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Antimicrobial characterisation combining spectrophotometric analysis of different oak species

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Abstract
The present study was carried out to evaluate the bioactive components and to investigate the antimicrobial efficiency of different *Quercus* species namely *Quercus suber, Quercus macrocarpa, Quercus montana, Quercus griffithii* and *Quercus serrata.* The methanol is used as a solvent to evaluate the antimicrobial properties of the leaf and stem parts of the different *Quercus* species against certain bacterial and fungal species by disc diffusion method, and the spectrophotometric analysis is carried out to characterize the bioactive constituents present in the different plant samples. The results of the study revealed that the leaf and stem extracts of these samples possess good antimicrobial activity and the bioactive component, Gallic acid is found to found to be maximum in leaf and stem extracts of the five different *Quercus species.*

Keywords: *Quercus suber, Quercus macrocarpa, Quercus montana, Quercus griffithii, Quercus serrata,* Spectrophotometric analysis, antimicrobial activity.

1. Introduction
In developing countries, medicinal plants were used in traditional medicine. Such plants have been investigated for better understanding of their medicinal properties [1]. The continuing emergence and development of resistance towards existing bacterial and fungal infections, has created the need for new antimicrobial compounds that exhibit activity against the resistant bacterial and fungal strains [2, 3].
Oak is a tree or shrub in the genus *Quercus,* belongs to the beech family, Fagaceae. The genus is native to the Northern Hemisphere and includes deciduous and evergreen species. Oak trees are usually large in size [4]. It has been reported that different *Quercus* species posses antibacterial and antifungal activity, that are used for suppressing the growth or reproduction of bacterial and fungal species [5]. Although the Oak trees are effective for treating certain diseases, it is prone to fungal diseases that may induce rotting of the inner part of the plant. Many species of oaks are under threat of extinction in the wild, largely due to land use changes, livestock grazing and unsustainable harvesting [6]. The present work deals with the detection of bioactive components and to analyze the antimicrobial activity of the leaf and stem extracts of different *Quercus species.*

2. Materials and Methods
2.1. Collection of plant sample
The leaf and stem samples of the five different *Quercus* species were identified and collected from Government Botanical Garden, Ooty, Tamil Nadu. The leaves and stem were separated, washed properly with distilled water and air dried at room temperature. The dried leaves and stem samples were powdered finely using a electric blender and stored in a clean glass container for further analysis.

2.2. Plant extracts
Vigorously washed leaf and stem samples of *Quercus suber, Quercus macrocarpa, Quercus montana, Quercus griffithii* and *Quercus serrata* were dried and were powdered with the help of electric blender. 5g of the powdered plant materials were loaded in the inner tube of the soxhlet apparatus and then fitted into a round bottom flask containing 150ml of Methanol. The solvent was boiled gently and the extraction was continued until complete extraction was effected (24 hours) and then the solvent was removed at reduced pressure, to yield a viscous dark green (or) brown residue.

1mg of the crude extracts of leaf and stem samples were taken and mixed with 1ml methanol. From this stock solution, different concentrations (80, 100 and 120 µg/ml) were prepared which were used for antimicrobial tests [7].

3. UV Spectrophotometric Analysis

Spectrophotometric analysis of the methanolic leaf and stem extracts was performed using Shimadzu UV-1800 Series Spectrophotometer. The leaf and stem extracts obtained from soxhlet extraction were filtered through Whatmann No.1 filter paper and the extracts were diluted with the same solvent (5µg/ml), which was used for extraction process. The extracts were scanned at the wavelength 260nm and the characteristic peaks were detected. The biomarker, standard Gallic acid was dissolved in methanol at different concentrations (0, 2, 4, 6, 8 and 10 µg/ml) for proximate analysis and the peak values were recorded. Each and every analysis was repeated thrice for the spectrum confirmation [8, 9].

