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## Pharmacognostic Evaluation, Preliminary Phyto-Chemical Screening and Antioxidant Potential of Bark of *Quercus oblongata* D. Don



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### ABSTRACT

**Background:** *Quercus oblongata* D. Don is utilized in traditional healing as many of its members have been utilizing to prevent and treat various disorders in humans such as hemorrhoids, gastric ulcers, asthma and healing of wound. **Aim:** Thus, the current study was performed to investigate and study the pharmacognostical and phytochemical estimation of *Quercus oblongata* D. Don. **Material and Methods:** Root samples of *Quercus oblongata* D. Don were put through organoleptic and microscopical studies. Various physicochemical variables were also calculated and initial phytochemical determination with different solvents like ethyl acetate, acetone, petroleum ether, methanol and aqueous extract of plant leaf part were carried out in accordance to polarity. Leaf powder of plant material was accommodated with various laboratory reagent used for powder microscopy and also subjected for florescence analysis under UV light of short and long wave length and visible light. **Results:** Transverse section of root reveals that bark shows cork (phellem) layer containing 2-5 layers of radially flattened or isodiametric cells which were arranged in less or more radial pattern. Medullary rays were uni or biseriate, having radially long cells, which were rectangular in shape. *Quercus oblongata* D. Don, Methanol, Phytochemical, Physicochemical, Chloroform, Microscopical, solvents. Bark powdered microscopy showed the existence of medullary rays which were uni to biseriate, along with fibers with sclereids, parenchyma and vessels which were pitted. Pitted vessels, fibers and sclereids were stained with phloroglucinol hydrochloric acid and were stained pink due to the confirmation of lignin present in them. Preliminary phytochemical analysis of bark denotes the existence of some therapeutic constituents such as alkaloids, flavonoids, tannins, saponins, steroids and proteins which in coming future will play vital role in herbal medicine. Antioxidant activity of the leaf extract of acetone and methanol showed that the plant contains potent antioxidant activity.



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## INTRODUCTION

The word herbal medicine indicates the utilization of therapeutic plants for cure and treatment of different ailments which extends from conventional and commercial medicines of each and every country. Around 80 % people all over the world are believing on herbal medicine over the past 3 decades as supplements and herbal-based medicinal supplements tremendously increased<sup>1</sup>. In India use of herbal medicine is mentioned in the different system of health viz. yoga, Siddha, Unani, Ayurveda, Naturopathy and Homeopathy which are continuous in use and are safe. Most of the population in India use conventional drugs on regular basis as home-remedies, as spices, as self-medication, as health food as well as over the counter or as drug mentioned in the various non allopathic systems<sup>2</sup>. Basically, herbal remedies are derived from plants and occurs naturally and are used to treat ailments or diseases with regional or local healing applications. These compounds or row morphological part of an herb having organic chemicals with complex nature<sup>3</sup>.

Ethanopharmacological or traditional medicine is defined as practices, knowledge and skill which are based on society's traditional culture, beliefs, presumptions, and experience so as to regulate their hygiene and health. In various ruler areas or indigenous peoples in various underdeveloped countries traditional treatment with herbs are highly effective<sup>4</sup>. System of medicine of health in India which is recognized officially share a large part of herbal drugs including yoga, Unani, Siddha, Ayurvedic, Naturopathy and Homeopathy system. Population of India around 1.1 billion which is around more than 70% uses these systems of medicine<sup>5</sup>. Since primitive time period for the treatment of different diseases various herbs have been utilized as healing agents in numerous formulations.

Of the world population around 60 to 80% which resides in developing nations depend essentially on healing or therapeutic plants for their initial health management according to World Health Organization<sup>6</sup>. The foundation of human disease treatment has been the natural compounds from animals, plant and minerals. For primary health care it is approximated that in countries which are developing about 80% of people depends on conventional remedy which is hugely based on species of different animals and plants. At present herbal medicines are in great demand and their popularity is gaining so much faster. At present herbal medicines are in great demand and their popularity is gaining so much faster. In ancient literature around 500 herbs are mentioned for their therapeutic use while in indigenous medicine systems around 800 herbs have been utilized<sup>7</sup>. Plant gives a huge and multiple

varieties of organic compounds, out of this large majority do not utilize by plants for their development and growth. These constituents conventionally referred to as active constituents or secondary metabolites which are distributed among restricted and taxonomic group in the plant kingdom and have great pharmacological effect<sup>8</sup>.

Fagaceae plant members are generally described by deciduous and economically evergreen wood plants, which are mostly found in tropical Southeast Asian part. Meanwhile identifying the distinctiveness and distribution of family Fagaceae, 35 species were found growing in various agro-climatic domain of tropical, subtropical and temperate area of northeast states in India<sup>9</sup>. The family Fagaceae covers over 1000 trees and shrubs species such as oaks (genus *Quercus*), chestnuts (*Castanea*) and beeches (*Fagus*) which are widely distributed in Northern Hemisphere<sup>10</sup>.

Fagaceae plant species are economically very important as they provide various benefits to both man and nature. Wood from this family has been used economically and is used for different purposes including fuel and timber<sup>11</sup>.

Family naming Fagaceae is distributed abundantly in forests regions of Northern Hemisphere having temperate and suitable tropical climatic conditions and areas. In traditional healing various its members have been utilizing to prevent and treat various disorders in humans which are gastric ulcers, hemorrhoid, asthma and healing of wound. Various biological activity of the family includes antibacterial, anti-inflammatory, anticancer, antidiabetic, antioxidant, and gastro protective activities due to the presence of important chemical constituents such as Phenolic acids, Flavonoids and triterpenoids<sup>12</sup>.

