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Phytochemical screening of Acacia polyacantha Roots Using Bilola technique as Green Extraction Method.

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Abstract:

Acacia polyacantha is a medicinal plant commonly used in The Sudanese traditional medicine. This study aimed to evaluate the qualitative phytochemical composition of its aqueous root extracts and sediments under hot, room temperature, and cold extraction conditions, followed by centrifugation. The sediments were re-dissolved in acetone to enhance the detection of semi polar compounds.Screening revealed tannins, flavonoids, phenols, and Carbohydrates, with intensity influenced by extraction temperature and centrifugation speed. Higher centrifugation improved compound separation, while hot and room-temperature extractions yielded stronger responses than cold extraction. Acetone-dissolved precipitates showed higher concentrations of phenolic and Carbohydrates; with improved flavonoid detection.The study demonstrates that combining aqueous extraction, centrifugation, and acetone re-dissolution of precipitates is an effective, low-cost, and eco-friendly method for phytochemical investigation, supporting the medicinal value of *Acacia polyacantha*.

Introduction:

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Plant products have been part

of phytomedicine s since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances..(Yadav, R *et al.*, 2011)

Medicinal plants have been used since ancient times as a rich source of therapeutic agents for the prevention and treatment of diseases. Also, they provide the main source of useful structures for the development of novel therapeutic agents.(Hammad , A *et al.*, 2022)

Many modern drugs have been stated to show resistance in bacterial infections. Also, these drugs are more expensive. At the same time most of African population survive under poverty line and cannot find the money for the expensive modern drugs. These challenges call for improved strategies on treatment, particularly in the development of antimicrobials. According to WHO, medicinal plants can offer the best alternative sources to obtain variety of drugs, so they can give new choices for solving antimicrobial resistance. The Sudan is a promising area for phytomedicine research because it contains a mixture of Islamic African and Arabic traditions, moreover the diversity of climates in The Sudan results in a rich variety of plant species.(Abubakr, A, H *et al.*, 2022)

Native to Africa, India and Asia but it introduced to the Caribbean .The root of A. polyacantha have chemical compounds that repel animals including rats, snakes and crocodiels. (Ahamed, A**.** *et al.*, 2022.) *Acacia polyacantha* subsp.campylacanthaWilld.(Leguminosae) known as hook thorn or white-stem thorn a tree with one stem bear branches commencing fairly high up, and a well-ordered flattish crown. The tree is spread through-out tropical Africa. In The Sudan A. polyacantha (called Kakamut) different parts have been used for treatments of various illnesses; gum for general health tonic, antidote for snake bite, and cure for venereal diseases. The roots and the bark are used for medicinal treatments, like a general health tonic, antidote for snakebite, cure for venereal diseases and stomach disorders.A preparation from the bark is used for stomach disorders, anti-healing and to bathe children who are restless at night.(Daffalla, M, H *et al.*,2018).As per earlier study, A. polyacantha contains many secondary metabolites such as saponins, catechic tannins, alkaloids, leuco anthocyanin, coumarins, sterols flavonoids, anthraquinones and terpenes(Ahamed, A *et al.*, 2022.)

Materials and Method

Sample Collection:

The roots of Acacia*polyacantha*plant, were collected on 10/04/2025 from Bazourah villages in Gedaref State, eastern The Sudan.

Sample Preparation:

The samples were cleaned and dried at room temperature, then finely ground, weighed, and stored in clean, sterile, tightly sealed plastic containers until analysis. The extraction process was conducted using water under different conditions.They were sample drying at room temperature, after that grinding by mortar and pestle and kept for extraction.

Extraction technique:

Bilola Technique for Chemical Separation:

In which one solvent used in different condition for extraction following by centrifugation in different speed to fractionate the constituents due to the molecular weight as follows.

All extraction procedures followed a consistent solid-to-solvent ratio (1:15 w/v). The primary variable was temperature (cold, room temperature, and hot conditions), while centrifugation was applied at two speeds (2000 rpm and 4000 rpm for 15 minutes).

Following extraction, all samples were filtered and subjected to centrifugation. Both supernatant and precipitate fractions were collected and analyzed separately. The precipitates were further dissolved in acetone to improve the detection of semi-polar phytochemicals.

