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Research Article

# Pharmacognostic Evaluation, Preliminary Phyto-Chemical Screening and Antioxidant Potential of Leaves 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 utilizes to prevent and treat various disorders in humans such as hemorrhoid, gastric ulcers, asthma and healing of wound. Various biological activity of the family includes antibacterial, anti-inflammatory, anti-cancer, antidiabetic, antioxidant, and gastro protective activities due to the presence of important chemical constituents such as Phenolic acids, Flavanoids and triterpenoids. Despite the known traditional utilization of this plant as a healer still there has been no decisive experimental outcomes mentioned related to phytochemical and pharmacognostic investigation of the plant.

**Aim:** Thus, the current study was performed to investigate and study the pharmacognostical and phytochemical estimation of *Quercus Oblongata* D. Don.

**Material and Methods:** Leaf 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 wavelength and visible light.

**Results:** In the lower and upper epidermis in leaf microscopical studies reveals various cell constants such as epidermis, glandular trichome, covering trichome, collenchymas, vascular bundle, xylem and palisade cells. Various physicochemical determinations such as extractive value, loss on drying, swelling, foaming index and ash value were evaluated for leaf part. Different range of fluorescence color was analyzed by Fluorescence analysis for powdered crude drug. Phytochemical determination shows the presence of chemical constituents such as alkaloids, flavanoids, tannins, glycosides and proteins in leaf. Antioxidant potential was determined with the help of DPPH assay method in which ethyl acetate and methanol extract showed maximum antioxidant activity.

**Keywords:** *Quercus Oblongata* D.Don, Methanol, Phytochemical, Physicochemical, Chloroform, Microscopical, solvents.

## INTRODUCTION

Flora is making a comeback and floral rebirth is spreading all around the world. Around 75% of the population of world depends and believes on plants and plant extracts for health care. On an average round around 30% of the whole plant species, at present time or at other was used for healing purposes. It is believed that out of the 2, 50,000 high families of plant species, which are more than in 80,000 amounts, have medicinal values. India is in the center for 12 biodiversity middle of the Universe with the presence of round around 45000 various plant species. Out of them, around 15000-20000 plants possess excellent medicinal activities. Only around 7000 to7500 species of plants are used for their healing and curing properties by conventional practitioners and communities[1]. In ancient times mankind almost depends on nature for both health and illness. Primitive peoples treated illness by using plant, animals and mineral parts[2].

Herbal medicines occur naturally, basically they are derived from plant substances and used to treat disease or illness within regional or local healing practices. Herbal medicinal products are mixture of complex organic compounds that is derived from any row or processed part of a particular plant. Herbal medicines have firmed roots

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in various cultures around the world[3].In the current years there has been wide development in the floral medicine field and these particular drugs acquiring vogue in developed and still developing countries, as they are obtained from natural and their toxic and side effects are less. Traditionally in utilization of these medicines are mainly obtained from plant, animals, organic matter and minerals[4]. In Indian system of medicine most of the traditional healers and practitioners formulate their own preparation and dispense them. The World Health Organization (WHO) has mentioned in their list around 21,000 plants, which are using worldwide for medicinal purpose. Out of these around 2500 plant species are present in India, out of which 150 plant species are utilized on a rich scale. Our country India is among the biggest promoter of healing herbs and it is known as herbal garden of the world[4].

Herbal medicines have been utilized and registered in India, Egyptian, Chinese, roman and Greek system of medicines for round about 5000 years as demonstrated by earliest as well as traditional literatures. The record of classical traditional medicine system in India includes Atherveda, Rigveda, Charak Samhita and Sushruta Samhita. Tribal (folk) medicines are main source for the native healthcare system. India is amongst to be a rich warehouse of medicinal plant from the ancient civilization. The various forests in India are happy hunting ground as principle origin of huge number of various medicinal and ethereal plants[5-6].

Traditional medicines immerse a beneficial role in the wellness program system especially of developing kingdoms. The World Health organization (WHO) reported that in these countries more than 80% of healthcare needs are fulfilled by conventional healthcare traders. People in countries which are developing mainly depend upon traditional medicines as they are cheaper and approachable than orthodox medicine[7].

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[8]. The family Fagaceae covers over 1000 trees and shrubs species such as oaks (genus Querqus), chestnut (Castanea) and beeches (Fagus) which are widely distributed in Northern Hemisphere[9].

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[10].

Fagaceae family is distributed widely in forests of Northern Hemisphere having temperate and tropical climatic conditions and areas. In traditional healing many of its members have been utilizes to prevent and treat various disorders in humans such as hemorrhoid, gastric ulcers, asthma and healing of wound. Various biological activity of the family includes antibacterial, anti-inflammatory, anti-cancer, antidiabetic, antioxidant, and gastro protective activities due to the presence of important chemical constituents such as Phenolic acids, Flavanoids and triterpenoids[11].

The Fagaceae family is subdivided into ten genera: *Formanodendron* Nixon et Crepet, *Colombobalanus* Nixon et Crepet, *Trigonobalanus* Forman, *Castanopsis* (D. Don) Spach, *Notholithocarpus* Manos, *Castanea* Miller, Cannon et S. Oh, *Quercus* L., *Lithocarpus* Bl., *Chrysolepis* Hjelmqvist and *Fagus* L. *Fagus, Castanopsis, Formanodendron, Castaneaa, Quercus* and Lithocarpus, are found mostly in South and Southwest China.

Fagaceae family contains various distinct characters which help to define the family and its genera. The diagnostic characters of the family are simple leaves that contains diagonally positioned tertiary vein, complexes of cycloptic and anomocytic stomata and specific trichome. One of the important diagnostic characteristics of Fagaceae family is trichome which is divided into intermediate, glandular and non-glandular trichome[12].