## **MATERIALS AND METHODS**

### **PLANT COLLECTION AND AUTHENTICATION**

Specimens of morphological plant were gathered from field areas, forest, and adjacent region of Kasardevi, Almora Uttarakhand during July. Sample was identified for different samples taxonomically by Botanical Survey of India botanist, 192, Kaulagarh Road, Northern Regional Center, and Dehradun-248195. Each sample one set was accumulated in the record of the Botanical Survey of India herbarium. The plant materials (leaves bark, root) were dried in air completely at normal temperature under shade and were then powdered to a fine quality

by using a mixer grinder or laboratory grinding mill. These shaded dried parts of the plant material are finally packed in air tight plastic bag until use.

### **MORPHOLOGICAL EVALUATION**

Ocular visualization gives the easier and fastest way by which quality, possibly and identity purity of a sample can be established. If there is significant difference in the sample with regard to consistency, color, taste or odor, through the identification, it is regarded that it is not confirming the official necessities. Macroscopical recognition of herbs having medicinal value is depend on color, size, shape, surface characters, fracture, surface characteristics and emergence of the cutter surface. Moreover, the features are calculated subjectively and are adulterants and substituents may nearly resemble the original or officially fulfilled required raw material as it is frequently necessary to prove the results by physiochemical or microscopy evaluations.

### **MICROSCOPICAL EVALUATION**

Plant materials using for medicine utilization are classified according to their organoleptic and microscopical characteristics. Visible inspection is the quickest and simplest means by which, purity, identity, and most probable drug quality can be established. Microscopical evaluation is a move forward towards identification and authentication of interior structural of crude plant sample to establish original identification by studying the arrangement of tissues. This is performed by recognizing internal cell constants such as vascular bundles, epidermis, collenchymas, schelernchyma, trichomes etc. For this procedure there is a transverse or longitudinal sectioning either by free hand or using microtome may be performed. For the present research work free hand sectioning was performed<sup>13, 14, 15</sup>.

### **POWDER MICROSCOPY**

Dried root powder microscopy of plants was performed. The powders of plants were placed on different clean glass slides. Drop of glycerol was added to plant material which were powdered and kept on the glass slide and it was covered with a cover slip was placed over it. The glass slide was then examined below the microscope and various images were clicked at required magnification. For good results various stains were also used to differentiate cellular structure. Each powder was treated and stained with Iodine, Phloroglucinol, Sudan III, Ruthenium red stain and studied by microscope<sup>13, 14, 16, 17</sup>.

## **PHYSICO-CHEMICAL PARAMETERS**

Dried powder of root was put through to physicochemical examination. Physicochemical constants such as loss on drying, extractive value, ash value, swelling index and foaming index was performed and studied<sup>19</sup>.

## **FLUORESCENCE ANALYSIS OF POWDERS**

Fluorescence analysis is among one of the methods used in pharmacognostic procedures which are useful in the identification of genuine samples and identifying adulterants<sup>20</sup>. In the process of fluorescence analysis, the morphological part of the plant or crude drug may be examined as such, or in their solution or as extract or in their powdered form. Although, in most of the samples the actual constituents which is responsible for the fluorescence activity has not been identified, the merits of the process are rapidity and simplicity which builds it a precious analytical tool for the recognition of various plant samples and crude drugs<sup>21</sup>.

## **EXTRACTION OF PLANT MATERIAL**

The powdered root of plant which were shade dried undergo successive extraction with various solvents according to the polarity. Plant material which was coarsely powdered around 50g was thoroughly extracted for 3 hours with solvent petroleum ether (50-70°C) in Soxhlet apparatus. Obtained extract was concentrated and solvent is recovered by recovery unit. The plant material which was extracted was then air dried and again packed in soxhlet apparatus and extracted exhaustively with ethyl acetate for 3 hours. Then the extract obtained device was filtered and was evaporated using Rota vapor or solvent under reduced pressure and is then recovered through recovery unit. The plant material which was extracted was air dried and is again packed in the Soxhlet apparatus and was extracted with acetone, methanol and lastly with water and finally filtered, evaporated using Rota vapor<sup>22</sup>.

## **PHYTOCHEMICAL ANALYSIS**

A concentration of stock concentration of 1 % (W/ V) of individual successive extract obtained using petroleum ether, ethyl acetate, acetone, water and methanol was got ready using the particular solvent. Extracts which were having negative and positive controls were tested for the identification of chemical constituents viz: alkaloids, tannins, triterpenoids,

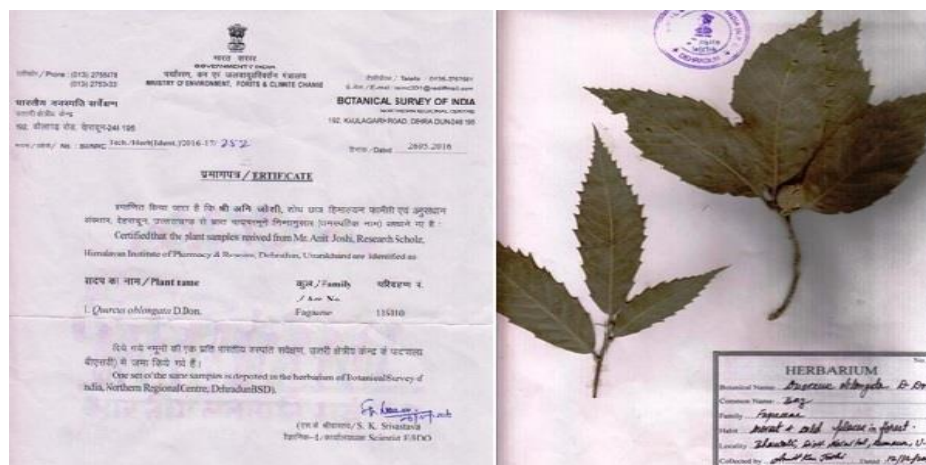
phytosterols, flavonoids, cardiac glycosides, anthraquinone glycosides, saponins glycosides, carbohydrates, proteins, amino acids, fats and fixed oils following standard methods<sup>22, 23</sup>.