Qualitative phytochemical Tests:

Flavonoids:

Alkaline Test:

A very small amount of sodium hydroxide solution was used to treat the extract. The presence of flavonoids is indicated by the development of an outstanding yellow coloring that becomes dull with the expansion of diluted acid*(R. Sowbaraniga., et al., 2019)*

Lead acetate Test:

Extract was given a light lead acetate treatment, consisting of a few drops. The yellow shading solution quickly revealed the presence of flavonoids. *(Divya P.V., et al., 2018)*

Ferric chloride Test:

A few drops of ferric chloride solution were added to the extracts. Flavonoidswere present as evidenced by the creation of A green precipitate(*Junaid R Shaikh., et al., 2020)*

Aluminium chloride test:

the addition of aluminium chloride solution induces the light-yellow colour, the existence of flavonoid is observed*(Godlewska . et al., 2023)*

NH4OH Test:

About 3 mL of dilute ammonia was added to 2 mL aqueous filtrate of each plant extract. This was followed by addition of 1 mL concentrated sulphuric acid (NH_4Cl + H_2SO_4). Yellow coloration in each extract showed the presence of flavonoids*(Ekwueme, F.N.,. et al.,* 2015)

Phenol and Tannins:

Lead acetate test:

Extract was given a light lead acetate treatment, consisting of a few drops. Dark green/bluish black colour the presence of Phenol (*Junaid R Shaikh., et al., 2020)*

Ferric chloride Test:

A few drops of ferric chloride solution were added to the extracts. Phenols were present as evidenced by the creation of blurry black color(Laxmi**.** *et al.,* 2022)

Carbohydrates:

Fehling's Test:

Perform Fehling's Test by combining 1 ml of each Fehling's A and B solution, then adding a bubble for one instant. Add an equal volume of the test extract mixture. Place the test tube in a pan of bubbling water for five to ten minutes. A yellow, black, and red PowerPoint presentation is initially viewed. (https://doi.org/10.37358/Rev.Chim.1949)

Qualitative Chemical Test:

The results of the qualitative phytochemical test for the roots extracts and Precipitate of *Acacia polyacantha*plant are shown in the flowing Tables.

Table (1): Phytochemical screening of the Root of *Acacia polyacantha* (kakmute) Plant Crude Aqueous Extract in Different Conditions

(+) = Phytochemical detected

(-) = Phytochemical detected

Phytochemicals	Test	Cold Conditions	At Room Temperature	Hot Conditions
Flavanoids	NaoH +HCL	++	++	+++
	$Pb(CH_3COO)_2$	+	+	++
	$FeCL_3$	++	++	+++
	NH_4CL + H2SO4 Conc	++	+	+++
	NH_4OH + HCL	+	+	+++
Phenolies	$Pb(CH_3COO)_2$	+	+	++
Tannins	$FeCL_3$	++	++	+++
Carbohydrates	Fehling +Heating	+++	+++	++

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Table (2) the phytochemical screening superannuate of Root of *Acacia polyacantha* (kakmute) Aqueous Extracts, after centrifuged

Phytochemicals	Test	Cold Conditions		At Room Temperature		Hot Conditions	
Flavanoids		2000 rpm for 15 min	4000 rpm for 15min	2000 rpm for 15 min	4000 rpm for 15min	2000 rpm for 15 min	4000 rpm for 15min
	NaoH +HCL	+	+	++	++	++	+++
	$Pb(CH_3COO)_2$	++	++	++	+++	+++	++
	$FeCL_3$	+++	++	++	+	++	++
	NH_4CL + H2SO4 Conc	+++	+	++	++	++	+
	NH_4OH + HCL	++	+	+++	++	++	+
Phenolies	$Pb(CH_3COO)_2$	++	++	++	+++	+++	++
Tannins	$FeCL_3$	+++	++	++	+	++	++
Carbohydrates	Fehling +Heating	++	+++	++	+++	++	+++

Table (3): the phytochemical screening Precipitate of Root of *Acacia polyacantha* (kakmute) Aqueous Extracts, after centrifuged.

Phytochemicals	Test	Cold Conditions	At Room Temperature	Hot Conditions

Flavanoids		2000 rpm for 15 min	4000 rpm for 15min	2000 rpm for 15 min	4000 rpm for 15min	2000 rpm for 15 min	4000 rpm for 15min
	NaoH +HCL	++	+++	++	++	++	++
	$Pb(CH_3COO)_2$	+	++	++	+++	++	++
	$FeCL_3$	++	+++	+	+++	++	+++
	NH_4CL + H2SO4 Conc	+	++	+	+	+	++
	NH_4OH + HCL	+	+	+	++	+	++
Phenolies	$Pb(CH_3COO)_2$	+	++	++	+++	++	++
Tannins	$FeCL_3$	++	+++	+	+++	++	+++
Carbohydrates	Fehling +Heating	+++	+	++	+++	++	++

Discussion of Results:

The qualitative Phytochemical Screening of Acacia polyacantha root normal aqueous extracts (Table 1) and aqueous extracts (Table 2) and their corresponding precipitates (Table 3), dissolved in both water and acetone, demonstrated that extraction efficiency and phytochemical distribution are strongly influenced by temperature, centrifugation speed, and duration .

