

Antioxidant Potential Calotropis Procera leaves, flowers, and latex, Zanthoxylum Armatum Dried Seeds, and Syzygium Aromaticum Dried Flower Buds

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ABSTRACT

Objectives: This study evaluates the antioxidant potential of Calotropis procera leaves, flowers, and latex, Zanthoxylum armatum dried seeds, and Syzygium aromaticum dried flower buds.

Methodology: The extracts were prepared using methanol, acetone, and chloroform as solvents. Antioxidant activity was assessed using the DPPH (1,1-Diphenyl-2-picryl-hydrazyl) free radical scavenging method, with ascorbic acid serving as the standard. Phytochemical screening identified secondary metabolites such as flavonoids, terpenoids, glycosides, and more.

Results: The results revealed that C. procera leaves and flowers exhibited the highest antioxidant activities, with Methanolic extracts showing the most significant radical scavenging potential. Methanolic extracts of S. aromaticum dried flower buds also demonstrated substantial antioxidant activity.

Conclusion: This comparative analysis underscores the therapeutic potential of these plants in managing oxidative stress-related diseases.

Keywords: Antioxidant potential, Calotropis Procera Leaves, Dried Flower Buds.

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Introduction

Plants are invaluable sources of bioactive compounds and have been integral to traditional medicine. Plants are powerful biological factories and have been constituents of phytomedicine since times ancient.¹ Antioxidants from plants mitigate the effects of free radicals, reducing the risk of chronic conditions like cardiovascular diseases and cancer. This study focuses on three medicinal plants: Calotropis procera, Zanthoxylum armatum, and Syzygium aromaticum, which are known for their rich phytochemical profiles and potential antioxidant properties.

Calotropis procera or milk weed is a xerophytic evergreen shrub about 6 m high belonging to the family Asclepiadaceae. It is extensively dispersed in Asia, Africa,

South America and northeast of Brazil.² It is erect, large, tall, much branched and perennial with milky latex throughout.³ It has great hairy shrub, leaves decussate, inflorescence extra axillary umbellate panicle, corolla purple, lobes erect.⁴ The fruits are inflated about 10 cm in diameter, grey green in color and release flat, brown seeds with a tuft of white hair at one end.⁵

The bark of C. procera is fibrous, scaly, deeply fissured when old, grey to light brown.⁶ Flowers are arranged in terminal, having five deep lobes and dirty white sepals with purple tips and white base.⁷ It has deep (3-4 m) taproot and secondary root system having lateral roots which may rapidly regenerate adventitious shoots when plant is injured. The stems are crooked and covered with a fissured corky bark. The gray-green leaves are 15 to 30 cm long and 2.5 to 10 cm broad.⁸ The plant is famous due to

presence of abundant latex in its green parts that is easily collected when the plant is cut.⁹

Whole plant of *C. procera* was traditionally used to treat the common diseases such as rheumatism, fever, cold, eczema, indigestion, jaundice and diarrhea. The root was used to treat eczema, leprosy, asthma, rheumatism, cough, cold, elephantiasis and diarrhea. The stem was used for the treatment of skin diseases, leprosy, intestinal worms and cure leucoderma. It is also used in ulcer, boils, antidote for snake poisoning, tumors, piles, liver disorder, spleen disorder.¹⁰ The whole plant was dried and taken as tonic, antihelmintic and expectorant.¹¹

The latex of *C. procera* is used in various conditions as analgesic, expectorant, leucoderma, anticonvulsant, tumors, leprosy, piles, anti-inflammatory, asthma, enlargement of spleen and liver, joint swelling.¹² Latex is also useful in the treatment of baldness, tooth ache, vertigo, and hair fall, paralysis, intermittent fever and for the treatment of ring worms. Its flowers are used as therapeutic agents to treat inflammation, cholera, asthma, piles and wound. Different phytochemicals have been reported i.e. cardenolides from latex and leaves.¹³ Chemical screening of the latex showed that this plant has cardenolides such as calotropin, calotoxin, uscharin, uscharidin and voruscharin.¹⁴

Zanthoxylum armatum DC is a branched shrub, sub deciduous aromatic tree (6 m height), belongs to family Rutaceae. The plant can be familiar by its shrubby habit, dense foliage, with pungent aromatic taste, prickled trunk and branches, and small red, subglobose fruits. It is widely spread in the hot valleys of Himalayas from Jammu to Bhutan, Nepal and Pakistan.¹⁵

Its leaves and fruits are used for mouth fresh and tooth care. Bark is used for intoxicating the fishes. Plant is essential oils locally used as fragrances and flavoring agents for food and beverages. It can be taken orally with warm water to treat stomach pain, cold and constipation.¹⁶

