers in addition to antioxidants[2].

Antibiotics in contemporary medicinal organization have marvellous outcome in controlling the infectious diseases [3]. On the former hand, the appearance of escape mechanism customized by generally of the pathogens certainly needs a suitable alternative of the currently obtainable antibiotics [4,5]. Furthermore, a lot of antibacterial as well as antifungal agents are recognized to demonstrate solemn annoying effects on host tissues foremost to the system toxicity [6].

Quercus infectoria Olivier (Fagaceae) is a miniature tree or a shrub chiefly present in Greece, Asia Minor, Syria in addition to Iran. The tree capitulate galls with the intention of materialize on its shoots as a significance of stabbing of gall wasp, Cypnis gallae tincotoriae [7]. The galls of *Quercus infectoria* have a immense therapeutic value in addition to pharmacologically been deciphered to be astringent, antidiabetic, antitremorine, local anaesthetic, antipyretic also antiparkinsonian [8,9]. In Asian countries, the galls of *Quercus infectoria* have been used for centuries in oriental conventional medicines for treating provocative diseases [10,11]. Gargle of hot water extract of galls is very effectual adjacent to inflamed tonsils, although unswerving application of boiled along with bruised galls on skin effectively cures several swelling or inflammation [12].

Thus current study aims to calculate the antioxidant potential of methanolic extract of *Quercus infectoria*. Plant extracts were tested for different free radical scavenging activities including the 1,1-diphenyl 2-picryl hydrazyl (DPPH) and their total antioxidant capacity. Also in this study, extensively claimed basic drugs of therapeutic system of science, have been used for those symptoms that mainly appears owing to the bacterial infections [13]. They have been screened for their antibacterial activity against *Shigella flexneri, Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris, Enterobacter aerogenes, Chryseobacteriu gleum, Bacillus subtilis* and antifungal activity against *Candida albicans, Aspergillus niger, Aspergillus fumigates, Aspergillus flavus.* The crude drug was the *Quercus infectoria* used as antimicrobial drug. The antimicrobial activity of this plant extract was compared with standard antibacterial drug Tetracycline.

MATERIALS AND METHODS

Collection:

Authentic samples: Various market samples of *Quercus infectoria* L. were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of March, 2010.

Identification:

All the samples were authenticated and were given identification number. The identification was as follows:

These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Processing of plant materials:

During the course of the study each sample was screened for its foreign matter and milled, before use.

Experimental details:

Present studies were performed on *Quercus infectoria* L. for the following studies-.

1. Phytochemical test of plant extract
2. Antioxidant Potentials of Methanolic extract of plant
3. Antimicrobial activity

Phytochemical Screening

Phytochemical screening was performed using standard procedure:

Test for Reducing Sugars (Fehlings Test)

The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for Terpenoides (Salkowski Test)

To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

Test for Flavonoides

4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

Test for Tannins

About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously. And observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Alkaloids

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent.

The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

Antioxidant Activity

Preparation of test extracts

All the test plant sample and their adulterants were milled and refluxed in ethanol for 36 h, filtered, concentrated to dryness *in vacuo*. A portion of ethanolic extract was further successively extracted in pet. ether, benzene, chloroform, alcohol and water, concentrated and stored at minimum temperature, until used.

Preparation of DPPH

DPPH (1, 1'-diphenyl-2-picrylhydrazl, $C_{18}H_{12}N_5O_6$; Hi media) 0.8 mg was dissolved in 10 ml methanol to obtain a concentration of 0.08 mg/ml for antioxidative (qualitative and quantitative) assay.

Qualitative assay

Each successive extract (10 mg) was dissolved in 10 ml of its suitable solvent to get a concentration of 1 mg/ml and from this, 0.25µl was taken with the help of micropipette, applied on silica gel G coated plates. These circular spots were sprayed with DPPH solution, allowed to stand for 30 min. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced, and the changes in colour (from deep- violet to light- yellow on white) were recorded at 517 nm on a UV spectrophotometer (Varian Cary PCB 150, Water Peltier System).