Table 1:

Under cold conditions and at room temperature normal extraction showed strong detection of Carbohydrates compound, indicating that lowering temperature enhances cell wall disruption and facilitates the release of polar phytochemicals into the aqueous medium. However, the moderate intensity of Flavanoids, tannins, and Phenoliesin some tests suggests partial thermal sensitivity of these compounds.

Under hot conditions normal extraction showed strong detection of Flavanoids, tannins, compounds, indicating that elevated temperature enhances cell wall

disruption and facilitates the release of polar phytochemicals into the aqueous medium. However, the moderate intensity of Phenolies and Carbohydrates in some tests suggests partial thermal sensitivity of these compounds.

Table 2:

Increasing the centrifugation speed to 2000 rpm for 15 minutes significantly improved phytochemical detection, particularly for Flavanoids, tannins, and Phenolies. This enhancement can be attributed to improved phase separation and concentration of dissolved compounds. Furthermore, increasing the centrifuge speed to 4000 rpm for 15 minutes resulted in the highest overall phytochemical intensity, especially for Carbohydrates., confirming that prolonged centrifugation at high speed maximizes extraction efficiency. Nevertheless, slight reductions in flavonoid intensity under these conditions may indicate thermal or oxidative degradation.

Under cold conditions, the lowest concentrations of phytochemicals for most compounds were observed at 4000 rpm for 15 minutes, indicating limited solubility and diffusion at low temperatures. Although reducing the centrifugation speed (2000 rpm for 15 minutes) resulted in a slight improvement, the extraction efficiency remained lower compared to hot and room temperature conditions

At room temperature, extraction at 2000 rpm for 15 minutes yielded a moderate presence of phytochemicals, reflecting the reduced extraction efficiency in the absence of heat. Increasing the centrifugation speed to 4000 rpm for 15 minutes improved the detection of compounds without compromising their stability, making these conditions particularly suitable for heat-sensitive phytochemicals such as flavonoids

Table 3:

Analysis of the sediment showed a relatively high concentration of Flavanoid and Tannins compounds, indicating that these components tend to aggregate and precipitate during centrifugation. Notably, under heated conditions at 4000 rpm for 15 minutes, the precipitate exhibited the highest density of phytochemicals, demonstrating an effective concentration of the active compounds.

Dissolution of the precipitate in acetone significantly enhanced the detection of semi-polar compounds such as flavonoids, whereas dissolution in water favored the identification of polar compounds. This highlights the importance of solvent polarity in phytochemical analysis.

At room temperature, the precipitate maintained a balanced phytochemical profile with improved stability, while cold conditions resulted in comparatively weaker responses.

Overall, the results demonstrate that hot extraction combined with high-speed centrifugation (4000 rpm for 15 minutes) provides optimal conditions for maximizing phytochemical yield, while the inclusion of acetone as a secondary solvent improves the detection of a broader range of compounds.

Results:

- Room temperature normal extraction showed strong detection of Carbohydrates compound.
- The moderate intensity of Flavanoids, tannins, and Phenols in some tests suggests partial thermal sensitivity of these compounds.
- Centrifugation, centrifuge speed and temperature show affective results as factors of chemical separation.
- Bilola Technique for Chemical Separation an innovative method that produced promising outcomes.

Conclusion:

This study confirms that the efficiency of phytochemical extraction from *Acacia polyacantha* is highly dependent on extraction conditions, particularly temperature, centrifugation speed, and time. Hot extraction at 4000 rpm for 15 minutes yielded the highest concentration of bioactive compounds, especially flavonoids and phenolics. The precipitate fraction was found to be richer in concentrated phytochemicals, and its dissolution in acetone enhanced the detection of semi-polar constituents such as flavonoids.

The combination of aqueous extraction, centrifugation, and dual-solvent analysis (water and acetone) represents an effective, low-cost, and environmentally friendly approach for phytochemical investigation. These findings support the medicinal value of Acacia polyacantha and its potential application in natural product research and pharmaceutical development so Bilola Technique for Chemical Separation as green extraction, an innovative method that produced promising outcomes.

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