4. Antimicrobial Assay

4.1. Preparation of growth media

Nutrient agar and Potato Dextrose agar (PDA) was used in the preparation of medium for the growth of bacterial and fungal organisms. 20ml of the nutrient agar and 15 ml of the PDA medium was poured in sterile petri plates and these were used for testing the antibacterial and antifungal susceptibility of the isolated strains.

4.2 Growth and maintenance of microorganisms

Bacterial cultures of *Bacillus subtilis, Streptococcus pneumonia, Escherichia coli* and *Staphylococcus aureus,* and the fungal cultures of *Aspergillus niger, Penicillium sp* and *Fusarium oxysporum* were collected from the central repository of Department of Microbiology and Department of Agricultural Biotechnology, Orissa university of Agriculture and Technology, Bhubaneswar. The Bacterial strains were maintained in nutrient broth and the fungi culture was maintained on PDA broth at 37 ºC.

4.3 Antibacterial susceptibility testing using disc diffusion method

A single colony of the bacterial organisms were aseptically transferred with an sterile inoculating loop to 10ml of sterile nutrient broth, which was incubated at 37 ºC for the growth of bacterial cultures. 100µl of the inoculums were aseptically transferred to petri plates and spread thoroughly using sterile L-rod. The sterile paper discs (Whatmann No.1 filter paper(5mm)) were soaked in the leaf and stem extracts of different concentrations (80,100 and 120µg/ml) and placed on the inoculated plates and incubated at 37 ºC for overnight in an inverted position. Zone of inhibition was measured for each leaf and stem samples. Negative controls were performed using paper discs loaded with methanol [10].

4.4 Antifungal susceptibility testing using disc diffusion method

The PDA medium was inoculated with 100µl of fungal spores and spreaded thoroughly with a sterile L- shaped spreader. The sterile paper discs were soaked in leaf and stem extracts, and were placed in the plates. The plates were incubated at 37 ºC for 24-48 hours. A disc soaked in methanol was used as negative control and the zone of inhibition was indicated near the respective disc [11].

5. Results and Discussion

The UV-Spectrophotometric analysis showed good correlation between the plant extract and standard (Gallic acid). The Gallic acid component is found to be maximum in the leaf and stem extracts of *Quercus montana* at different concentrations 2.922 and 2.975 respectively. The profile showed different absorption values for the leaf and stem extracts at 260nm.

Table 1.1: Spectrophotometric analysis of standard Gallic acid

Concentration (µg/Ml)	Absorbances (At 260nm)
0	0
2	0.144
4	0.273
6	0.418
8	0.521
10	0.616

Table 1.2: Spectrophotometric analysis of different *Quercus* species

S. No	Plant Sample	Plant Parts Used	Concentration (µg/Ml) (At 260 Nm)
1.	*Quercus suber*	Leaf	2.278
		Stem	2.018
2.	*Quercus macrocarpa*	Leaf	0.542
		Stem	1.382
3.	*Quercus montana*	Leaf	2.922
		Stem	2.795
4.	*Quercus griffithii*	Leaf	1.351
		Stem	0.833
5.	*Quercus serrata*	Leaf	2.912
		Stem	1.270

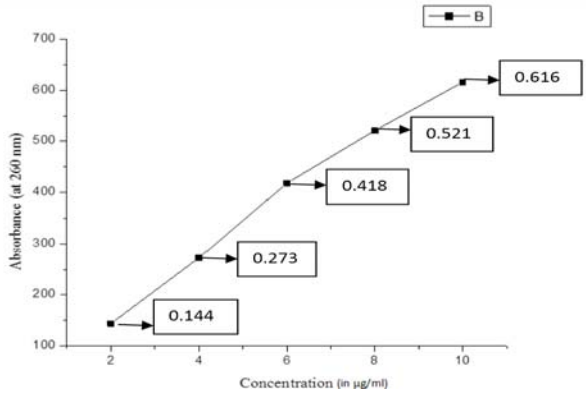

Fig 1: Standard graph for Gallic acid in UV spectrophotometric analysis

In the antibacterial assay, it was observed that the leaf and stem extracts of *Quercus montana* showed maximum zone of inhibition against the bacterium *Bacillus subtilis*, *Streptococcus pneumonia* and *Escherichia coli*. The leaf extracts of *Quercus montana* and the stem extracts of *Quercus macrocarpa* exhibited maximum zone of inhibition of against the bacterium *Staphylococcus aureus.*