## MATERIALS AND METHODS

### Plant Collection and Authentification

Specimens of morphological plant were gathered from field areas, forest, and adjacent region of Kasardevi, Almora Uttarakhand during the month of 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 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 airtight 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 recognization of herbs having medicinal value depends on color, size, shape, surface characters, fracture, surface characteristics and emergence of the cutted 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 authentification 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[13, 14, 15].

**Powder Microscopy**

Dried leaves and root powder microscopy of plants were 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 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[13, 14, 16, 17].

**Leaf Constants Determination**

Various leaf invariable parameters were used to differentiate those plant species which were closely related and are identified not easily by universal microscopy[18].

**Physico-Chemical Parameters**

Dried powder of leaves and root were put through to physicochemical examination. Physiochemical constants such as loss on drying, extractive value, ash value, swelling index and foaming index was performed and studied[19].

**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[20]. 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 recognization of various plant samples and crude drugs[21].

**Extraction of Plant Material**

The powdered leaves 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 (50-70ºC) 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[22].

**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, falvonoids, cardiac glycosides, anthroquinone glycosides, saponins glycosides, carbohydrates, proteins, amino acids, fats and fixed oils following standard methods[22, 23].

**Antioxidant Activity by Dpph Methods**

With the help of DPPH (1, 1-diphenyl-2picryl hydrazyl) different extracts of plants morphological parts (leaves, barks and root) 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 concentration (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 sand for around time 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 was performed in triplicate and the compound which was used as standard was ascorbic acid[24, 25, 26].

**RESULT AND DISCUSSION**

**Collection of Plant Material**

**Figure 1: Herbarium specimen and plant authentification certificate of *Quercus Oblongata* D.Don**


The unknown sample of plants were 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**

**Microscopical Evaluation**

**Microscopical Evaluation of *Quercus Oblongata* D.Don**

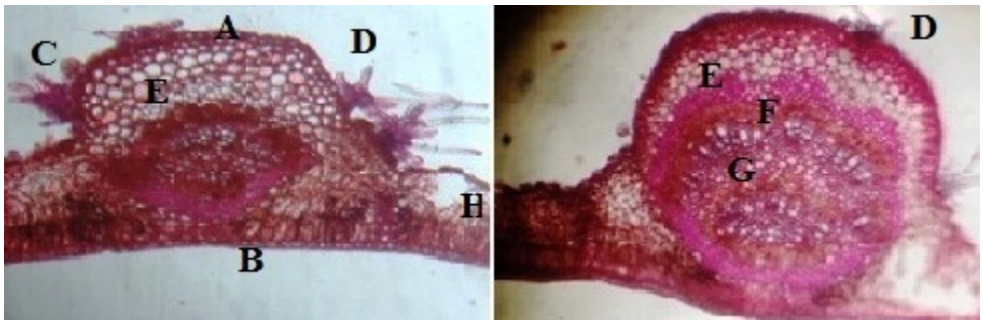


**Figure 3: T. S. of *Quercus Oblongata* D.Don leaf showing lower epidermis, upper epidermis, glandular trichome, covering trichome, collenchymas, vascular bundle, xylem and palisade cells**

Transverse section of the oak leaf showed highly domed mid rib in the abaxial position. Upper epidermal layer consists of cells which were oval-square in shape and is neighbored by cuticle layer which was thick. 5-10 rows of parenchymatous cells were filled at the base of epidermis layer. Vascular bundles were completely surrounded by a ring of sclerenchymatous cells in the mid rib. Phloem and xylem vessels are present in a circle form. Vascular bundle were in dorsal side in the mid rib contains parenchymatous cells having 8-15 rows. Structure of lower epidermal cells was oval square shaped and as compared to upper epidermal cells it is smaller. Stellate hairs are present in upper and lower epidermal cells.

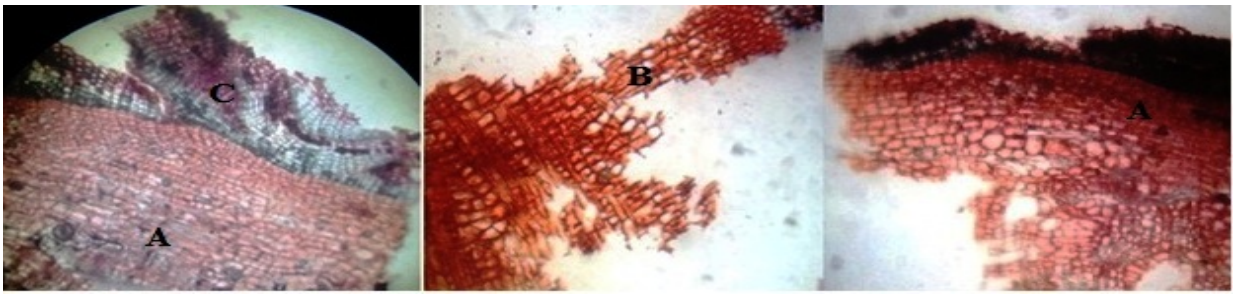


**Figure 4: T. S of *Quercus Oblongata* D. Don bark showing cortex, medullary rays and cork portion**

Stem bark transverse section reviles 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 unlignified 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.

**Powder Microscopy**

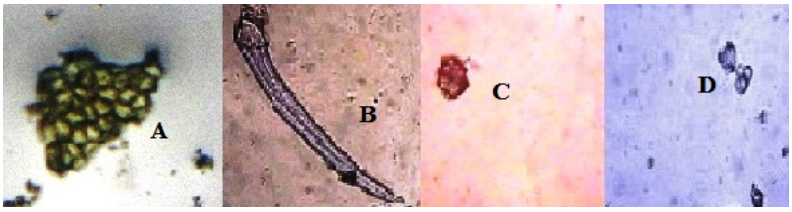


**Figure 5: Powder photomicrograph of *Quercus Oblongata* D. Don leaf showing epidermal cells, covering trichome, rosette calcium oxalate crystals, starch grains**

The plant material which was powdered was dark green in color, consisting of fragments of palisade cells, parenchyma, and stomata along with epidermal cells fragments. Lignified vessels having simple pits were observed. The shape of the epidermal cells was irregular. Trichome was multicellular, uniseriate covering trichomes. Phloem fibers are lignified long and slender. Xylem vessels contain spiral, annular thickening vessels. Starch grains are present in the form of small or large granules. Rosette shaped calcium oxalate crystals were observed.