### ANTIOXIDANT ACTIVITY BY DPPH METHODS

With the help of DPPH (1, 1-diphenyl-2-picryl hydrazyl) different extracts of plants morphological part (barks) were measured and calculated for free radical scavenging activity. In this method in ethanol, 0.1mM solution of compound DPPH was prepared. 3 ml of different plant extracts of various morphological parts was added to 1 ml of DPPH solution at distinct concentrations (2, 4, 6, 8, 10, 12 µg/ml). By the use of dilution method distinct concentration of only those extracts which were having solubility in ethanol were prepared. The mixture was then permitted to stand for around period of 30 minutes with strong shaking at room temperature and with the help of spectrophotometer (Shimadzu) absorbance of distinct solvents were measured at 517 nm. The experiment and method were performed in triplicate and the compound which was used as standard was ascorbic acid<sup>24, 25, 26</sup>.

### RESULT AND DISCUSSION

#### COLLECTION OF PLANT MATERIAL



**Figure 1: Herbarium specimen and plant authentication certificate of *Quercus oblongata* D. Don**

The unknown sample of plants was collected from the field areas, forest, and adjacent region of Kasardevi, Almora Uttarakhand during the month of July and herbarium of the sample were made in 2 sets and were submitted to Botanical Survey of India botanist, 192, Kaulagarh Road, Northern Regional Center, Dehradun-248195 Sample of the plants were

identified taxonomically by taxonomist as *Quercus oblongata* D. Don and one set was accumulated in the herbarium record of Botanical Survey of India. Certificate and identified herbarium sample is mentioned in the above figure.

### MACROSCOPICAL EVALUATION



**Figure 2: Macroscopical characters of leaf and bark part of *Quercus oblongata* D. Don**

Bark is flat with a smooth inner surface and outer surface is rough. The size of the bark varies from 2-4 cm in length and 1 to 2 cm in width, inner surface of bark is brown and outer surface is whitish black. Bark part having no odor with slightly bitter and acrid taste.

### MICROSCOPICAL EVALUATION

#### Microscopical Evaluation of *Quercus oblongata* D. Don

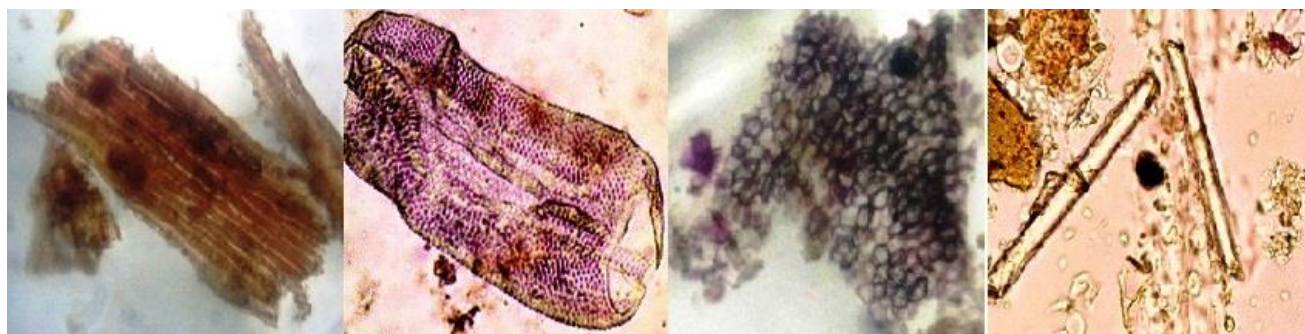


**Figure 3 T.S of *Quercus oblongata* D. Don bark showing cortex, medullary rays and cork portion**

Stem bark transverse section reveals that bark shows cork (phellem) layer containing 2-5 layers of radially flattened or isodiametric cells which were arranged in less or more radial pattern. Medullary rays were uni or biseriate, having radially long cells, which were rectangular in shape. In the transverse section the starting area of secondary phloem was

distinguished by the presence of multiseriate bands tangential in shape of axial parenchyma cells with which sieve tubes elements were alternating with row of fibers which were tangential and 3-5 cells wide.

Secondary phloem rays crossed these rows. The portion of phelloderm was not developed well, was composed of thin-walled cells with 2-3 radial rows looking like adjoining parenchyma cells. Sieve tube elements were having an irregular to round shape containing undignified thin walls which were having tangential arrangement. Sieve tube elements were present in groups of 2 to 3 and were solitary which were having tangential arrangement.



**Figure 4: Powder photomicrograph of *Quercus oblongata* D. Don bark showing medullary rays, fibers, cork cells and xylem vessels**

Bark powdered microscopy showed the existence of medullary rays which were uni to biseriate, along with fibers with sclereids, parenchyma and vessels which were pitted. Pitted vessels, fibers and sclereids were stained with phloroglucinol hydrochloric acid and were stained pink due to the confirmation of lignin present in them. The representation of fiber is accountable for the noticed granular or striated fracture. Calcium oxalate crystals were present in abundant amount in bark, especially in axial parenchyma adjoining to sieve tube elements. In the secondary phloem Phenolic compounds in abundant quantity were observed by dark color.



**PHYSICO-CHEMICAL PARAMETERS**



**Figure 5: Physico-chemical parameters of *Quercus oblongata* D. Don bark mentioning type loss on drying, ash value, swelling index and foaming index**

**Loss on drying**

The value of loss on drying for the bark sample of *Quercus oblongata* D. Don was found to be 5.424%.

