

PHYTOCHEMICAL ANALYSIS AND ANTI-OXIDANT POTENTIAL OF ETHANOLIC EXTRACT OF *POLYALTHIA LONGIFOLIA* LEAVES

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ABSTRACT

Medicinal herbs because of their therapeutic effect have been used for ages to treat a different type of diseases. *Polyalthia longifolia* has a wide range of pharmacological activity including anti-inflammatory, anti-oxidant, anti-cancerous and anti-microbial properties. The main objective of this study was to use the *Polyalthia longifolia* ethanolic leaves extract and to evaluate their phyto-chemistry and anti-oxidant activity. In this study, in vitro DPPH assay has been utilized to know about anti-oxidant potential of *Polyalthia longifolia* leaves. Tannins, Alkaloids, Terpenoids, Flavonoids, Steroids, and Cardiac glycosides were found in the *Polyalthia longifolia* leaves during phytochemical analysis. However, Phlobatannin, Saponins, and Anthraquinone were not present. The ethanolic extract of *P. longifolia* leaves showed considerable anti-oxidant action. *Polyalthia longifolia* leaves have the ability to treat oxidative stress and can be utilized as an analgesic and anti-oxidant medication.

Keywords: Anti-oxidant activity, *Polyalthia longifolia* leaves, phytochemicals, UV-vis spectrophotometer.

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INTRODUCTION

Men utilized herbal remedies to cure ailments in the past. With the expansion of scientific understanding, ethnobotanical pharmacology is quickly expanding. These plant-based medications are both safe and widely available (Modak *et al.*, 2007). Traditional systems such as Hikmat, Ayurveda, Unani, Siddha, and homoeopathy advocated for the use of 95% diverse medicinal herbs in treatments (Satyavati *et al.*, 1976). According to the World Health Organization survey, 60-80% of the world's population relies on the herbal medicines as they possess specific physiology that have beneficial effect on the mankind (World Health Organization, 2002).

Polyalthia longifolia is an Asian tree of the Annonaceae family. *Polyalthia* (Annonaceae) is a subtropical and tropical plant genus with over 120 species of shrubs and trees. Because of the presence of several phytochemicals (Zarga and Shamma, 1982; Kijoa *et al.*, 1990), this genus is thought to be medicinally significant. *Polyalthia* have been used for ages for different ailments such as scorpion stings, excessive blood pressure, and as a respiratory stimulant (Padmaa and Khosa, 2009). *Polyalthia longifolia* is a plant that is used to cure fever, skin illnesses, gonorrhoea, uterine sickness, diabetes, and hypertension (Raghunathan and Mitra, 1982; Kirthikar and Basu, 1998).

The Mast Tree is grown. It is often cultivated in Sind as an avenue tree or in gardens, and it is also planted

in Punjab on occasion. In Karachi, it seldom blossoms. This evergreen tree may grow to be over 10 meters tall and is often planted owing to its efficiency in reducing noise pollution. The pattern of growth of *Polyalthia longifolia* is symmetrical pyramidal pattern, with long thin lanceolate leaves. Tables 1 and 2 show the vernacular names and classification of *Polyalthia longifolia*, respectively.

Table 1: Vernacular names of *Polyalthia longifolia*

Hindi	Ashok
English	Mast tree, False Ashok, Asoka tree
Bengali	Debdaru
Sanskrit	Ashoka

Table 2: Classification of *Polyalthia longifolia*

Domain	Eukaryota
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Magnoliales
Family	Annonaceae
Genus	<i>Polyalthia</i>

MATERIALS AND METHODS

Target plant collection: Leaves of *Polyalthia longifolia* were collected from the canal's bank in the Gulberg 5 district of Lahore (Figure 1).

Cleaning of leaves: Cleaning the plant's leaves is required to eliminate any dust or other impurity agents that might interfere with the testing results. For cleaning, dip the leaves in fresh-water to remove impurity agents. Wash at least 4-5 times with water to ensure the purity of leaves. Cleaning should be done to yield better results.

Drying of leaves: After cleaning process, leaves should be dried to remove water. Drying the leaves of the plant should be done under the shade. Avoid drying of leaves of the plant in direct sunlight, oven etc. otherwise this would be led to the degradation of phytochemicals which effects on our experimental results.

Obtaining leaves extract: The Soxhlet apparatus is used to obtain a leaves extract of *Polyalthia longifolia*. The known number of dried leaves to about 70g is subjected in the Soxhlet apparatus. The solvent used in the extraction process is ethanol.