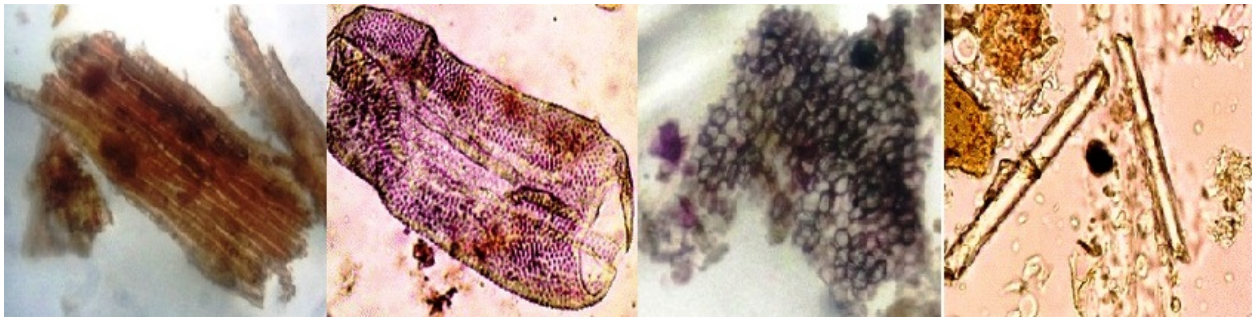

**Figure 6: Powder photomicrograph of *Quercus Oblongata* D. Don bark showing medullary rays, fibers, cork cells and xylem vessels**

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. 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.

**Determination of Leaf Constants**

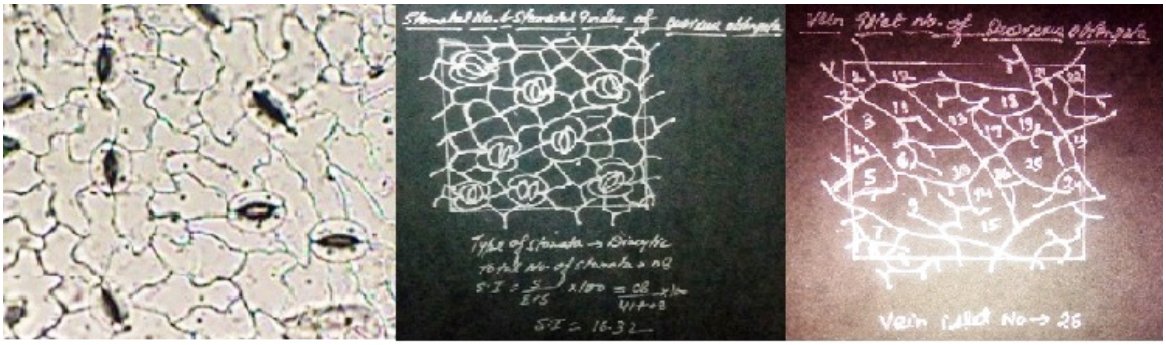

**Figure 7: Leaf constant parameters of *Quercus Oblongata* D.Don leaf mentioning stomata type, index of stomata and vein islet number**

The stomata type present was anomocytic, values of stomatal index, stomatal number and vein islet number of upper and lower epidermis of leaf was calculated and results were tabulated in below mentioned table.

**Table 1: Stomatal no. and stomatal index of *Quercus Oblongata* D. Don leaf**

| S. No. | Surface Type | No. of stomata (Per mm$^2$) | Epidermal cells total in number | Index of Stomata (I= S÷E+S x 100) (Value in 1mm$^2$ area) | Vein islet no. (Value in 1mm$^2$ area) |
|---|---|---|---|---|---|
| 1 | Lower | 61 | 246 | 19.869 | 27 |
| | | 67 | 256 | 20.743 | 32 |
| | | 63 | 262 | 19.384 | 29 |
| | Mean ± S.E.M (n=3) | 63.666 ± 1.763 | | 19.998 ± 0.397 | 29.333 ± 1.452 |
| 2 | Upper | 46 | 189 | 19.574 | 21 |
| | | 42 | 167 | 19.138 | 24 |
| | | 37 | 128 | 22.424 | 20 |
| | Mean ± S.E.M (n=3) | 41.666 ± 2.603 | | 20.376 ± 1.030 | 21.666 ± 1.201 |

**Physico-Chemical Parameters**

**Figure 8: Physico-chemical parameters of *Quercus Oblongata* D. Don leaf mentioning type loss on drying, ash value, swelling index and foaming index**

**Loss on Drying**

The value of loss on drying for the leaf sample of *Quercus Oblongata* D. Don was found to be 7.940%.

**Table 2: Loss on drying of *Quercus Oblongata* D. Don leaf powder**

| S. No. | Drug wt. + porcelain dish wt. before drying A (g) | Drug wt. + porcelain dish wt. after drying B (g) | Loss on drying A-B (g) | % of loss on drying |
|---|---|---|---|---|
| 1 | 10.019 + 42.712 | 51.930 | 0.801 | 7.994 |
| 2 | 10.011 + 41.101 | 50.316 | 0.796 | 7.951 |
| 3 | 10.017 + 43.945 | 53.173 | 0.789 | 7.876 |
| Mean ± S.E.M (n=3) | | | | 7.940 ± 0.034 |

**Ash Value**

The values of ash i.e. Total ash, water soluble ash and acid insoluble ash were evaluated as per official process. 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 leaf was calculated as 10.297%. 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. Amount of silica present in given plant material is determined by acid insoluble ash. Value of acid insoluble ash was computed as 2.357%. 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 2.917%. The results were found to be almost within limits.