**Table no. 1: Loss on drying of *Quercus oblongata* D. Don bark powder**

S. No.	Drug wt. + porcelain dish before drying A (g)	Drug wt. + porcelain dish after drying B (g)	Loss on drying A-B (g)	% of loss on drying
1	10.039 + 42.649	52.142	0.546	5.438
2	10.041 + 40.992	50.501	0.532	5.298
3	10.043 + 44.502	53.989	0.556	5.536
<b>Mean ± S.E.M (n=3)</b>				<b>5.424 ± 0.069</b>

**Ash Value**

The values of ash i.e. Total ash, water-soluble ash and acid insoluble ash were evaluated as per official process. The existence of inorganic content in a raw drug is a measure of total ash. Total ash greater value gave indication that more inorganic matter is present in plant material. In the drug existence of inorganic matter is a measure of total ash. Large value shows the plant material consists of more inorganic matter. The total ash value of *Quercus oblongata* D. Don bark was calculated as 6.928%. To the total ash concentrated acid was added, the acid combines and reacts with calcium oxalate crystals. If calcium oxalate crystals are large in number in plant material, quantity of substance after acid treatment will remain quite less. Acid insoluble ash lower value denotes the existence of calcium oxalate crystals in

large number in plant material. The amount of silica present in given plant material is determined by acid insoluble ash. Value of acid insoluble ash was computed as 2.604%. Another part of total ash is water soluble ash, which dissolves in the drug and is excellent indicator of the water-soluble salts. Water soluble ash was computed as 2.027%. The results were found to be almost within limits.

**Table no. 2: Total ash value of *Quercus oblongata* D. Don bark powder**

S. No.	Drug wt. (g)	Wt. of empty china dish (g)	Crucible wt. + Wt. of ash (g)	Ash Wt. (g)	% of total ash
1	3.067	17.094	17.307	0.213	6.944
2	3.065	17.056	17.253	0.197	6.427
3	3.065	18.009	18.228	0.219	7.145
<b>Mean± S.E.M (n=3)</b>					<b>6.928 ± 0.285</b>

**Table no. 3: Acid insoluble ash value of *Quercus oblongata* D. Don bark powder**

S. No.	Drug wt. (g)	Wt. of empty china dish (g)	Crucible wt. + Wt. of ash (g)	Ash Wt. (g)	% of acid insoluble ash
1	3.032	18.873	19.336	0.076	2.506
2	3.035	17.927	18.456	0.082	2.701
3	3.030	17.652	18.450	0.079	2.607
<b>Mean ± S.E.M (n=3)</b>					<b>2.604 ± 0.056</b>

**Table no. 4: Water soluble ash value of *Quercus oblongata* D. Don bark powder**

S. No.	Drug wt. (g)	Wt. of empty china dish (g)	Crucible wt. + Wt. of ash (g)	Ash Wt. (g)	% of total ash
1	3.008	16.435	17.281	0.063	2.094
2	3.007	17.345	18.143	0.059	1.962
3	3.010	17.129	17.774	0.061	2.026
<b>Mean ± S.E.M (n=3)</b>					<b>2.027 ± 0.038</b>

### Extractive Value

The extractive value of *Quercus oblongata* D. Don bark root was determined by the hot extraction method and cold extraction method. The extractive values were calculated for

different plant extracts. In this study it was investigated that in hot extraction procedure, methanolic extract shows maximum extractive value of 10.404% while extractive value of ethyl acetate was found to be 1.751% which was lower as compared to other extracts. In case of cold extraction method water soluble extract shows the peak extractive value of 12.218% while chloroform was less effective with the extractive value of 1.319% as compared to other solvent extracts. The results are tabulated as follows:

**Table no. 5: Petroleum ether soluble extractive value of *Quercus oblongata* D. Don bark powder (Hot Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.0013	57.4591	57.4933	0.0342	3.418
2	4.0011	56.5492	56.5829	0.0337	3.369
3	4.0013	57.2390	57.2708	0.0318	3.178
<b>Mean ± S.E.M (n=3)</b>					<b>3.321 ± 0.073</b>

**Table no. 6: Chloroform soluble extractive value of *Quercus oblongata* D. Don bark powder (Hot Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.0032	56.2381	56.2654	0.0273	2.727
2	4.0031	57.0914	57.1183	0.0269	2.687
3	4.0031	56.7893	56.8170	0.0277	2.767
<b>Mean ± S.E.M (n=3)</b>					<b>2.727 ± 0.023</b>

**Table no. 7: Ethyl acetate soluble extractive value of *Quercus oblongata* D. Don bark powder (Hot Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.0028	58.0192	58.0370	0.0178	1.778
2	4.0027	57.8901	57.9068	0.0167	1.668
3	4.0028	57.9012	57.9193	0.0181	1.808
<b>Mean ± S.E.M (n=3)</b>					<b>1.751 ± 0.042</b>

**Table no. 8: Methanol soluble extractive value of *Quercus oblongata* D. Don bark powder (Hot Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.0006	58.0193	58.1247	0.1054	10.538
2	4.0007	59.0011	59.1038	0.1027	10.268
3	4.0007	58.9012	59.0053	0.1041	10.408
<b>Mean ± S.E.M (n=3)</b>					<b>10.404 ± 0.077</b>

**Table no. 9: Water soluble extractive value of *Quercus oblongata* D. Don bark powder (Hot Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.0004	59.8713	59.9604	0.0891	8.909
2	4.0004	58.2019	58.2897	0.0878	8.779
3	4.0006	57.7632	57.8515	0.0883	8.828
<b>Mean ± S.E.M (n=3)</b>					<b>8.838 ± 0.037</b>

**Table no. 10: Petroleum ether soluble extractive value of *Quercus oblongata* D. Don bark powder (Cold Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.0041	59.0283	59.0539	0.0256	2.557
2	4.0039	59.0547	59.0794	0.0247	2.467
3	4.0041	58.9602	58.9861	0.0259	2.587
<b>Mean ± S.E.M (n=3)</b>					<b>7.611 ± 0.036</b>

