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Comparative Study of Qualitative Phytochemical Constituents of Calotropis procera Leaves, Latex, and Flowers, Zanthoxylum armatum Dried Seeds, and Eugenia caryophyllus Dried Flower Buds

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ABSTRACT

Objective: This study aimed to conduct a comparative qualitative analysis of phytochemical constituents in various parts of Calotropis procera (leaves, latex, and flowers), Zanthoxylum armatum (dried seeds), and Eugenia caryophyllus (dried flower buds) using different organic solvents.

Methodology: Plant materials were collected from Kotli, Azad Jammu and Kashmir. Extracts were prepared using methanol, acetone, and chloroform. Seventeen standard phytochemical screening tests were performed to identify the presence of various bioactive compounds such as flavonoids, terpenoids, glycosides, coumarins, resins, fixed oils, quinones, proteins, and others.

Results: The methanolic extracts of C. procera leaves and E. caryophyllus flower buds exhibited the most diverse and abundant phytochemical profiles. Notably, flavonoids and terpenoids were consistently present across all plant species and solvent types, indicating a strong pharmacological potential. Z. armatum dried seeds also demonstrated notable concentrations of fixed oils, betacyanin, and proteins. Solvent efficacy followed the order: methanol > acetone > chloroform. The absence of saponins and emodins in all samples was also observed.

Conclusion: The studied medicinal plants, particularly C. procera and E. caryophyllus, are rich in bioactive phytochemicals and hold significant potential for pharmaceutical applications. Methanol proved to be the most effective solvent for extracting diverse phytochemical classes. These findings support the traditional use of these plants and warrant further studies for the isolation and pharmacological evaluation of individual compounds.

Keywords: Phytochemical Screening, Calotropis procera, Zanthoxylum armatum, Eugenia caryophyllus, Methanolic Extracts, Flavonoids, Terpenoids, Medicinal Plants.

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Introduction

Plants are powerful biological factories and have been constituents of phytomedicine since times ancient. Man is capable to get from the astonishing variety of industrialized chemicals.[1] Phytochemicals are primary and secondary metabolites that are present in vegetables, leaves and roots.[2] Reactive oxygen species (ROS) are the free radicals

like superoxide anion (O^{2-}) and hydroxyl (OH). Exogenous consumption of antioxidant s can help the body scavenge free radical radicals efficiently.[3]

Calotropis procera, commonly known as milkweed, is a xerophytic, evergreen shrub that can grow up to 6 meters in height and belongs to the family Asclepiadaceae**.** It is widely distributed across Asia, Africa, South America, and the northeastern region of Brazil.[4] It is an erect, tall, and extensively branched perennial plant characterized by the presence of milky latex throughout its tissues. The shrub has densely hairy stems; its leaves are decussate, and the inflorescence is extra-axillary, forming umbellate panicles. The corolla is typically purple, with erect lobes.[5] The fruit is inflated, approximately 10 cm in diameter, grey-green in color, and contains flat brown seeds each bearing a tuft of white hairs at one end to aid in dispersal.[6]

The bark of *C. procera* is fibrous, scaly, deeply fissured when old, grey to light brown.[7] Flowers are arranged in terminal, having five deep lobs and dirty white sepals with purple tips and white base. 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. The plant is famous due to presence of abundant latex in its green parts that is easily collected when the plant is cut. [8]

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. The whole plant was dried and taken as tonic, antihelmintic and expectorant.[9, 10]

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.[11] 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 [12]. Its flowers are used as therapeutic agents to treat inflammation, cholera, asthma, piles and wound [13].

Different phytochemicals have been reported i.e. cardenolides from latex and leaves, triterpenoids [14] anthocyanins from flowers and hydrocarbons. Chemical screening of the latex showed that this plant has cardenolides such as calotropin, calotoxin, uscharin, uscharidin and voruscharin. [15]

Eugenia Caryophyllus: Eugenia caryophyllus is an evergreen tree (10 to 20 m high), belonging to the family Myrataceae and native to India, Zanzibar, Indonesia, Ceylon and Mauritius.[16] It is used as a flavoring agent and spice for scenting. It is commonly used against bad breath due to their amazing aroma. It contains 13 percent, 15 to 20 percent volatile and 10 percent fixed oil. It is used in mouth chewing and is boiled in water and drink. It is commonly used in dentistry.[17]