**Table 3: Total ash value of *Quercus Oblongata* D. Don leaf 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 | 3.009 | 16.452 | 16.764 | 0.312 | 10.368 |
| 2 | 3.012 | 18.120 | 18.417 | 0.297 | 9.860 |
| 3 | 3.010 | 17.782 | 18.103 | 0.321 | 10.664 |
| Mean ± S.E.M (n=3) | | | | | 10.297 ± 0.234 |

**Table 4: Acid insoluble ash value of *Quercus Oblongata* D. Don leaf 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 | 3.026 | 17.442 | 17.516 | 0.074 | 2.445 |
| 2 | 3.029 | 16.872 | 16.941 | 0.069 | 2.277 |
| 3 | 3.022 | 17.289 | 17.360 | 0.071 | 2.349 |
| Mean ± S.E.M (n=3) | | | | | 2.357 ± 0.048 |

**Table 5: Water soluble ash value of *Quercus Oblongata* D. Don leaf powder**

| S. No. | Drug wt. (g) | Wt. of empty china dish (g) | Crucible wt. + Wt. of ash (g) | Ash Wt. (g) | % of water-soluble ash |
|---|---|---|---|---|---|
| 1 | 3.017 | 18.238 | 18.329 | 0.091 | 3.016 |
| 2 | 3.014 | 17.904 | 17.993 | 0.089 | 2.952 |
| 3 | 3.017 | 17.780 | 17.864 | 0.084 | 2.784 |
| Mean ± S.E.M (n=3) | | | | | 2.917 ± 0.069 |

**Extractive Value**

The extractive value of *Quercus Oblongata* D.Don leaf 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 12.474% while extractive value of ethyl acetate was found to be 1.619% which was lower as compared to other extracts. In case of cold extraction method water soluble extract shows the peak extractive value of 9.314% while chloroform was less effective with extractive value of 1.584% as compared to other solvent extracts. The results are tabulated as follows:

**Table 6: Petroleum ether soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 4.0037 | 59.3621 | 59.3992 | 0.0371 | 3.708 |
| 2 | 4.0017 | 58.7621 | 58.7932 | 0.0311 | 3.108 |
| 3 | 4.0017 | 58.4298 | 58.4650 | 0.0352 | 3.518 |
| Mean ± S.E.M (n=3) | | | | | 3.444 ± 0.177 |

**Table 7: Chloroform soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 57.5621 | 57.5852 | 0.0231 | 2.309 |
| 2 | 4.0013 | 58.0862 | 58.1101 | 0.0239 | 2.389 |
| 3 | 4.0017 | 57.9431 | 57.9709 | 0.0278 | 2.779 |
| Mean ± S.E.M (n=3) | | | | | 2.492 ± 0.144 |

**Table 8: Ethyl acetate soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 4.0007 | 56.7234 | 56.7390 | 0.0156 | 1.559 |
| 2 | 4.0009 | 57.0213 | 57.0375 | 0.0162 | 1.619 |
| 3 | 4.0007 | 56.9621 | 56.9789 | 0.0168 | 1.679 |
| Mean ± S.E.M (n=3) | | | | | 1.619 ± 0.034 |

**Table 9: Methanol soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 4.0031 | 59.0128 | 59.1380 | 0.1252 | 12.510 |
| 2 | 4.0029 | 58.7532 | 58.8768 | 0.1236 | 12.351 |
| 3 | 4.0031 | 58.4271 | 58.5525 | 0.1257 | 12.561 |
| Mean ± S.E.M (n=3) | | | | | 12.474 ± 0.063 |

**Table 10: Water soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 4.0009 | 57.3762 | 57.4504 | 0.0742 | 7.418 |
| 2 | 4.0010 | 56.9021 | 56.9789 | 0.0768 | 7.678 |
| 3 | 4.0010 | 57.2319 | 57.3031 | 0.0712 | 7.118 |
| Mean ± S.E.M (n=3) | | | | | 7.404 ± 0.161 |

**Table 11: Petroleum ether soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 4.0004 | 56.8632 | 56.8845 | 0.0213 | 2.129 |
| 2 | 4.0003 | 57.5632 | 57.5869 | 0.0237 | 2.369 |
| 3 | 4.0004 | 57.0024 | 57.0249 | 0.0225 | 2.249 |
| Mean ± S.E.M (n=3) | | | | | 2.249 ± 0.069 |

**Table 12: Chloroform soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 4.0132 | 58.2123 | 58.2279 | 0.0156 | 1.554 |
| 2 | 4.0132 | 58.2456 | 58.2618 | 0.0162 | 1.614 |
| 3 | 4.0132 | 57.9862 | 58.0021 | 0.0159 | 1.584 |
| Mean ± S.E.M (n=3) | | | | | 1.584 ± 0.017 |

**Table 13: Ethyl acetate soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 4.0043 | 59.0021 | 59.0555 | 0.0534 | 5.334 |
| 2 | 4.0045 | 58.7689 | 58.8251 | 0.0562 | 5.613 |
| 3 | 4.0043 | 58.8732 | 58.9256 | 0.0524 | 5.234 |
| Mean ± S.E.M (n=3) | | | | | 5.393 ± 0.113 |

**Table 14: Methanol soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 4.0007 | 56.7653 | 56.8296 | 0.0643 | 6.428 |
| 2 | 4.0007 | 57.6759 | 57.7398 | 0.0639 | 6.388 |
| 3 | 4.0004 | 58.3465 | 58.4113 | 0.0648 | 6.479 |
| **Mean ± S.E.M (n=3)** | | | | | 6.431 ± 0.026 |