**Table no. 11: Chloroform soluble extractive value of *Quercus oblongata* D. Don bark powder (Cold Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.0019	58.9012	58.9144	0.0132	1.319
2	4.0019	56.9035	56.9172	0.0137	1.369
3	4.0018	58.2912	58.3039	0.0127	1.269
<b>Mean ± S.E.M (n=3)</b>					1.319 ± 0.028

**Table no. 12: Ethyl acetate soluble extractive value of *Quercus oblongata* D. Don bark powder (Cold Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.0027	57.0091	57.0488	0.0397	3.967
2	4.0027	57.2971	57.3372	0.0401	4.007
3	4.0028	56.8076	56.8463	0.0387	3.867
<b>Mean± S.E.M (n=3)</b>					3.947 ± 0.041

**Table no. 13: Methanol soluble extractive value of *Quercus oblongata* D. Don bark powder (Cold Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.0021	60.0012	60.1077	0.1065	10.644
2	4.0022	59.0234	59.1285	0.1051	10.504
3	4.0022	59.3201	59.4257	0.1056	10.554
<b>Mean± S.E.M (n=3)</b>					10.567 ± 0.040

**Table no. 14: Water soluble extractive value of *Quercus oblongata* D. Don bark powder (Cold Extraction Method)**

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter(g)	% of extractible matter
1	4.0037	58.0638	58.1849	0.1211	12.098
2	4.0037	58.6651	58.7883	0.1232	12.308
3	4.0037	59.0981	59.2206	0.1225	12.238
<b>Mean± S.E.M (n=3)</b>					<b>12.214 ± 0.061</b>

### Swelling Index

Various healing plants possess a definite healing value due to the presence of fluctuating constituents of hemicelluloses or pectin, gum and mucilage which leads to different swelling properties of diverse plant material. The swelling index parameter was identified and computed to determine the amount of plant crude material that shows swelling after treatment with water and to calculate that the plant material contains some content of mucilage. The swelling index of *Quercus oblongata* D. Don bark was found to be 6.233. The results are tabulated as follows:

**Table no. 15: Swelling index value of *Quercus oblongata* D. Don bark powder**

S. No	Powdered drug wt. (gm)	Stock Volume (in ml)	Swelling factor
1	1.0	25	6.1
2	1.0	25	6.7
3	1.0	25	5.9
<b>Mean± S.E.M (n=3)</b>			<b>6.233 ± 0.240</b>

### Foaming Index

In 10 test tubes decoction of plant material and water was taken in different ratio, foam was measured with the help of scale after shaking the test tube and when the foam in the test tube becomes persistent. Foam height in every test tube was measured above 1cm. So the foaming

index of *Quercus oblongata* D. Don bark was found to be more than 1000 indicating good amount or presence of saponins. The results are tabulated as follows:

**Table no. 16: Foaming index value of *Quercus oblongata* D. Don bark powder**

S. No.	Powdered drug wt. (gm)	Stock Volume (in ml)	Dilution of the test solution (in ml)										Foaming Index
			1	2	3	4	5	6	7	8	9	10	
1	1.0	100	1.2	1.6	1.7	1.8	1.9	1.6	2.3	2.5	2.1	2.7	≤ 1000
2	1.0	100	1.5	1.3	1.2	1.5	2.0	1.9	2.5	2.3	2.6	2.2	≤ 1000
3	1.0	100	1.4	1.1	1.5	1.5	2.2	1.6	2.0	2.4	1.9	2.2	≤ 1000
<b>Mean± S.E.M (n=3)</b>													1000 ± 0.000

### FLUORESCENCE ANALYSIS OF POWDERS

The powder of (mesh size 40) of morphological part of *Quercus oblongata* D. Don was examined under daylight and UV light. Fluorescence analysis of plants various morphological parts showed various coloration by utilizing distinct chemical reagents under UV and visible light. In case of different natural products UV light produce a fluorescent nature, which is important character of fluorescence analysis. The outcomes of fluorescence determination revealed that in visible light various shades were exhibited by plant powder such as yellow, green, cream and brown fluorescence while different shades of yellow, green, cream, brown, black and light red fluorescence were observed in short and long UV. The results are tabulated as follows:

**Table No. 17: Fluorescence analysis of *Quercus oblongata* (Bark)**

S. No.	<i>Quercus oblongata</i> (Bark)	Visible	Short UV-254 nm	Long UV-365 nm
1	Powder + 1N NaOH in water	Amber	Amber	Amber green
2	Powder + 1N NaOH in alcohol	White	White	White
3	Powder + acetic acid	Light brown	Light green	Light green
4	Powder + methanol	Brown	Light brown	Light green
5	Powder + H <sub>2</sub> SO <sub>4</sub>	Black	Black	Black
6	Powder + petroleum ether	No-color	No-color	No-color
7	Powder + HCl	Amber	Amber	Amber green
8	Powder + water	Light-brown	Cream	Cream
9	Powder + nitric acid	Amber	Brown	Light brown
10	Powder + acetone	Cream	Cream	No-color

#### **EXTRACTION OF PLANT MATERIAL**

500 gm coarse powders of bark part of *Quercus oblongata* D. Don was subjected to successive extraction with different solvents like petroleum ether, chloroform, acetone, ethyl acetate, methanol and water in for around 3 hours per solvent. Results are tabulated in below mentioned table.