Traditionally *E.caryophyllus* has been used for the preservation of food as it has antiseptic and disinfectant potential. They are used in the treatment of respiratory disorders, dental disorders, sore throat and headache. In Ayurveda and Chinese medicine, it is utilized for the treatment of several infections and diseases.[18]

E. caryophyllus have many therapeutic uses, like to control nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distension and gastrointestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves.[19] Beside this cloves are highly antiseptic antimutagenic, anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic, antifungal[20], antiparasitic antiviral. [21]

Phytochemical constituents of the plant include trans-caryophyllene, eugenol, α-humulene, syzygin B, eugenol acetate, syzygin A, ferulic acid, caffeic acid and ellagic acid.[22]

Zanthoxylum Armatum: DC is a branched shrub, sub deciduous aromatic tree (6 m height), belongs to the 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.[23]

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.[24]

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, cramps 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.[25]

Various phytochemical constituents like lignins, alkaloids, sterols, phenolics, terpenoids, coumarins, flavonoids, glycosides, benezenoids, 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. [26]

The current study is designed to examine the comparative analysis of phytochemical screening of *Calotropis procera* leaves, flowers and latex,*Zanthoxylum armatum* dried seeds and *Syzygium aromaticum* dried flower buds. 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

District Kotli, one of the ten administrative districts of Azad Jammu and Kashmir (AJK), Pakistan, is recognized for its diverse flora, including numerous economically and medicinally significant plant species. Despite advancements in modern medicine, a large portion of the local population continues to rely on medicinal plants for the treatment of various ailments.

This study focused on three selected medicinal plants: Calotropis procera (commonly known as Aak), Zanthoxylum armatum (locally referred to as Timber), and Eugenia caryophyllus (Clove). Specimens of C. procera—including leaves, flowers, and latex—were collected from multiple locations within District Kotli, including Sehnsa, Barali, Sarda, and Panjera. Dried seeds of Z. armatum were obtained from Gulhar and Panjera, while dried flower buds of E. caryophyllus were procured from a local grocery shop in Kotli city.

Phytochemical Screening: Methanolic, acetone, and chloroform extracts were prepared from the selected plant materials. A total of 17 standard qualitative phytochemical assays were conducted to identify the presence of various bioactive constituents.

Saponins: Detected by vigorous shaking of 2 ml of extract with 2 ml of distilled water; stable foam formation indicated presence.

Fixed Oils and Fats: A drop of extract was pressed between filter papers; an oil stain confirmed the presence.

Quinones: Addition of concentrated sulfuric acid to 1 ml of extract; red coloration indicated quinones.

Steroids: Mixing of 2 ml extract with 2 ml chloroform and concentrated sulfuric acid; a red lower layer indicated steroids.

Carbohydrates: Iodine test with 1 ml iodine solution; blue/purple coloration indicated presence.

Starch: Addition of iodine solution to extract; black color indicated starch.

Resins: Extract treated with acetone and distilled water; turbidity confirmed presence.

Gums and Mucilage: Addition of 2 ml absolute alcohol to 1 ml extract with stirring; a cloudy precipitate indicated presence.

Proteins: Xanthoprotein test using concentrated nitric acid; yellow coloration indicated presence.

Amino Acids: Ninhydrin test with boiling; blue coloration confirmed presence.

Betacyanin: Addition of 2N NaOH to 1 ml extract; yellow color change confirmed presence.

Glycosides: Chloroform and ammonia were added to the extract; pink coloration indicated glycosides.

Emodins: Extract treated with benzene and 10% NH_3; red coloration indicated presence.

Coumarins: Addition of 10% NaOH to aqueous extract; yellow color indicated presence.

Terpenoids: Extract mixed with chloroform and heated, followed by sulfuric acid; reddish-brown coloration indicated terpenoids.

Test for Leucoanthocyanins: Five milliliters of isoamyl alcohol were added to five milliliters of extract. The appearance of a red coloration in the upper layer was considered a positive indication of leucoanthocyanins.

Test for Flavonoids: One milliliter of plant extract was treated with lead acetate solution. The formation of a yellow precipitate confirmed the presence of flavonoids.

