

Comparative Study of Qualitative and Quantitative Phytochemical Analysis in Leaves of Selected Medicinal Plants

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Abstract: The traditional system of medicine in India has shown much better improvement, has fewer side effects and is less expensive than modern synthetic drugs in the treatment of many diseases. The objective of the present study was to comparatively evaluate the qualitative and quantitative phytochemical analysis of leaves of *Andrographis paniculata* (Burm.f.) Nees, *Syzygium cumini* (L.) Skeels, *Aegle marmelos* (L.) Correa, *Caesalpinia crista* Thunb. Phytochemical screening is an essential procedure that leads to the identification of new and unique molecules. The shade-dried leaves were powdered and extracted with methanol, acetone, water and chloroform using the soxhlet extraction technique. The phytochemical study of selected plant will identify the different types of secondary metabolites found in the plant's leaves. Phytochemical screening showed the presence of alkaloids, flavonoids, tannins, terpenoids, steroids, phenols, protein, amino acid, *Syzygium cumini* (L.) Skeels leaf shows the highest phenol, tannins and flavonoid contents in methanolic extract. *Aegle marmelos* (L.) Correa leaf shows the highest alkaloids contents in methanolic extract. *Caesalpinia crista* Thunb. leaf shows the highest content of steroid in methanolic extract.

Key word: Comparison, Medicinal plant, Phytochemical, Secondary metabolites, Soxhlet.

Introduction :

Plants have been utilized for thousands of years to make medications, cosmetics, flavors, and dyes that enhance people's health and quality of life. Herbal medicine is based on the idea that plants have natural healing qualities that can be used to cure disease and promote health. Medicinal plants have been recognized for centuries as an abundant reservoir of therapeutic compounds utilized in the prophylaxis of diseases and health disorders on a global scale (Sofowora, *et al.*, 2013). As per the World Health Organization, as much as 80% of the global population continues to depend on conventional remedies, including herbal treatments for their primary health care needs (Ekor, 2014). The Food and Agricultural Organization has elucidated that nearly one billion persons, especially within developing nations, depend on indigenous plant species to fulfill their medicinal needs (Bharucha, 2010). In the Indian subcontinent, three conventional therapeutic systems have been embraced Ayurveda, Siddha, Unani. In the Ayurveda and Unani medical systems, plants and herbal remedies are mostly used to cure a variety of illnesses (Gunawardana, *et al.*, 2019). Some people still choose to employ traditional folk remedies because they have fewer adverse effects, despite the fact that synthetic drugs are widely accessible and effective in treating a wide range of illnesses in modern society (Yuan, *et al.*, 2016). About 28,000 plant species with known medicinal uses have been cataloged by Bob Allkin of the Royal Botanic Gardens at Kew (Yoo, 2018). The therapeutic plants have yielded almost 13,000 secondary metabolites. In plants, the secondary metabolites carry out specific tasks or act as defense chemicals. These secondary metabolites have therapeutic qualities, such as antioxidant and anticancer effects (Shehadeh, 2021). Plants have medical value because they contain chemical compounds that have a specific physiological effect on the human body. Alkaloids, flavonoids, tannins, and phenolic compounds are essential bioactive molecules found in plants (Tungmunthum, *et al.*, 2018). Phytochemicals are naturally occurring compounds in various parts of the plants which can protect the body by providing medicinal value or nutrients. These phytochemical compositions of the different medicinal plants mainly depend on the region where those are cultivated, the climate of that particular region, and the method and period of collection. Therefore, the present study was to comparatively evaluate the qualitative and quantitative analysis of phytochemical constituents of leaves of above mentioned five medicinal plants.

Material and Method

Collection of plant sample:

The leaves of selected specie collected from the city area and farm in Visnagar, Mehsana District during the period extending from June to October in the year 2024. These botanical specimens were authenticated within the Department of Botany at Sheth M. N. Science College, Patan.

Processing of plant samples:

The collected leaves were thoroughly washed several times with tap water to remove dust particles and then allowed to air-dry at room temperature for three weeks to prevent any direct

loss of phytochemical components from exposure to sunlight. A grinder mixture was then used to grind the shade-dried plant components, which were subsequently sealed in air tight plastic zip bags.