Plants are used to treat diseases, i.e., asthma, bronchitis, cholera, fever, fibrosis, indigestion, rheumatism, skin diseases, toothache, and varicose veins. Prickly ash is used in many chronic problems such as rheumatism and skin diseases; cramp in the leg, ulcers. It is also used for low blood pressure, fever, and inflammation. The plant showed antimicrobial and antioxidant potential against pathogenic bacteria as well as fungi.¹⁷

Various phytochemical constituents like lignins, alkaloids, sterols, phenolics, terpenoids, coumarins,

flavonoids, glycosides, benzenoids, alkenic acids, amino acids, fatty acids have been isolated from this plant. Essential oil contains linalool and limonene. Seeds contain hydroxylic (4Z) enolic acid and different volatile compounds.¹⁸

The current study is designed to examine the comparative analysis of antioxidant activity and phytochemical screening of *Calotropis procera* leaves, flowers and latex, *Zanthoxylum armatum* dried seeds. This study might be helpful to explore the potential of these above discussed plants in AJK and Pakistan to introduce these plants as a vital tool to initiate the economic activities for their commercial use that might be helpful to treat different health problems.

Methodology

The current study was based on two plants i.e. *Calotropis procera* (Aak) and Timber (*Zanthoxylum armatum*). The plant *C. procera* (Aak) leaves, flowers and latex were collected from different places of District Kotli Azad Kashmir. Fresh leaves, flowers and latex were collected from Sehnsa, Barali, Sarda and Panjera. *Z. armatum* (Timber) dried seeds were collected from Gulhar and Panjera.

Fresh leaves were collected in plastic bags from the plant. Leaves were washed under tap water and dried under shade for 07 days. Dried leaves were ground into fine powder with the help of the mechanical grinder and stored in polythene bags at room temperature. This method was reported Singh JS.¹⁹ Then this sample was used for the preparation of methanol, acetone and chloroform extract.

Fresh flowers were collected in July 2019 from Panjera. Flowers were plucked out and collected in a zipper bag. Flowers were washed properly with tap water and shade dried for 2 weeks. The dried flowers were then crushed into fine powder with the help of grinder and stored in polythene bags at room temperature. This method was described by.²⁰ Then this sample was used for the preparation of chloroform, acetone and methanol extract.

C. procera plant latex was collected between June and July 2019 from Panjera village of District Kotli. Latex was collected directly from plant into falcon tube and subjected to oven drying at 60 °C for 12 hours. Then it was used for further extract preparation. This method was stated by.²¹

Fruits of locally growing *Z. armatum* DC., were collected in a zipper bag and then washed well with tap water and

air dried in the shade until constant weight was gained. The dried fruits were then ground to an abrasive powder in a grinder at short spruts of 30 second each. Further it was used for extract preparation. This method was used quantified by Abid M et al.²²

For the determination of antioxidant activity of powdered leaves, latex and flowers of *C. procera*, seeds of *Z. armatum* were extracted by using three organic solvents i.e. Methanol, Acetone and Chloroform. For this 12.5 gram of dried powdered *C. procera* were soaked in 50 ml of methanol, 12.5 gram in 50 ml acetone and 12.5 ml in 50 ml chloroform in separate sterile conical flasks. And 6.25 gram of dried crushed flowers of *C. procera* was soaked in 25 ml methanol, 6.25 gram in 25 ml acetone and 6.25 gram in 25 ml of chloroform in disinfected narrowed flasks. And 700 milligrams of dried latex powder was extracted by using 7.0 ml of organic solvent acetone. And 12.5 gram of desiccated ground *Z. armatum* seeds were soaked in 50 ml methanol, 12.5 gram in 50 ml acetone and 12.5 gram in 50 ml chloroform. All above extracts were loaded on an orbit shaker at the speed of 120 rpm for 48 hours.

After 48 hours the mixtures were filtered using Whatman filter paper number 1. Filtrate was collected in pre-weighed beakers. The filtrate was dried in hot drier by setting the temperature of drier slightly lesser than the boiling point of each organic solvent so that the solvent would be evaporated from the filtrate. The extracts were observed periodically untill fully dried.

After the complete evaporation of solvent from the filtrate the dried extract was obtained and each extract was weighed again of each sample. For the antibacterial activity this dried extract of each sample is to be dissolved in Dimethyl-sulfoxide (DMSO).