Quantitative assay

A concentration of 1 mg/ml of ethanolic extract of each test sample was prepared to obtain different concentrations (10^2µg to 10^{-3} µg/ ml). Each diluted solution (2.5 ml each) was mixed with DPPH (2.5ml). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The UV absorbance was recorded at 517 nm. The experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that cause 50% reduction in absorbance (RC_{50}) was calculated. The same procedure was also followed for the standards-quercetin and ascorbic acid.

Antimicrobial Activity

Sources of test organisms

Bacteria-Pure culture of all test organisms, namely *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri, Proteus vulgaris, Enterobactor aerogenes* and fungi *Candida albicans, Aspergillus niger, Trichophyton rubrum* were obtained through the courtesy of Mahatma Gandhi Institute of applied Sciences (MGiaS), Jaipur, which were maintained on Nutrient broth media. Culture of test microbes: For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar test plates were prepared pouring approximately 15 ml of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial\ cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 h. To prepare the test plates, in bacteria, 10-15 ml of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of test extracts

Crushed powder (50 g) of all the species were successively soxhlet extracted with Methanol. Later, the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extract was pooled. The filtrate was concentrated to dryness *in vitro* and re dissolved in respective solvents, out of which 80mg/10disc i.e. 8mg/disc concentration were stored at 4°C in a refrigerator, until screened for antibacterial activity.

Bactericidal assay

For both, bactericidal *in vitro* Disc diffusion method was adopted (Gould and Bowie, 1952), because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whattman No.1 paper(6 mm in diameter), which were containing 8 mg of the text extracts, its control (of the respective solvent) and tetracycline as reference drugs(standard disk) separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred.

The inhibition zone (IZ) in each case were recorded and the activity index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample / Inhibition zone of standard).

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of *Quercus infectoria* shows the occurrence of tannin, terpenoids and flavonoids whereas it shows the absence of Alkaloids Reducing sugar, saponin, respectively. The screening of the *Quercus infectoria* make only a a small amount of differences in the constituent of the toughened plants. The drug shows the confirmation of strong antioxidant activity in more or in a less important amount. The existence of flavonoids sin this plant is credible to be scrupulous for the free radical scavenging effects hardnosed.

Table 1: Showing phytochemical screening results of *Quercus infectoria*.

	Quercus infectoria					
Test	**Reducing Sugar**	**Saponin**	**Tannin**	**Terpenoides**	**Flavonoides**	**Alkaloides**
	-ve	-ve	+	+	+	-ve

Antioxidant Activity

In the present investigation it was showed that the maximum optical density comes out to be 1.597 nm which is at the concentration 10^{-3} µg/ml and the smallest optical density is 0.193 nm which is at the concentration 10^{3} µg/ml where as the other shows comparable O.D at different concentrations i.e. 1.568 nm at 10^{-2}µg/ml, 1.511 nm at 10^{-1} µg/ml, 0.968 nm at 1 µg/ml, 0.299 nm at 10^{1} µg/ml, 0.210 nm at 10^{2} µg/ml.

In the present investigations antioxidant activity of *Quercus infectoria* showed appreciable activity against the DPPH assay method where the regression line clear cut showed the effectiveness of it as it's have potentials which are comparable to ascorbic acid. The antioxidant activity of *Quercus infectoria* in methanolic extract using DPPH assay method shows appreciable activity comparable to standard ascorbic acid. The straight line showed y = -0.182x+1.776 & regression = 0.838 whereas, in above drug the straight line is y = -0.290x+2.069 & regression = 0.894.

Antimicrobial Activity

Antibacterial Activity of *Quercus infectoria*

When antibacterial activity of *Quercus infectoria* was performed against above seven microorganisms through the preparation of alcoholic extract of plant and disc of .01mg/ml, 1mg/ml & 2mg/ml was prepared, the results of *Quercus infectoria* antibacterial activity were quite good, *Proteus vulgaris* showed very good results (20 mm) of MIC & minimum of *Enterobacter aerogenes* (9 mm).