**Table 15: Water soluble extractive value of *Quercus Oblongata* D. Don leaf 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 | 4.0035 | 56.2190 | 56.3132 | 0.0942 | 9.411 |
| 2 | 4.0039 | 56.7601 | 56.8539 | 0.0938 | 9.370 |
| 3 | 4.0035 | 57.0089 | 57.1006 | 0.0917 | 9.161 |
| Mean± S.E.M (n=3) | | | | | 9.314 ± 0.077 |

**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 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 leaf was found to be 6.900. The results are tabulated as follows:

**Table 16: Swelling index value of *Quercus Oblongata* D. Don leaf powder**

| S. No | Powdered drug weight (gm) | Stock Volume ( in ml) | Swelling factor |
|---|---|---|---|
| 1 | 1.0 | 25 | 7.1 |
| 2 | 1.0 | 25 | 6.9 |
| 3 | 1.0 | 25 | 6.7 |
| Mean± S.E.M (n=3) | | | 6.900 ± 0.115 |

**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 below 1cm. So the foaming index of *Quercus Oblongata* D.Don leaf was found to be 120.37 indicating average amount or presence of saponins. The results are tabulated as follow:

**Table 17: Foaming index value of *Quercus Oblongata* D. Don leaf 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 | 0.4 | 0.2 | 0.6 | 0.7 | 0.4 | 0.5 | 0.9 | 1.7 | 0.9 | 0.8 | 125.000 |
| 2 | 1.0 | 100 | 0.1 | 0.3 | 0.5 | 0.4 | 0.7 | 0.5 | 0.8 | 0.4 | 1.5 | 0.6 | 111.111 |
| 3 | 1.0 | 100 | 0.2 | 0.4 | 0.5 | 0.7 | 0.6 | 0.3 | 0.7 | 1.2 | 0.8 | 0.7 | 125.000 |
| Mean ± S.E.M (n=3) | | | | | | | | | | | | | 120.370 ± 4.629 |

**Fluorescence Analysis of Powders**

The powder of (mesh size 40) of various morphological parts of *Premna barbata* Wall. Ex Schauer, *Lactuca dissecta* D.Don and *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:

***Quercus Oblongata* (Leaf)**

**Table 18: Fluorescence analysis of *Quercus Oblongata* D. Don leaf powder powders**

| S. No. | *Quercus Oblongata* (Leaf) | Visible | Short UV-254 nm | Long UV-365 nm |
|---|---|---|---|---|
| 1 | Powder + 1N NaOH in water | Dark brown | Brown | Amber, green |
| 2 | Powder + 1N NaOH in alcohol | Light green | Light green | Green |
| 3 | Powder + acetic acid | Light green | Greenish yellow | Green |
| 4 | Powder + methanol | Green | Light green | Green |
| 5 | Powder + $H_2SO_4$ | Black | Black | Black |
| 6 | Powder + petroleum ether | Pale yellow | Light red | Light green |
| 7 | Powder + HCl | Amber | Brown | Amber, green |
| 8 | Powder + water | Pale yellow | Pale yellow | Pale yellow |
| 9 | Powder + nitric acid | Brick red | Brick red | Amber, green |
| 10 | Powder + acetone | Light green | Light red | Green |

**Extraction of Plant Material**

500 gm coarse powders of various morphological parts of *Premna barbata* Wall. Ex Schauer, *Lactuca dissecta* D.Don and *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 hrs per solvent. Results are tabulated in below mentioned table.

**Table 19: Data showing successive solvent extraction values and nature of extract of *Quercus Oblongata* D.Don leaf**

| S. No | Solvent Used | Wt. of drug (gm) | Yield (gm) | % Yield | Extract color | Property | Mean ± S.E.M |
|---|---|---|---|---|---|---|---|
| 1 | Pet. ether | 120 | 2.164 | 1.803 | Dark Green | Crystalline | 1.868 ± 0.037 |
| | | | 2.243 | 1.869 | | | |
| | | | 2.319 | 1.932 | | | |
| 2 | Chloroform | 120 | 4.276 | 3.563 | Greenish brown | Sticky | 3.497 ± 0.035 |
| | | | 4.129 | 3.440 | | | |
| | | | 4.187 | 3.489 | | | |
| 3 | Acetone | 120 | 3.194 | 2.661 | Greenish Yellow | Sticky | 2.764 ± 0.064 |
| | | | 3.413 | 2.844 | | | |
| | | | 3.297 | 2.747 | | | |
| 4 | Ethyl acetate | 120 | 1.946 | 1.621 | Light Green | Sticky | 1.464 ± 0.082 |
| | | | 1.734 | 1.445 | | | |
| | | | 1.679 | 1.339 | | | |
| 5 | Methanol | 120 | 5.679 | 4.732 | Yellowish Green | Sticky | 4.637 ± 0.047 |
| | | | 5.521 | 4.600 | | | |
| | | | 5.498 | 4.581 | | | |
| 6 | Water | 120 | 6.729 | 5.607 | Brownish Green | Sticky | 5.495 ± 0.063 |

**Qualitative Phytochemical Analysis**

With the help of screening chemical test the tested extract of *Quercus Oblongata* D.Don leaves revels the existence of chemical constituents such as alkaloids, flavanoids, tannins, saponins, steroids and few proteins while the bark extract contains few alkaloids, flavanoids, tannins, saponins, steroids and proteins.