Each of the qualitative tests was carried out in triplicate to ensure the reliability and reproducibility of the

observations. The selection of methanol, acetone, and chloroform as extraction solvents was based on their varying polarity profiles, which allow for a comprehensive evaluation of phytochemical diversity. These solvents enabled the extraction of a broad range of phytoconstituents, from highly polar to non-polar compounds.

The presence or absence of specific phytochemical classes was recorded for each solvent and plant part. The comparative profiling aimed to highlight which combinations of plant material and solvent yielded the richest spectrum of secondary metabolites.

Ethical Considerations:As the study involved plant material only, ethical approval was not applicable. However, all plant collection and identification procedures were conducted in accordance with local biodiversity conservation guidelines.

Test for Leucoanthocyanins: 5 ml of isoamyl alcohol was added to 5 ml of extract, appearance of red color in upper layer indicated the presence of Leucoanthocyanin.

Test for Flavonoids: To 1 ml of extract, lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for the presence of flavonoids.

Discussion

Calotropis procera, Zanthoxylum armatum and *Eugenia caryophyllus* are medicinal plants and are enriched with bioactive compounds like antioxidants, phenolics, saponins, carbohydrates, proteins, fixed fat and oil, quinone, steroids, starch, resins, gums and mucilage, amino acid, betacyanin, glycosides, terpenoids, emodin, coumarins, leucoanthocyanins and flavonoids. The important aim of the current study was to find out the phytochemical activity of Calotropis procera leaves, flowers and latex, Zanthoxylum armatum dried seeds and dried flower buds of Eugenia caryophyllus.

Phytochemical Screening: The results of phytochemical screening of methanol, acetone and chloroform extracts of Calotropis procera leaves, flowers and latex, Zanthoxylum aramatum dried seeds and dried flower buds of Eugenia carophyllus were carried out using standard procedures to detect antioxidant, phenolics, saponins, carbohydrates, proteins, fixed fat and oil, quinone, steroids, starch, resins, gums and mucilage, amino acid, betacyanin, glycosides, terpenoids, emodin, coumarins, leucoanthocyanins and flavonoids. Their concentrations vary amongst the extracts evaluated. The presence of components in these plants is an indication that perhaps these plants may have some medicinal potential. The phytoconsituents detected in the extracts could be responsible for the antioxidant.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties like flavonoids have a lot of beneficial uses due to their anti-inflammatory, antifungal, antifungal, antioxidant and wound healing properties.

Phytochemicals Analysis: A total of 17 qualitative phytochemical tests were performed. The data showed that extracts of Calotropis procera leaves, flowers and latex, Zanthoxylum armatum dried seeds and dried flower buds of Eugenia caryophyllus had different phytochemical present in them.

Test for saponins: Methanol, acetone and chloroform extracts of Calotropis procera leaves, flowers and latex, Zanthoxylum aramatum dried seeds and dried flower buds of Eugenia carophyllus showed negative results for saponins.

Fixed oils and fats: All methanolic, acetone and chloroform extracts of Calotropis procera leaves, flowers and latex (acetone only), Zanthoxylum aramatum dried seeds and dried flower buds of Eugenia carophyllus subjected to test for Fixed oils and fats showed positive result.

Test for Quinones: All extracts showed positive results for quinones except CP leaves and acetone and chloroform extract of ZA.

Test for steroids: Methanolic flower extract of CP, ZA and EC showed positive results. The Acetone extract of EC also showed positive results for steroids while remaining were found negative.

Test for Carbohydrates: All extracts of CP, ZA and EC subjected to tests for Carbohyrates showed negative results.

Test for amino acid: Methanolic and chloroform extracts of CP leaves and flowers, acetone extract of CP latex and methanolic extract of ZA seeds showed positive results when subjected to Ninhydrin test. The remaining extracts appeared to be negative for amino acid.

Tests for proteins: Acetone extracts of ZA seeds and chloroform extracts of CP leaves, flowers, ZA seeds and EC dried flower buds showed positive results while methanolic extracts of CP leaves, flowers, ZA dried seeds

and dried flower buds of EC showed negative results. Acetone extracts of CP leaves, flowers, latex and EC dried flower buds also showed negative results.