Table 2: Showing Optical density of *Quercus infectoria* on different concentrations.

Concentration (µg/ml)	O.D (nm)
0.001	1.597
0.01	1.568
0.1	1.511
1	0.968
10	0.299
100	0.210
1000	0.193

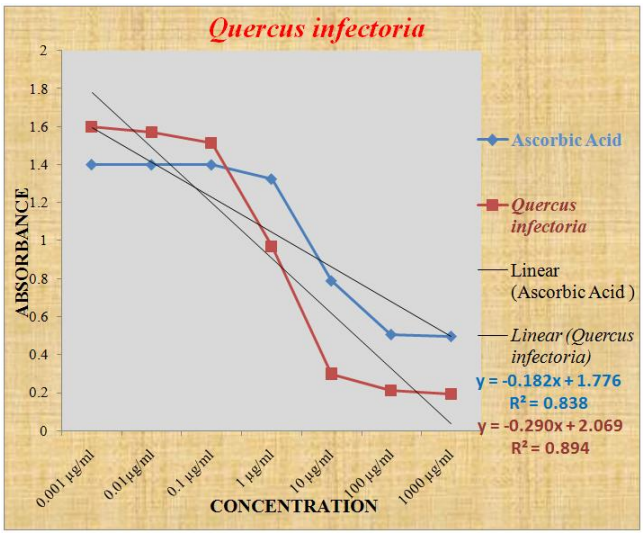

Fig. 1: Graph showing Antioxidant Activity of *Quercus infectoria* at different concentration.

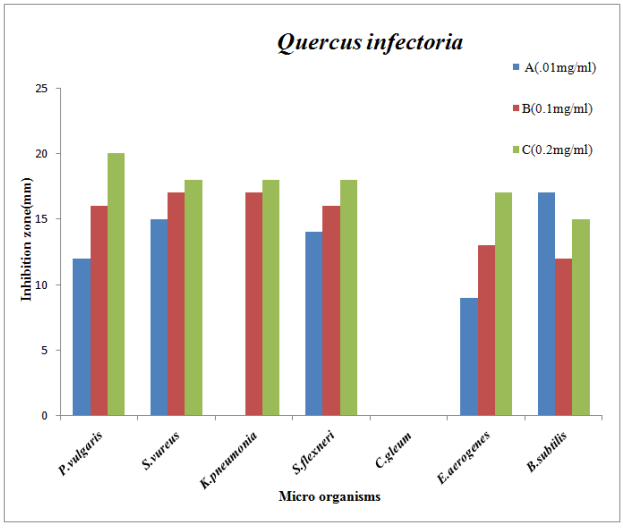

Fig. 2: Graph showing Antibacterial Activity of methanolic extract of *Quercus infectoria.*

Table 3: Showing Inhibition zone of *Quercus infectoria* on different concentrations.

	Inhibition zone(mm)		
	A(.01mg/ml)	**B(0.1mg/ml)**	**C(0.2mg/ml)**
Proteus vulgaris	12	16	20
Staphylococcus aureus	15	17	18
Klebsiella pneumoniae	-	17	18
Shigella flexneri	14	16	18
Chryseobacteriu gleum	-	-	-
Enterobacter aerogenes	9	13	17
Bacillus subtilis	17	12	15

Table 4: Showing Inhibition zone of *Quercus infectoria* on different concentrations with standard.