**Table no. 18: Data showing successive solvent extraction values and nature of extract of *Quercus oblongata* D. Don bark**

S. No	Solvent Used	Wt. of drug (gm)	Yield (gm)	% Yield	Extract color	Property	Mean $\pm$ S.E.M
1	Pet. ether	120	1.982	1.651	Yellow brown	Sticky	1.520 $\pm$ 0.068
			1.792	1.493			
			1.701	1.417			
2	Chloroform	120	2.719	2.265	Brownish black	Sticky	2.203 $\pm$ 0.031
			2.598	2.165			
			2.617	2.180			
3	Acetone	120	4.412	3.367	Brownish Black	Sticky	3.561 $\pm$ 0.113
			4.306	3.558			
			4.512	3.760			
4	Ethyl acetate	120	3.194	2.661	Light Brown	Sticky	2.693 $\pm$ 0.031
			3.094	2.578			
			3.217	2.680			
5	Methanol	120	11.013	9.177	Reddish brown	Sticky	9.228 $\pm$ 0.025
			11.098	9.248			
			11.112	9.260			
6	Water	120	8.796	7.330	Reddish brown	Sticky	7.197 $\pm$ 0.069
			8.512	7.093			
			8.602	7.168			

### QUALITATIVE PHYTOCHEMICAL ANALYSIS

The tested extract of the bark extract contains few alkaloids, flavonoids, tannins, saponins, steroids and proteins.

**Table no. 19: Preliminary phytochemical investigation of various extracts of barks of *Quercus oblongata* D. Don bark**

Chemical Constituents	Tests	PEPB	CFPB	ACPB	EAPB	MEPB	WAPB
<b>Alkaloids</b>	Mayer's test	-ve	-ve	-ve	-ve	-ve	-ve
	Wagner's test	-ve	+ve	-ve	-ve	-ve	-ve
	Hager's test	-ve	-ve	-ve	-ve	-ve	-ve
	Dragendroff's test	-ve	-ve	-ve	+ve	+ve	-ve
<b>Flavanoids</b>	Alkaline reagent test	-ve	+ve	-ve	+ve	+ve	+ve
	Shinoda test	-ve	+ve	-ve	-ve	+ve	+ve
<b>Tannins</b>	Ferric Chloride test	+ve	-ve	+ve	+ve	+ve	+ve
	Lead acetate test	+ve	+ve	-ve	+ve	-ve	+ve
<b>Cardiac glycosides</b>	Keller Killiani test	-ve	-ve	-ve	-ve	-ve	-ve
	Legal Test	-ve	-ve	-ve	-ve	-ve	-ve
<b>Anthraquinone glycosides</b>	Borntrager's test	-ve	-ve	-ve	-ve	-ve	-ve
<b>Saponin glycosides</b>	Foam test	-ve	-ve	+ve	-ve	-ve	+ve
<b>Steroids</b>	Salkowski test	-ve	-ve	+ve	+ve	-ve	-ve
	Liebermann Burchard	+ve	-ve	-ve	-ve	+ve	+ve
<b>Proteins</b>	Biuret test	-ve	+ve	-ve	-ve	+ve	+ve
	Millon's test	-ve	+ve	-ve	-ve	+ve	-ve
	Xanthoproteic test	+ve	-ve	+ve	+ve	-ve	-ve

[PE=Petroleum ether, CF= Chloroform, AC=Acetone, EA=Ethyl acetate, ME=Methanol, WA=Water]

**ANTIOXIDANT ACTIVITY (Free radical scavenging activity using DPPH)**

**Antioxidant Activity of *Quercus oblongata* D. Don bark extract**

In-vitro antioxidant activity of various extract *Quercus oblongata* D. Don bark extract was determined and was compared with standard ascorbic acid with the help of DPPH assay. Absorbance of control sample was found to be 0.288. The absorbance of the sample at

various concentrations was calculated and measured and inhibition of percentage was computed by drawing and plotting a calibration curve between percentage inhibition and concentration. IC<sub>50</sub> value were obtained as 17.296, 16.255, 20.568 and 19.332. It means that ethyl acetate and acetone extract of root at elevated concentration encapsulate increase number of free radicals produced by DPPH resulting into decrease in absorbance and elevation in IC<sub>50</sub> value. Results are tabulated as:

**Table no. 20: Percentage scavenging activity of Standard Compound (Ascorbic Acid)**

S. No.	Concentration (µg/ml)	Absorbance at 517 nm	% Inhibition	Mean ±S.E.M. (n=3)
1	2	0.248, 0.244, 0.245	13.88, 15.27, 14.93	14.69 ± 0.418
2	4	0.234, 0.236, 0.236	18.75, 18.05, 18.05	18.28 ± 0.233
3	6	0.222, 0.226, 0.220	22.91, 21.52, 23.61	22.68 ± 0.614
4	8	0.211, 0.209, 0.211	26.73, 27.43, 26.73	26.96 ± 0.233
5	10	0.196, 0.199, 0.196	31.94, 30.90, 31.94	31.59 ± 0.346
6	12	0.174, 0.176, 0.176	39.58, 38.88, 38.88	39.11 ± 0.233