In the antifungal assay, it was observed that the leaf and stem extracts of *Quercus serrata* and *Quercus macrocarpa* showed the maximum zone of inhibition against the fungal species *Aspergillus niger, Penicillium sp* and *Fusarium oxysporum.* The crude leaf and stem extracts of *Quercus montana* possessed very good antibacterial activity, whereas the leaf and stem extracts of *Quercus macrocarpa* and *Quercus serrata* showed significant antifungal property. The negative control, methanol showed very less activity against both the bacterial and fungal species.

Table 2.1: Antibacterial activity at different concentration of plant extracts.

Sl. No	Plant Species	Plant Part Used	Concentration (µg/ml)	Zone of inhibition (in mm)			
				Bacillus subtilis	*Streptococcus pneumonia*	*E.coli*	*S.aureus*
1.	*Quercus Suber*	Leaf	0	NA	NA	NA	NA
			80	10	10	11	11
			100	11	11	12	11
			120	12	11	13	12
		Stem	0	NA	NA	NA	NA
			80	11	14	16	13
			100	12	14	17	14
			120	12	15	25	16
2.	*Quercus macrocarpa*	Leaf	0	NA	NA	NA	NA
			80	10	11	12	11
			100	12	13	20	13
			120	13	13	21	14
		Stem	0	NA	NA	NA	NA
			80	10	11	14	12
			100	11	13	16	13
			120	12	14	19	22
3.	*Quercus montana*	Leaf	0	NA	NA	NA	NA
			80	10	12	19	18
			100	11	12	21	20
			120	13	19	23	21
		Stem	0	NA	NA	NA	NA
			80	10	10	13	12
			100	11	11	14	13
			120	13	12	26	13
4.	*Quercus griffithii*	Leaf	0	NA	NA	NA	NA
			80	11	11	13	13
			100	12	12	16	14
			120	12	15	17	15
		Stem	0	NA	NA	NA	NA
			80	10	10	12	12
			100	11	11	13	13
			120	12	11	19	15
5.	*Quercus Serrata*	Leaf	0	NA	NA	NA	NA
			80	11	10	15	13
			100	11	11	16	15
			120	13	12	22	16
		Stem	0	NA	NA	NA	NA
			80	10	10	12	11
			100	12	11	12	12
			120	12	12	18	15

*NA- No activity

Table 2.2: Antifungal activity at different concentrations of plant extract

Sl. No	Plant Species	Plant part Used	Concentration (µg/ml)	Zone of Inhibition (in mm)		
				Aspergillus niger	*Penicillium sp*	*Fusarium oxysporum*
1.	*Quercus Suber*	Leaf	0	NA	NA	NA
			80	11	10	12
			100	13	11	13
			120	14	12	15
		Stem	0	NA	NA	NA
			80	11	11	12
			100	11	12	14
			120	14	14	20
2.	*Quercus macrocarpa*	Leaf	0	NA	NA	NA
			80	11	11	11
			100	14	12	13
			120	15	14	24
		Stem	0	NA	NA	NA
			80	14	12	13
			100	15	16	18
			120	22	17	21
3.	*Quercus Montana*	Leaf	0	NA	NA	NA
			80	12	11	13
			100	15	12	19
			120	15	13	20
		Stem	0	NA	NA	NA
			80	12	10	12
			100	13	11	15
			120	14	12	17
4.	*Quercus griffithii*	Leaf	0	NA	NA	NA
			80	10	10	10
			100	11	12	12

			120	12	14	14
		Stem	0	NA	NA	NA
			80	10	12	12
			100	12	13	13
			120	13	14	15
5.	*Quercus Serrata*	Leaf	0	NA	NA	NA
			80	11	11	11
			100	11	13	12
			120	16	15	17
		Stem	0	NA	NA	NA
			80	12	12	12
			100	12	13	14
			120	18	15	23