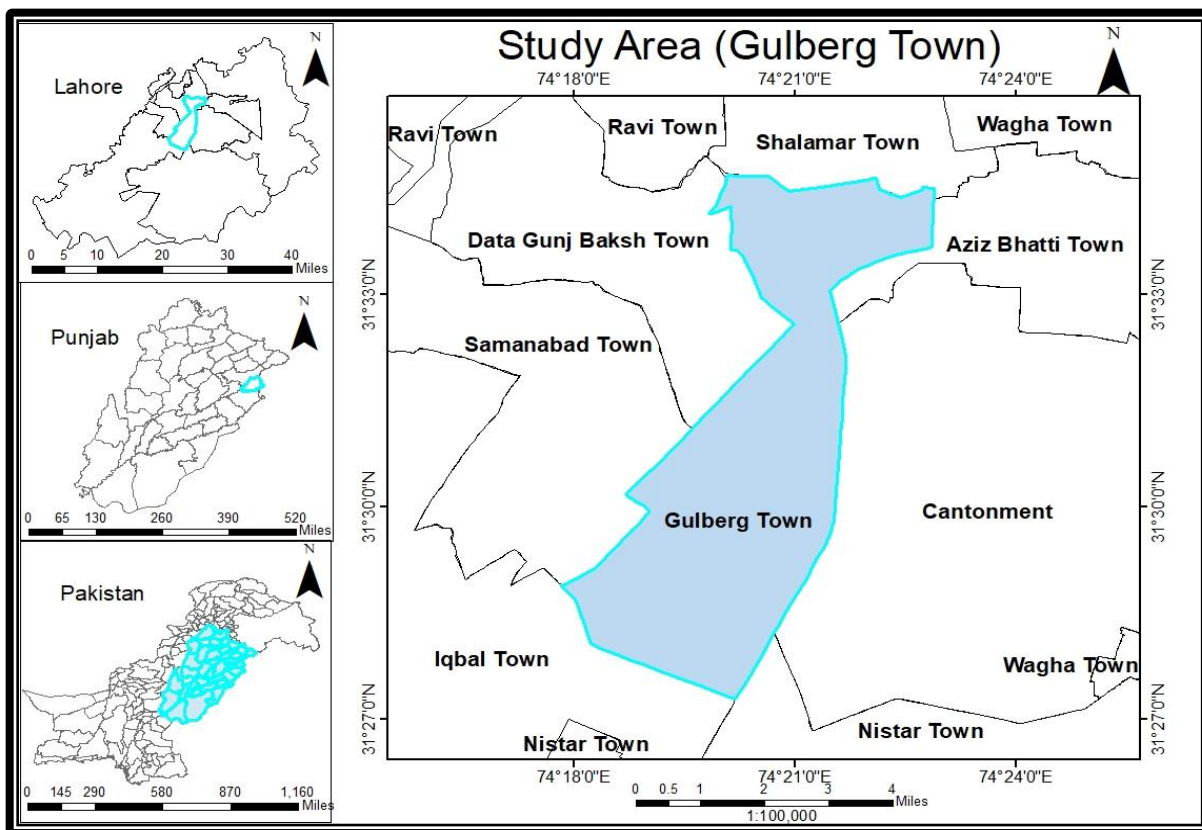


Figure 1. Map of the study area

Soxhlet method: A thimble containing 70g of *Polyalthia longifolia* leaf powder is placed in a Soxhlet extractor. The distillation flask was filled with ethanol and placed in the heating chamber. The reflux condenser is kept at the top of the Soxhlet. The solvent boils in the flask, evaporates, and condenses in the condenser after passing through the extractor's side tube. Hot condensed solvents enter the thimble and dissolve the necessary constituents. When the solvent filled to the mark, it is immediately drained and the solvent runs back to the chamber. The cycle might be repeated an unlimited number of times. The procedure took 15 hours to complete. Soxhlet equipment is shut off when the side tube is full with

colorless liquid. The purified extract was then concentrated on a hot plate till 100ml of extract remain.

Plant Extract Phytochemical Analysis: For the phytochemical evaluation, leaves extract of *Polyalthia longifolia* dissolved in minute quantity of ethanol.

Test for Tannins: When few drops of FeCl_3 solution were combined with a little volume of extract, then dark green color appeared which indicated tannins in the extract (Uzama *et al.*, 2011).

Test for Flavonoids: The addition of sodium hydroxide to the extract resulted in a yellow color, which was

removed following the addition of dilute acid (Zohra *et al.*, 2012).

Test for Alkaloids:

Hager’s test: A little amount of extract was combined with Hager's reagent and the yellow coloring was observed (Nyamai *et al.*, 2016).