Micro organism	**I.Z OF STANDARD**	***Quercus infectoria***		
		Inhibition zone(mm)		
		A(.01mg/ml)	**B(0.1mg/ml)**	**C(0.2mg/ml)**
		IZ AI	**IZ AI**	**IZ AI**
Proteus vulgaris	19	12 0.63	16 0.84	20 1.05
Staphylococcus aureus	22	15 0.68	17 0.77	18 0.81
Klebsiella pneumoniae	14	-	17 1.21	18 1.28
Shigella flexneri	13	14 1.07	16 1.23	18 1.38
Chryseobacteriu gleum	18	-	-	-
Enterobacter aerogenes	16	9 0.56	13 0.81	17 1.06
Bacillus subtilis	21	17 0.80	12 0.57	15 0.71

Antifungal Activity of *Quercus infectoria*

When antifungal activity of *Quercus infectoria* was performed against above four fungus it was found that activity was appreciable but showed positive activity against *Aspergillus fumigates* (12 mm) & no activity was shown against *Candida albicans, Aspergillus niger* & *Aspergillus flavus.*

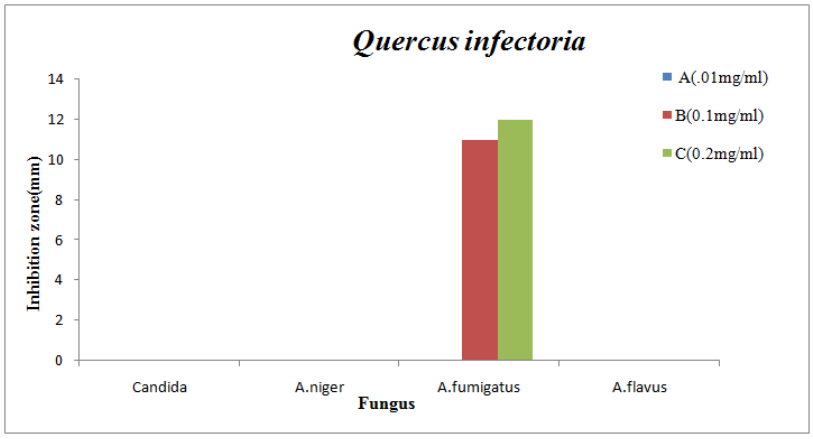

Fig. 3: Graph showing Antifungal Activity of methanolic extract of *Quercus infectoria.*

Table 5: Showing Inhibition zone of *Quercus infectoria* on different concentrations.

	Quercus infectoria		
	Inhibition zone(mm)		
	A(.01mg/ml)	**B(0.1mg/ml)**	**C(0.2mg/ml)**
Candida albicans	-	-	-
Aspergillus niger	-	-	-
Aspergillus fumigates	-	11	12
Aspergillus flavus	-	-	-

Table 6: Showing Inhibition zone of *Quercus infectoria* on different concentrations with standard.

		Quercus infectoria		
		Inhibition zone(mm)		
		A(.01mg/ml)	**B(0.1mg/ml)**	**C(0.2mg/ml)**
	I.Z OF STANDARD	**IZ AI**	**IZ AI**	**IZ AI**
Candida albicans	12	-	-	-
Aspergillus niger	16	-	-	-
Aspergillus fumigates	19	-	11 0.57	12 0.63
Aspergillus flavus	9	-	-	-

CONCLUSION

From the above results and discussion it can be concluded that the methanolic extract of *Quercus infectoria* possesses the effective antioxidant & antimicrobial substances and a which may be justify on the basis of using this plant's extract as folkloric remedies. The significant test systems, wholly free radical scavenging next to with reducing power, were used for the chemical analysis. Further in the present study indicates that the flavonoids are present in *Quercus infectoria.* The occurrence of flavonoids in huge quantity is rationally proportional to the antioxidant activity so it is evidently show that occurrence of flavonoids will prove the antioxidant activity and promote a drug for treatment of various infectious disease. Further, the results of antibacterial activity were quite good, *Proteus vulgaris* showed very good results (20 mm) of MIC & minimum of *Enterobacter aerogenes* (9 mm), Whereas antifungal activity of *Quercus infectoria* was found that activity was appreciable but showed positive activity against *Aspergillus fumigates* (12 mm).

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