Free radical scavenging (RSA) activity was measured by DPPH method using the procedure described by Blais. The antioxidant activity of *C. procera* leaves, flowers and latex, *Z. armatum* dried seeds and *E. caryophyllus* dried flower buds were measured by using the DPPH method. Measurements in the measuring cuvettes were performed 30 min after addition of DPPH in order to give enough time for the reaction of the cellular antioxidants with DPPH. During this 30 min, measuring cuvettes was kept in the dark at room temperature. Ascorbic acid was used as a standard. Absorbance of each of the solution was taken at 517 nm using UV- spectrophotometer. Concentration of test extract was: 75 µg/ml, 150 µg/ml and 225 µg/ml. The experiment was repeated triplicate.

Antioxidant activity of *Calotropis procera* leaves, flowers and latex, *Zanthoxylum armatum* dried seeds and dried flower buds of *Eugenia caryophyllus* were determined at different solvent concentrations. Results were showed in terms of percentage scavenging activity. Each plant exhibited remarkable scavenging potential with different solvents. The percentage of DPPH radical scavenging activity presented in Table I. The DPPH radical scavenging activity of the extract increases with increasing concentration. Leaves and flowers of *C. procera* showed highest activity than latex. Present study showed that *Calotropis procera* leaves, flowers and latex has different antioxidant potential from 5 percent to 76 percent which increases with increasing sample concentrations. The methanolic extract of *C. procera* leaves was found to be most effective than acetone and chloroform extracts. With different solvents it showed different percentages of scavenging activity. Free radicals are involved in many diseases i.e. neurodegenerative diseases, AIDS and cancer. Antioxidants manage all these diseases through their scavenging ability. DPPH is an easy, rapid and sensitive method to perform the antioxidant activity of a specific plant extracts.²³ DPPH solution decolorized from purple to light yellow color, showing that antioxidant potential is due to proton donating ability. Table II represents scavenging activity of *Zanthoxylum armatum* dried seeds and dried flower buds of *Eugenia caryophyllus* that ranges from 4 percent to 45 percent. Methanolic extract of dried flower buds of *Eugenia caryophyllus* showed higher activity. The change in results may be due to environmental effects and various concentrations or due to genetic variations.

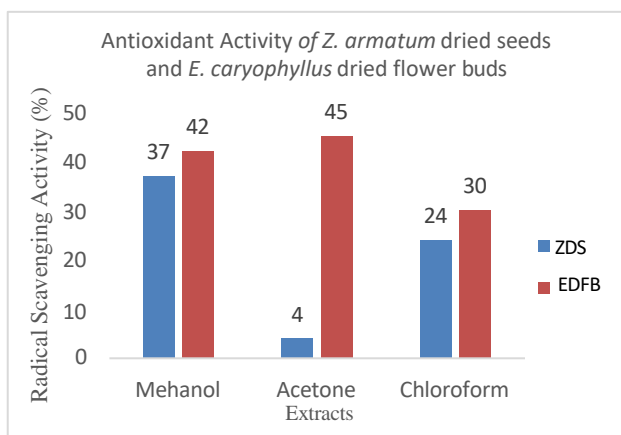
Table I: Antioxidant Activity of *C. procera* Leaves, Flowers and latex.

Extracts	Leaves	Flowers	Latex
Methanol	76	72	–
Acetone	11	5	15
Chloroform	30	20	–

Methanolic extracts consistently outperformed acetone and chloroform in antioxidant assays, likely due to the higher solubility of phenolic compounds in methanol. The study confirms that the antioxidant efficacy varies based on plant part, species, and solvent used.

Table II: Antioxidant Activity of *Z. armatum* Dried Seeds and *E. caryophyllus* Dried Flowers Buds.

Extracts	ZDS	EDFB
Methanol	37	42
Acetone	4	45
Chloroform	24	30



Conclusion

This comparative study highlights the antioxidant and phytochemical richness of *C. procera*, *Z. armatum*, and *S. aromaticum*. *Calotropis procera*, *Zanthoxylum armatum* and *Eugenia caryophyllus* are medicinal plants and are the best source of antioxidants and phytochemical constituents. There is great impact of these plants in the treatment of many diseases. *Eugenia caryophyllus* plant oil and extracts not only have the potential to kill the pathogens but they are also easy to excess, feasible to use and less expensive. So, there is a need to search for new, inexpensive and effective drugs from plants with possible antioxidant properties. In the present study antioxidant of *Calotropis procera* leaves, flowers and latex, *Zanthoxylum armatum* dried seeds and dried flower buds of *Eugenia caryophyllus* observed. There are significant antioxidant effects of *C. procera* leaves, flowers and latex, dried seeds of *Z. armatum*. The superior antioxidant activity of *C. procera* leaves and *S. aromaticum* dried flower buds underscores their potential as natural sources of therapeutic antioxidants. Further research could explore the isolation and characterization of specific bioactive compounds for pharmaceutical applications.

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