Test for Cardiac Glycosides: The test was performed by adding a small amount of sample extract in 5ml of water, along with the addition of 2ml of glacial acetic acid with one drop of FeCl₃ solution then by adding 1ml of concentrated H₂SO₄, a brown ring at the interface will indicate the existence of cardenoloids deoxy sugar properties. A violet ring may form under the brown ring, whereas a greenish ring may form slightly above the brown ring and eventually expand across the acetic acid layer (Lalrinzuali *et al.*, 2015).

Test for Saponins:

Froth test: In a test tube, a little amount of extract was combined with 5ml of distil water. The solution was forcefully agitated to produce a stable froth, which shows the presence of saponins.

Phlobatannin testing: When a little quantity of extract was heated with 1% HCl, red precipitate deposition shows the existence of Phlobatannins (Tepal, 2016).

Test for Steroids:

Salkowaski test: Salkowaski test were performed by the addition of 2ml concentrated H₂SO₄ and 2ml chloroform to a small amount of extract. The acid layer appears to be fluorescently greenish-yellow and the chloroform layer appears fluorescently red which confirmed the existence of steroids (Nyamai *et al.*, 2016).

Test for Terpenoids: Terpenoids test were performed by the addition of 2ml of chloroform and 3ml of conc. H₂SO₄ to a small amount of extract, a reddish-brown coloring at the interface, indicated the existence of terpenoids (Hossain *et al.*, 2013).

Anthraquinone test: 10ml of dilute H₂SO₄ was added to a small amount of sample extract and then heated the solution and filtered it, while it was still hot. Then by adding 5ml of chloroform to the filtrate and shaken. In another test tube the chloroform layer was pipette out and 1ml of dilute ammonia was added. The resultant solution changed to violet, indicating the presence of anthraquinone (Gul *et al.*, 2017).

DETERMINATION OF ANTI-OXIDANT ACTIVITY (IN VITRO ASSAY)

DPPH Assay: 1,1-Diphenyl-2-picrylhydrazil (DPPH) is a molecule containing stable free radical. DPPH is reduced to a stable diamagnetic molecule by accepting electrons

from the antioxidant. DPPH assay is used to determine the free radical scavenging ability of the extract. To assess the antioxidant property of a leaf extract, mix a leaf extract and the free radical DPPH in a 1:3 ratio. Prepare various concentrations of extract and take 3ml of each in separate test tubes, then add 1ml of DPPH solution to each tube. Shake vigorously and leave them in the dark for at least one hour and then note the absorbance at 517nm, which corresponds to the extract's free radical scavenging activity. Monitoring was used to determine how much the DPPH-radical was reduced.

The RSA was estimated in percentage by using the following formula:

$$\text{Radical Scavenging Percentage (\% Protection)} = [A_{\text{DPPH}} - A_{\text{Sample}} / A_{\text{DPPH}}] \times 100$$

A_S denotes the absorbance of the solution when a certain level of sample extract is added and A_{DPPH} denotes the absorbance of the DPPH solution (Saradha *et al.*, 2013; Ramchoun *et al.*, 2015).

RESULTS

Phytochemical Evaluation: The presence of phytochemicals in an ethanolic extract of *Polyalthia longifolia* leaves was investigated. The phytochemical analysis revealed that Flavonoids, Alkaloids, Tannins, Terpenoids, Anthraquinone, and Cardiac glycosides were present, but Saponins, Phylobatannins, and Steroids were absent (Table 3).

Table 3: showing the presence of Phytochemicals in *Polyalthia longifolia*

Phytochemicals	Result
Tannins	+
Alkaloid	+
Terpenoid	+
Flavonoid	+
Saponins	—
Phlobatannins	—
Anthraquinone	+
Steroids	—
Cardiac Glycosides	+

Present = +, Absent = —

ANTI-OXIDANT ACTIVITY

Preparation of samples: By using the stock solution, five solutions of different concentration were prepared. Five samples were made from the stock solution by dissolving 0.5ml, 1ml, 1.5ml, 2.0ml, and 2.5ml in test tubes containing 9.5ml, 9.0ml, 8.5ml, 8.0ml, and 7.5ml

ethanol. The current solutions are 5%, 10%, 15%, 20%, and 25%, respectively.

RSA Assay: A DPPH-in-methanol solution helps in the assessment of the RSA (radical scavenging activity) of *Polyalthia longifolia* leaf extracts of varying concentrations. The dark purple color of the newly generated DPPH solution, which has a maximum absorbance at 517nm, diminishes in the presence of antioxidant. This is due to the antioxidant supplying electrons or reducing the free radical DPPH to a colorless molecule known as 2,2-diphenyl-1-hydrazine. The

quenching of DPPH molecules causes a decrease in absorbance at 517nm. As a result, the greater the reduction in absorbance, the greater the antioxidant action on it (Manandhar *et al.*, 2019).