**Table no. 21: Percentage scavenging activity of methanolic extract of *Quercus oblongata* bark**

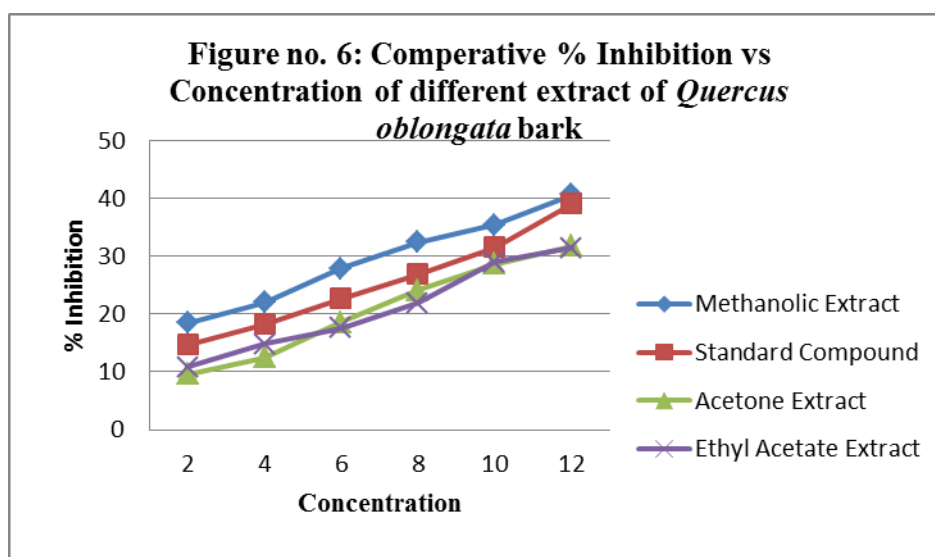
S. No.	Concentration (µg/ml)	Absorbance at 517 nm	% Inhibition	Mean ±S.E.M. (n=3)
1	2	0.238, 0.235, 0.231	17.36, 18.40, 19.79	18.51 ± 0.703
2	4	0.227, 0.225, 0.222	21.18, 21.87, 22.91	21.98 ± 0.502
3	6	0.210, 0.208, 0.205	27.08, 27.77, 28.81	27.88 ± 0.502
4	8	0.197, 0.193, 0.194	31.59, 32.98, 32.63	32.40 ± 0.417
5	10	0.184, 0.186, 0.188	36.11, 35.41, 34.72	35.41 ± 0.401
6	12	0.171, 0.169, 0.173	40.62, 41.31, 39.93	40.62 ± 0.398

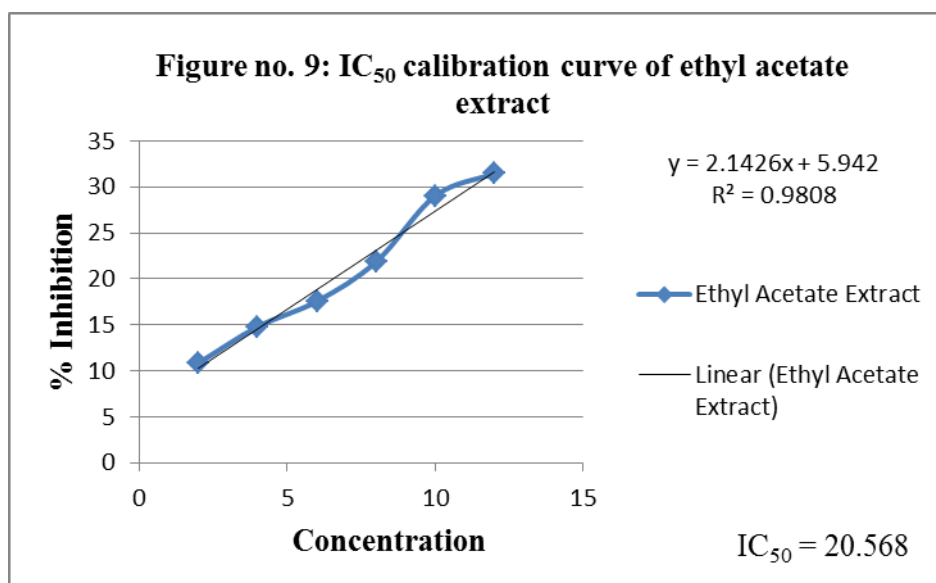
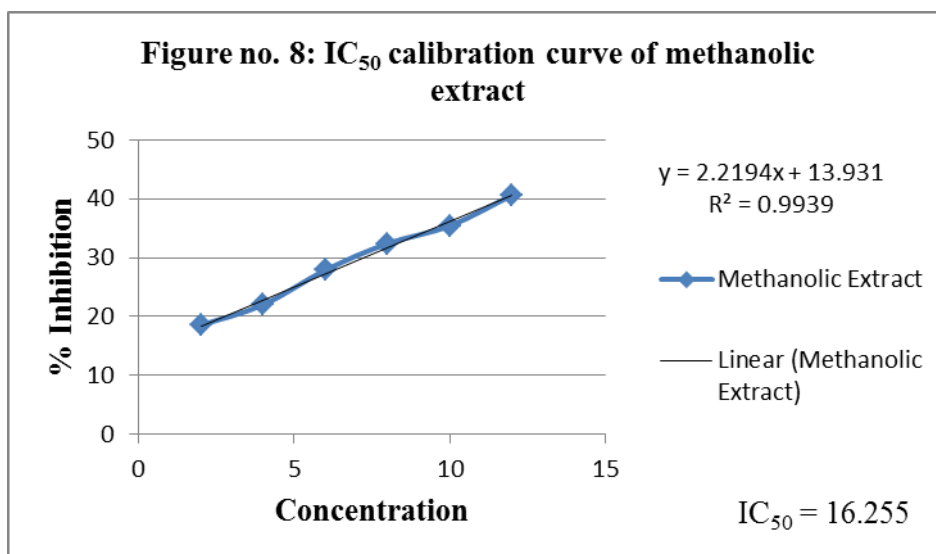
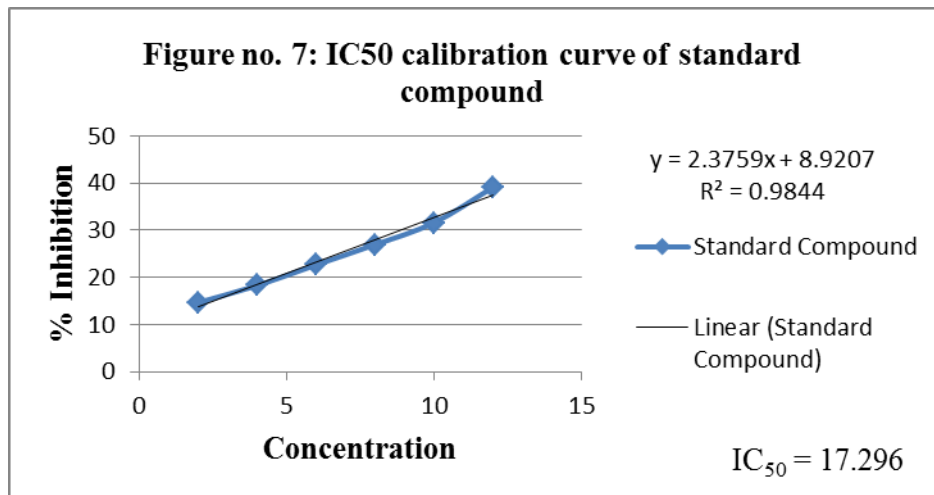
**Table no. 22: Percentage scavenging activity of acetone extract of *Quercus oblongata* bark**