Test for Flavonoids: The plant extracts, i.e. CP leaves, flowers and latex, ZA dried seeds and EC dried flower buds subjected to test for flavonoids indicated the presence of flavonoids as an important constituent. Methanolic extract of CP leaves and acetone extract of CP latex showed negative results.

Test for betacyanins: All flower extracts of CP, Acetone extract of CP latexand ZA seeds, chloroform extract of ZA seeds, CP leaves and EC dried flower buds showed positive results. All others were negative.

Test for starch: It was observed that all plants extracts were starching negatively.

Test for Resins: Acetone extracts of CP latex, Chloroform extract of CP leaves and methanolic, acetone and chloroform extracts of EC dried flower buds showed positive results for resins while all other were negative for resins.

Test for Glycosides: All extracts when tested for glycosides were found negative except the acetone extract of EC that appeared to be positive.

Test for Terpenoids: All methanolic extracts showed positive results. Chloroform extract of ZA dried seeds and Acetone extract of EU also indicated the presence of terpenoids while other extracts showed negative results.

Test for Emodins: Test results revealed that all plants extracts were devoid of Emodins.

Test for Coumarins: Methanolic, acetone and chloroform extracts of CP flowers, acetone extract of CP latex, chloroform extracts of CP leaves, ZA seeds and EC indicated the presence of coumarins. All other extracts showed negative results.

Test for Gums and Mucilage: Chloroform extract of CP leaves, acetone extract of CP latex, methanolic extract of EC and chloroform extract of EC showed positive results while remaining showed negative result.

Test for Leucoanthocyanins: All extracts showed negative results for leucoanthocyanins except methanolic extract of EC.

In this study, the qualitative analysis of *C. procera* leaf extracts (methanol, acetone and chloroform) as shown on Table I respectively, reveals the presence of active constituents such as fixed oils and fat, resins, gums and mucilage, protein, amino acids, betacyanin, terpenoids and coumarins mostly in chloroform extract and few in methanolic extract.[27] performed the phytochemical screening of the ethanolic extracts of flowers, young buds, mature leaves and stems of *C.procera* (Ait) and found that alkaloids, cardiac glycoside, saponins, phenolics, triterpenoids and tannins are present in almost all parts of which agreed with the present result. The different phytochemical constituents present in the different extracts may be attributed to the solvents used for extraction and for different solvents have different spectrum of solubility for the phytoconstituents of plants. Among phytochemical, alkaloids are one of the largest groups in plants having amazing effect on humans and this led to the development of powerful painkiller medicine. The leaves are used to treat joint pain and reduce swelling. It is also used as a homeopathic medicine. [28]

Table I: Qualitative Phytochemical constituents of C. procera leaf.

Constituents	Methanolic extract	Acetone extract	Chloroform extract
Saponins	–	–	–
Fixed oils and Fats	+	+	+
Quinone	–	–	–
Steroids	–	–	–
Carbohydrates	–	–	–
Starch	–	–	–
Resins	–	–	+
Gums and Mucilage	–	–	+
Proteins	–	–	+
Amino acid	+	–	+
Betacyanin	–	–	+
Glycosides	–	–	–
Terpenoids	+	–	–
Emodins	–	–	–
Coumarins	–	–	+
Leucoanthocyanins	–	–	–
Flavonoids	–	–	+

Table II Qualitative Phytochemical constituents of C.procera Flowers.

Constituents	Methanolic extract	Acetone extract	Chloroform extract
Saponins	–	–	–
Fixed oils and Fats	+	+	+
Quinone	+	+	+
Steroids	+	–	–
Carbohydrates	–	–	–
Starch	–	–	–
Resins	–	–	–
Gums and Mucilage	–	–	–
Proteins	–	–	+
Amino acid	+	–	+
Betacyanin	+	+	+
Glycosides	–	–	–
Terpenoids	+	–	–
Emodins	–	–	–
Coumarins	+	+	+
Leucoanthocyanins			
Flavonoids	+	+	+

Table II shows the presence of fixed oils and fat, resins, gums and mucilage, protein, amino acids, betacyanin, terpenoids, coumarins, flavonoids, quinones and steroids in different extracts of *C.procera* flowers. The presence of these components indicates that it may have some medicinal potential. Table III indicates the presence of fixed oils and fat, resins, gums and mucilage, amino acids, betacyanin, coumarins, quinones and steroids in the acetone extract of *C.procera* latex. Pervious phytochemical studies on aerial parts of the plant showed the presence of alkaloids, cardiac glycosides, flavonoids and sterol reported in *C.procera* .[29]

Table III: Qualitative Phytochemical constituents of C.procera Latex.