**Table 20: Preliminary phytochemical investigation of various extracts of leaves of *Quercus Oblongata* D. Don leaves**

| Chemical Constituents | Tests | PEPB | CFPB | ACPB | EAPB | MEPB | WAPB |
|---|---|---|---|---|---|---|---|
| **Alkaloids** | 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 |
| **Flavanoids** | Alkaline reagent test | -ve | +ve | -ve | +ve | +ve | +ve |
| | Shinoda test | -ve | +ve | -ve | -ve | +ve | +ve |
| **Tannins** | Ferric Chloride test | +ve | -ve | +ve | +ve | +ve | +ve |
| | Lead acetate test | +ve | +ve | -ve | +ve | -ve | +ve |
| **Cardiac glycosides** | Keller Killiani test | -ve | -ve | -ve | -ve | -ve | -ve |
| | Legal Test | -ve | -ve | -ve | -ve | -ve | -ve |
| **Anthraquinone glycosides** | Borntrager's test | -ve | -ve | -ve | -ve | -ve | -ve |
| **Saponin glycosides** | Foam test | -ve | -ve | +ve | -ve | -ve | +ve |
| **Steroids** | Salkowski test | -ve | -ve | +ve | +ve | -ve | -ve |
| | Liebermann Burchard | +ve | -ve | -ve | -ve | +ve | +ve |
| **Proteins** | 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]**

**Thin Layer Chromatographic Studies**

TLC *Quercus Oblongata* D.Don revealed the among chloroform, methanol and water extracts of leaves, methanolic and aqueous extracts of leaves gave an excellent observation and results which was directing towards the confirmation of various chemical constituents. At various solvent systems chemical constituents gave different value of $R_f$.

In the variation of $R_f$ in secondary metabolites gives important observation and rule for observing their polarity and also for suitable selection of particular solvent system which can be used by column chromatography for the dissociation of natural compounds which are pure. With different polarity at various ratios of solvent mixture is used for compounds which is to be separated and is pure can be further isolated from plant extract. By the use of various solvent systems compounds $R_f$ value was calculated by the selection of suitable system of solvent for a peculiar plant extract. The results for $R_f$ values of leaf extracts are tabulated in the table no.

**Figure 9: TLC profile of *Quercus Oblongata D.Don* leaf**

**Table 21: TLC solvent system along with spraying reagent and $R_f$ value for determination of secondary metabolites in *Quercus Oblongata* D.Don leaf**

| S.No | Secondary metabolites | Solvent System | Spraying reagent | Extract type | $R_f$ Value |
|---|---|---|---|---|---|
| 1 | Alkaloids | EtOAc:$CHCl_3$: $H_2O$ (5:3:1) | Mayer's reagent | Chloroform<br>Methanol<br>Water | 0.54, 0.61, 0.62<br>0.31,0.37, 0.42<br>0.54, 0.49, 0.57 |
| 2 | Flavanoids | Butan-1ol:EtOAc: $H_2O$ (5:10:15) | 3 % boric acid & 10 % oxalic acid | Chloroform<br>Methanol<br>Water | 0.58, 0.62, 0.64<br>0.57, 0.61, 0.68<br>0.34, 0.29, 0.36 |
| 3 | Tannins | $CHCl_3$: $H_2O$ (6:4) | Ferric Chloride | Chloroform<br>Methanol<br>Water | 0.31, 0.33,.0.26<br>0.47, 0.51, 0.43<br>0.56, 0.53, 0.57 |
| 4 | Saponins | $CHCl_3$:MeOH: $H_2O$ (60:30:4) | Antimony trichloride | Chloroform<br>Methanol<br>Water | 0.38, 0.19, 0.26<br>0.45, 0.42, 0.39<br>0.52, 0.56, 0.49 |
| 5 | Steroids | 1-Hexanol:PET:AcOH (65:35:1) | 3 % Sulphuric acid | Chloroform<br>Methanol<br>Water | 0.27, 0.31, 0.17<br>0.45, 047, 0.41<br>0.25, 0.21, 0.29 |
| 6 | Amino acids | Butan-1ol: AcOH: $H_2O$ (3:1:1) | Ninhydrine Reagent | Chloroform<br>Methanol<br>Water | 0.34, 0.27, 0.30<br>0.25, 0.27, 0.31<br>0.59, 0.63, 0.57 |

***Antioxidant Activity***

In-vitro antioxidant activity of various extract of *Quercus Oblongata* D.Don leaf 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. 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. $IC_{50}$ value obtained for standard (ascorbic acid), methanolic extract, ethyl acetate extract and acetone extract were obtained as 17.296, 19.529, 18.678 and 22.354 respectively. It means that acetone and methanol extract of crude plant material at elevated concentration encapsulate increased number of free radicals produced by DPPH resulting into decrease in

absorbance and elevation in $IC_{50}$ value. Results are tabulated as:

**Table 22: 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 23: Percentage scavenging activity of methanolic extract of *Quercus Oblongata* leaf**

| S.No. | Concentration (µg/ml) | Absorbance at 517 nm | % Inhibition | Mean ± S.E.M. (n=3) |
|---|---|---|---|---|
| 1 | 2 | 0.258, 0.250, 0.253 | 10.41, 13.19, 12.15 | 11.91 ± 0.811 |
| 2 | 4 | 0.246, 0.248, 0.248 | 14.58, 13.88, 13.88 | 14.11 ± 0.233 |
| 3 | 6 | 0.232, 0.230, 0.235 | 19.44, 20.13, 18.40 | 19.32 ± 0.502 |
| 4 | 8 | 0.222, 0.224, 0.222 | 22.91, 22.22, 22.91 | 22.68 ± 0.230 |
| 5 | 10 | 0.210, 0.212, 0.211 | 27.08, 26.38, 26.73 | 26.73 ± 0.202 |
| 6 | 12 | 0.186, 0.188, 0.186 | 35.41, 34.72, 35.41 | 35.18 ± 0.230 |