* NA- No activity

The MIC Values were calculated for the leaf and stem extracts at different concentrations, ranged from 80 to 120 µg/ml respectively. In antibacterial assay, methanolic leaf and stem extracts of *Quercus suber* exhibited low minimal inhibitory activity against *Bacillus subtilis, Streptococcus pneumonia, Escherichia coli* and *Staphylococcus aureus.* In antifungal assay, methanolic leaf and stem extracts of *Quercus griffithii* showed minimal inhibitory activity against *Aspergillus niger, Penicillium sp* and *Fusarium oxysporum.*

Results of the present study showed that the leaf and stem extracts of different *Quercus species* indicated a broad spectrum of bioactive nature against these bacterial and fungal culures. Future works could also be made possible to investigate the specific phytoconstituents present in the above mentioned *Quercus species,* which are responsible for these antimicrobial activities. Phytochemicals can serve as a valuable source of information and provide appropriate standards to establish the quality of these plant materials in medicinal and pharmaceutical studies.

6. Conclusion

In the present study, the UV Spectrophotometric analysis revealed the presence of a pure bioactive component, Gallic acid, which is found to be maximum in the leaf and stem extracts of the five different *Quercus species.* The antimicrobial activity of the methanolic leaf and stem extracts of *Quercus suber, Quercus macrocarpa, Quercus montana, Quercus griffithii* and *Quercus serrata* by disc diffusion method against certain bacterium *Bacillus subtilis, Streptococcus pneumonia, Escherichia coli* and *Staphylococcus aureus*, and fungal species *Aspergillus niger, Penicillium sp* and *Fusarium oxysporum,* revealed the antibacterial and antifungal activity exhibited by both the leaf and stem extracts, and the zone of inhibition was determined respectively.

7. Reference

1. Sathya Priya. Antibacterial activity of methanol extract of *Ruta chalepensis* (L), *Quercus infectoria* (Oliver) and *Canthium parviflorum* (Lam). Ancient Science of Life. 2009; 29(2):28-31.
2. Global survey of *Exsitu* Oak collections, Botanical Gardens Conservation International, 2009.
3. Khouzami. The role of methanolic extract of *Quercus infectoria* bark in lipemia, glycemia, gastric ulcer and bacterial growth. Journal of Medicinal Plants Research. 2009; 2(4):224-230.
4. Kiritikar KR, Basu BD. Indian Medicinal Plants" International Book Distributers, Deharadun, 2005; 2(1):649-651.
5. Graham G, Bhatia A. Healing Outcomes of MRSA infected wounds with a protocol combining Oakin dressing with elements of de-escalation theory. Journal of Wound Care, APWH, 2014, 23(2).
6. Abdessalem Abdessamad. Evaluation of Phenotypic diversity by use of variable analysis multi of various populations of Oak Cork (*Quercus suber* L.) in Tunisia. Open Journal of Ecology. 2014; 4:861-872.
7. Ghias Uddin, Abdur Rauf. Phytochemical screening, antimicrobial and antioxidant activities of aerial parts of *Quercus robur* L. Middle East Journal of Medicinal Plants Research. 2012; 1(1):01-04.
8. Vaidya. Preliminary phytochemical screening of *Quercus infectoria* Oliv for treatment of Skin diseases. Journal of Medicinal Plants Research. 2013; 7(27):2019-2027.
9. Boris. Antioxidant characterization of Oak extracts combining Spectrophotometric assays and Chemometrics. The Scientific World Journal. 2013; Article ID:134656-8.
10. Hashim. Identification of quantative chemical compounds of ethanolic extracts of *Quercus infectoria* and studies its inhibitory effect in some bacteria, Indian Journal of Research. 2013, 2(8).
11. Andrensck. Antimicrobial and antioxidative enrichment of Oak (*Quercus robur*) bark by rotation planar extraction using Extra Chrom, International Journal of Food Microbiology. 2004; 92:181-187.