Constituents	**Acetone extract**
Saponins	–
Fixed oils and Fats	+
Quinone	+
Steroids	–
Carbohydrates	–
Starch	–
Resins	+
Gums and Mucilage	+
Proteins	–
Amino acid	+
Betacyanin	+
Glycosides	–
Terpenoids	–
Emodins	–
Coumarins	+
Leucoanthocyanins	–
Flavonoids	–

Table IV indicates that fixed oils and fats, protein is present only in acetone and chloroform extracts of Z.armatum dried seeds. Quinones, steroids and amino acids are present only in methanol extract. Terpenoids is present in methanolic and chloroform extracts and coumarins are present in chloroform extract only.

Table IV: Qualitative phytochemical constituents of Z.armatum dried seeds.

Constituents	Methanolic extract	Acetone extract	Chloroform extract
Saponins	-	-	-
Fixed oils and Fats	–	+	+
Quinone	+	-	-
Steroids	+	-	-
Carbohydrates	-	-	-
Starch	-	-	-
Resins	-	-	-
Gums and Mucilage	-	-	-
Proteins	-	+	+
Amino acid	+	-	-
Betacyanin	-	+	+
Glycosides	-	-	-
Terpenoids	+	-	+
Emodins	-	-	-
Coumarins	-	-	+
Leucoanthocyanins	-	-	-
Flavonoids	+	+	+

Table V: Qualitative phytochemical constituents of E. cryophyllus.

Constituents	Methanolic extract	Acetone extract	Chloroform extract
Saponins	-	-	-
Fixed oils and Fats	+	+	+
Quinone	+	+	+
Steroids	+	+	-
Carbohydrates	-	-	-
Starch	-	-	-
Resins	+	+	+
Gums and Mucilage	+	-	+
Proteins	-	-	+
Amino acid	-	-	-
Betacyanin	-	-	+
Glycosides	-	+	-
Terpenoids	+	+	-
Emodins	-	-	-
Coumarins	-	-	+
Leucoanthocyanins	+	-	-
Flavonoids	+	+	+

Flavonoids are present in all extracts. Table V shows that fixed oils and fats and flavonoids are present in all extracts of dried flower buds of *E.caryophyllus.*

Quinones, steroids and terpenoids are present in methanolic and acetone extracts. Gums and mucilage are present only in methanolic and chloroform extracts. Protein, betacyanin and coumarins are present only in chloroform extract. Glycosides are present in acetone extract and flavonoids are present in all extracts of dried flower buds of *E.caryophyllus.*

Solvent Efficacy: Methanol consistently extracted the most diverse phytochemical profiles, followed by acetone and chloroform.

Plant Parts: Among the tested plant materials, C. procera leaves and E. caryophyllus flower buds demonstrated the richest phytochemical composition.

Bioactive Compounds: Flavonoids and terpenoids were prominent across all samples, highlighting their potential pharmacological significance.

Conclusion

This study highlights the phytochemical richness of ***C. procera***, ***Z. armatum***, and ***E. caryophyllus***, particularly in methanolic extracts. The diverse presence of bioactive compounds underscores their potential for therapeutic applications. Future studies should aim at isolating and characterizing specific phytochemicals to explore their medicinal properties. Calotropis *procera,Zanthoxylum*

armatum and *Eugenia caryophyllus* are medicinal plants and are the best source of 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. In the present study phytochemical constituents 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 and dried flower buds of E. caryophyllus were observed that the plants contain large amount of phytochemicals that contribute to its good antioxidant and free radicals scavenging activities, which might be helpful in preventing the progress of oxidative stress in various diseased conditions. The results obtained provide support for the use of this plant in traditional medicine. The result of present study might be used advantageously to improve the efficiency of drugs to treat the various diseases.

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