**Table 24: Percentage scavenging activity of Ethyl acetate extract of *Quercus Oblongata* leaf**

| S.No. | Concentration (µg/ml) | Absorbance at 517 nm | % Inhibition | Mean ±S.E.M. (n=3) |
|---|---|---|---|---|
| 1 | 2 | 0.252, 0.253, 0.250 | 12.50, 12.15, 13.19 | 12.61 ± 0.305 |
| 2 | 4 | 0.239, 0.236, 0.238 | 17.01, 18.05, 17.36 | 17.47 ± 0.305 |
| 3 | 6 | 0.226, 0.227, 0.224 | 21.52, 21.18, 22.22 | 21.64 ± 0.306 |
| 4 | 8 | 0.218, 0.216, 0.219 | 24.30, 25.00, 23.95 | 24.41 ± 0.308 |
| 5 | 10 | 0.201, 0.204, 0.203 | 30.20, 29.16, 29.51 | 29.62 ± 0.305 |
| 6 | 12 | 0.181, 0.186, 0.183 | 37.15, 35.41, 36.45 | 36.33 ± 0.505 |

**Table 25: Percentage scavenging activity of Ethyl acetate extract of *Quercus Oblongata* leaf**

| S.No. | Concentration (µg/ml) | Absorbance at 517 nm | % Inhibition | Mean ±S.E.M. (n=3) |
|---|---|---|---|---|
| 1 | 2 | 0.252, 0.253, 0.250 | 12.50, 12.15, 13.19 | 12.61 ± 0.305 |
| 2 | 4 | 0.239, 0.236, 0.238 | 17.01, 18.05, 17.36 | 17.47 ± 0.305 |
| 3 | 6 | 0.226, 0.227, 0.224 | 21.52, 21.18, 22.22 | 21.64 ± 0.306 |
| 4 | 8 | 0.218, 0.216, 0.219 | 24.30, 25.00, 23.95 | 24.41 ± 0.308 |
| 5 | 10 | 0.201, 0.204, 0.203 | 30.20, 29.16, 29.51 | 29.62 ± 0.305 |
| 6 | 12 | 0.181, 0.186, 0.183 | 37.15, 35.41, 36.45 | 36.33 ± 0.505 |