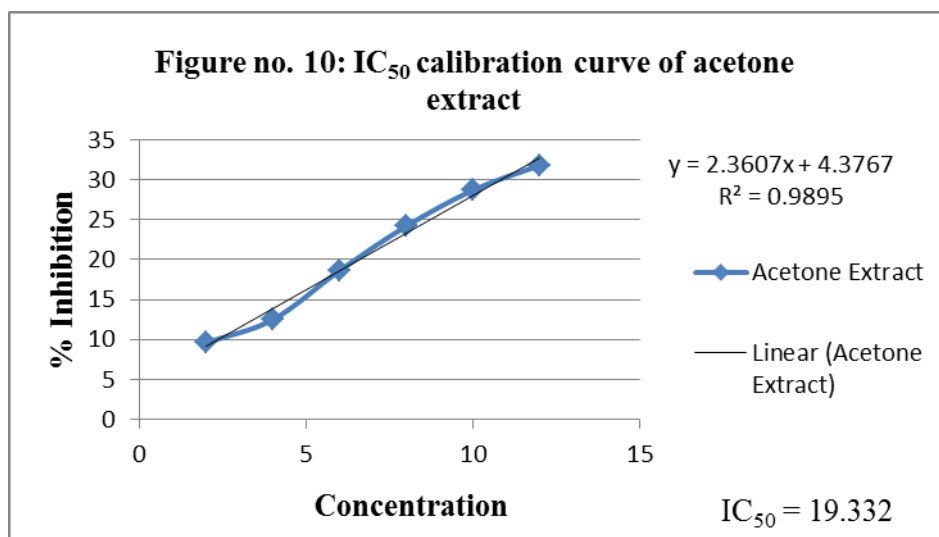
S. No.	Concentration (µg/ml)	Absorbance at 517 nm	% Inhibition	Mean ±S.E.M. (n=3)
1	2	0.261, 0.258, 0.262	9.37, 10.41, 9.02	9.60 ± 0.417
2	4	0.252, 0.254, 0.250	12.5, 11.80, 13.19	12.49 ± 0.401
3	6	0.237, 0.234, 0.232	17.70, 18.75, 19.44	18.63 ± 0.505
4	8	0.220, 0.218, 0.217	23.61, 24.30, 24.65	24.18 ± 0.305
5	10	0.204, 0.207, 0.205	29.16, 28.12, 28.81	28.69 ± 0.305
6	12	0.199, 0.194, 0.196	30.90, 32.63, 31.94	31.82 ± 0.502

**Table no. 23: Percentage scavenging activity of acetone extract of *Quercus oblongata* bark**

S. No.	Concentration (µg/ml)	Absorbance at 517 nm	% Inhibition	Mean ±S.E.M. (n=3)
1	2	0.261, 0.258, 0.262	9.37, 10.41, 9.02	9.60 ± 0.417
2	4	0.252, 0.254, 0.250	12.5, 11.80, 13.19	12.49 ± 0.401
3	6	0.237, 0.234, 0.232	17.70, 18.75, 19.44	18.63 ± 0.505
4	8	0.220, 0.218, 0.217	23.61, 24.30, 24.65	24.18 ± 0.305
5	10	0.204, 0.207, 0.205	29.16, 28.12, 28.81	28.69 ± 0.305
6	12	0.199, 0.194, 0.196	30.90, 32.63, 31.94	31.82 ± 0.502







## CONCLUSION

The current study is well-defined to traverse the herbal diversity of Kumaon and Garhwal region of Uttarakhand for healing value. In this present investigation it is focused for providing towards the knowledge database of therapeutic plants in terms of pharmacognostic, physicochemical and phytochemical efficacy of *Quercus oblongata* D. Don. Pharmacognostic study of root reveals cells and their arrangement which play important role in standardization of this crude drug which will be helpful in the prevention of substitution, adulteration and in identification of two or more species of similar Genus. Transverse section of root reveals that bark shows cork (phellem) layer containing 2-5 layers of radially flattened or isodiametric cells which were arranged in less or more radial pattern. Medullary rays were uni or biseriate, having radially long cells, which were rectangular in shape. In the transverse section the starting area of secondary phloem was distinguished by the presence of multiseriate bands tangential in shape of axial parenchyma cells with which sieve tubes elements were alternating with row of fibers that were tangential and 3-5 cells wide. Bark powdered microscopy showed the existence of medullary rays which were uni to biseriate, along with fibers with sclereids, parenchyma and vessels that were pitted. Pitted vessels, fibers and sclereids were stained with phloroglucinol hydrochloric acid and were stained pink due to the confirmation of lignin present in them. The representation of fiber is accountable for the noticed granular or striated fracture.

Preliminary phytochemical analysis of bark denotes the existence of some therapeutic constituents such as alkaloids, flavonoids, tannins, saponins, steroids and proteins which in coming future will play a vital role in herbal medicine. Antioxidant activity of the leaf extract

of acetone and methanol showed that the plant contains potent antioxidant activity. As not so much research is done and published and available on this plant, the result which comes out from this present research serves as a standard in further investigation of this plant.

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