The current study, observed the effect of *Polyalthia longifolia* leaf extract on DPPH free radical scavenging. The RSA % was computed as follows:

$$\% \text{ RSA (Protection \%)} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the samples of extract and standard (Ramchoun *et al.*, 2015).

able 4: % Protection of *Polyalthia longifolia* leaves against free radical scavenging

Treatment(s)	Concentrations (% V/V)	Absorbance (A)nm	Protection (%)
Control	—	1.517± 0.03	—
EEPLL	5%	1.383± 0.02	8.83
EEPLL	10%	1.253± 0.01	17.40
EEPLL	15%	1.121± 0.03	26.10
EEPLL	20%	0.984± 0.01	35.13
EEPLL	25%	0.838± 0.01	44.76

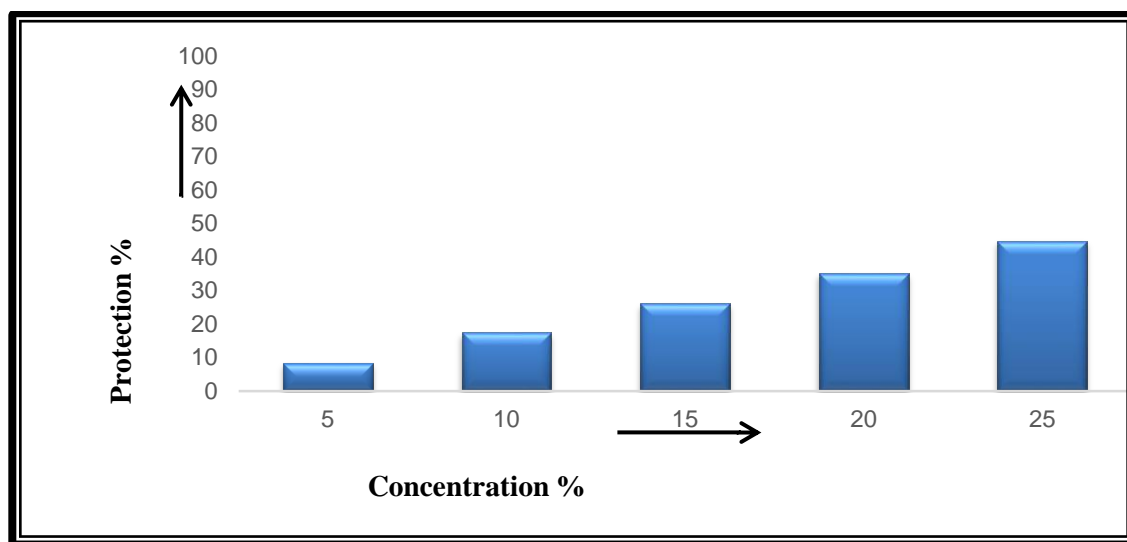


Figure 2. % Protection of *Polyalthia longifolia* against free radicals

DISCUSSION

Medicinal plants include a variety of phytochemicals that are responsible for the treatment of many ailments. Men used medicinal plants in the past because of their therapeutic potential, which allowed them to treat ailments. Nature has provided us with therapeutic plants that have healing properties (Ramchoun *et al.*, 2015). Different chemical methods were used to evaluate the phytochemicals in the ethanolic

extracts of *Polyalthia longifolia* leaves, which exhibited certain therapeutic potential (Ramchoun *et al.*, 2015).

The study was performed to evaluate the antioxidant activity [RSA (radical scavenging activity)] of different concentrations of *Polyalthia longifolia* leaves extract by using DPPH radical scavenging assay. The core principle of this assay is that, the dark purple colour of newly generated DPPH solution which has maximum absorbance at 517nm, diminishes, in the presence of antioxidant. This is due to the antioxidant supplying electrons or reducing the free radical DPPH.

The results of this study revealed that *Polyalthia longifolia* leaves ethanolic extract has strong anti-oxidant activity that increases with extract concentration. The *Polyalthia longifolia* ethanolic leaves extract showed the highest anti-oxidant potential of 44.76 %.

Conclusion: The present study revealed that *Polyalthia longifolia* ethanolic leaves extract had the potential to cure oxidative stress. At larger quantities, it can be employed in the production of anti-oxidant drugs because of their therapeutic importance.

List of abbreviation

EEPLL: Ethanolic Extract of *Polyalthia longifolia* leaves

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