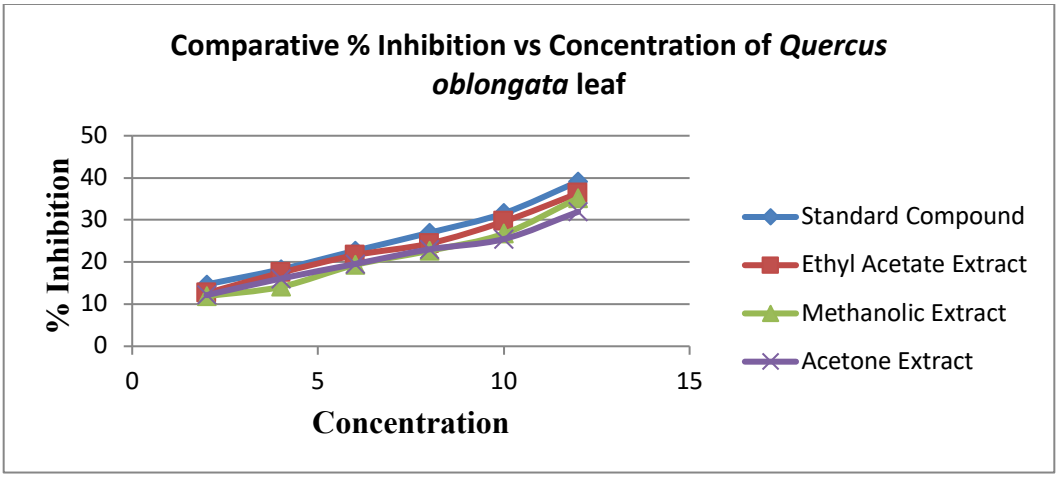


**Figure 10: Comparative % Inhibition vs Concentration of *Quercus Oblongata* leaf**

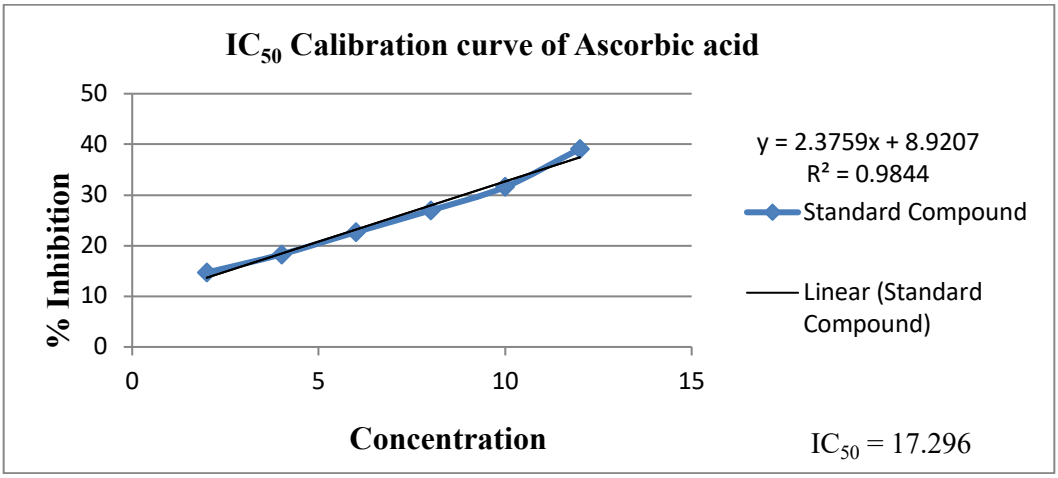


**Figure 11: Calibration curve for estimation value of $IC_{50}$ for Ascorbic acid**

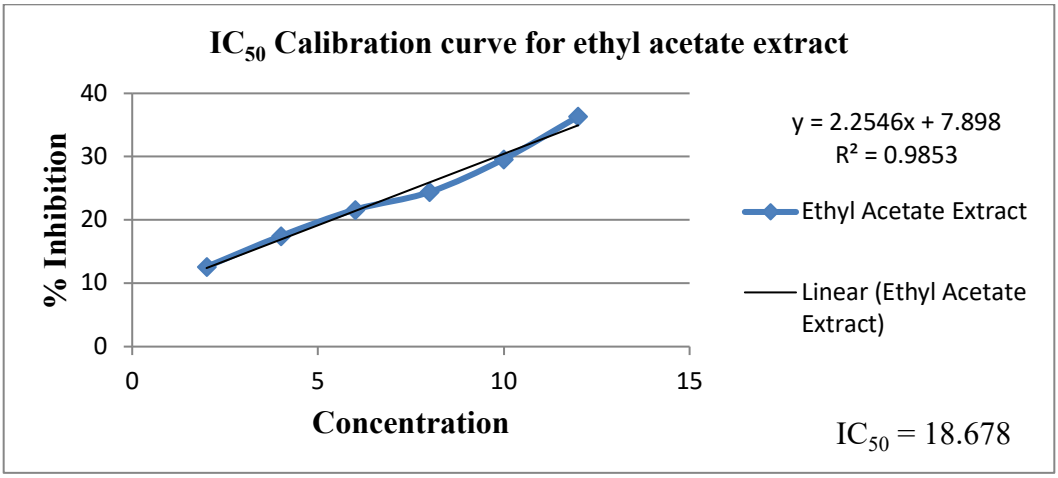


**Figure 12: Calibration curve for estimation value of $IC_{50}$ of ethyl acetate extract**

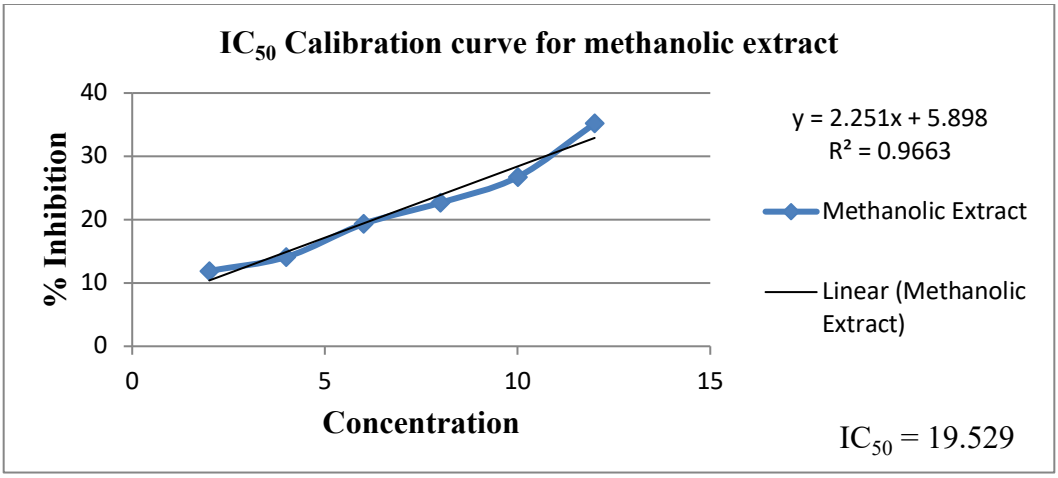


**Figure 13: Calibration curve for estimation value of $IC_{50}$ of methanol extract**

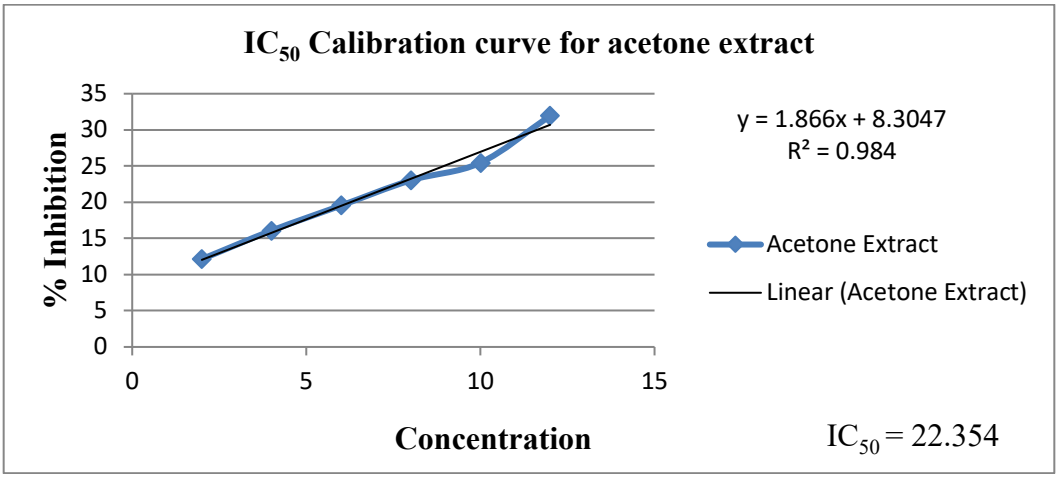


**Figure 14: Calibration curve for estimation value of $IC_{50}$ of acetone extract**

## CONCLUSION

The current study focuses to traverse the vegetation diversity of Garhwal and Kumaun region of Uttarakhand for therapeutic value. In this research work it is aimed at contributing towards the knowledge database of therapeutic plants in terms of pharmacognostic, physicochemical and phytochemical efficacy of *Quercus Oblongata* D.Don. Pharmacognostic study of leaf revels cells and their arrangement which play important role in standardization of this crude drug which will be helpful in prevention of substitution, adulteration and also in identification of two or more species of similar Genus. Initial phytochemical screening of leaf indicates the presence of some chemical constituents which in future will play essential 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 outcomes from this current study serve as a standard in further study of this plant.

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