Preparation of Extract:

Hot water Extract

During the hot water extraction procedure, 200 milliliters of distilled water were added to a beaker containing five grams of dried, fine-grained plant material. After that, the mixture was heated for 20 minutes at temperatures between 30 and 40 degrees Celsius while being constantly stirred on a hot plate. The aqueous extract was then filtered by filter paper and the filtrate designated for further phytochemical analysis. When not in use, the water extract was kept in a refrigerator for preservation.

Soxhlet Extract

The Soxhlet extraction technique was used to prepare the crude plant extract for solvent extraction. A thimble containing roughly 10 grams of the powdered plant material was filled evenly, and 130 ml of different solvents were used to extract it separately. Acetone, chloroform, and methanol were among the solvents used. The extraction procedure was continued for a full day, or until the solvent in the extractor's siphon tube turned colorless. After that, the extract was moved to a beaker and heated to 30 to 40 degrees Celsius on a hot plate until the solvent had completely evaporated. The resulting dried extract was then kept for use in phytochemical investigations subsequently at 4°C in a refrigerator.

Phytochemical analysis:

Qualitative phytochemical screening test (Harborne,1998).

A) Test for Carbohydrates:

Benedict's reagent was applied to the extract and it was then gently heated. The presence of reducing sugars is indicated by an orange red precipitate (Benedict's test).

B) Test for Alkaloids:

The extract were treated with Dragendorff's reagent (potassium bismuth iodide solution), the presence of alkaloids is shown by the production of a red precipitate (Dragendorff's test).

C) Test for Tannins:

A few drops of a 5% ferric chloride solution were added to two ml of extract solution as part of the detection process, which produced a blue color that indicated the presence of tannins (Ferric chloride test).

D) Test for Flavonoids:

Plant extract (0.5 g) was dissolved in a mild sodium hydroxide solution, and then a few drops of hydrochloric acid solution were added to detect the presence of flavonoids. A subsequent colorless reaction occurred in the yellowish solution.

E) Test for Cardiac Glycoside:

After adding 1-2 drops of 5% FeCl_3 and 1 ml of glacial acetic acid to 2 ml of extract, add 1 ml of concentrated H_2SO_4 . The presence of cardiac glycoside is shown by the green-blue color (Kelller-Killiani test).

F) Test for phenols:

To the extracted solution, add the Folin-Ciocalteu reagent. The presence of phenol is indicated by the color blue.

G) Test for Caumarine:

When 2 ml of extract is mixed with 3 ml of 10% NaOH, a yellow colour forms signifying the presence of caumarin.

H) Test for Steroids:

Each extract was weighed out at 0.3 g and mixed with 2 ml of H_2SO_4 , 2 ml of acetic anhydride, and 2 ml extract. When the color shifted from violet to blue or green, steroids were found in some of the samples.

I) Test for Reducing sugar:

0.5 ml of the extract, added 1ml water and 5-8 drops of Fehling's solution and observed for brick red precipitate indicate presence of reducing sugar.

Quantitative phytochemical screening test:**A. Estimation of phenol:**

The Folin-Ciocalteu reagent was used to quantify the total amount of phenolic compounds present in the extracts. The reference standard for the analytical process was gallic acid. Gallic acid solutions were prepared in methanol at concentrations of 0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml. Additionally, 1.5 ml of each sample was poured into test tubes and then mixed with 1.5 ml of distilled water to create concentrations of 0.1 and 1 mg/ml of the plant extract in a chosen solvent. This was followed by the addition of 0.5 ml of Folin-Ciocalteu reagent, and the mixture was then allowed to sit at room temperature for 4 minutes. Two milliliters of a 20% sodium carbonate solution were then

added. Before the spectrophotometric measurement of absorbance at 765 nm, the test tubes were sealed and allowed to incubate in a water bath for one minute (Harborne,1998).

B. Estimation of Flavonoid:

The determination of total flavonoid content was accomplished through a aluminium chloride colorimetric assay. A 0.5-mL aliquot of the appropriately diluted sample solution was amalgamated with 4 mL of distilled water, followed by the addition of 0.3 mL of a 5% NaNO₂ solution. After a duration of 5 minutes, 0.3 mL of a 10% AlCl₃ solution was incorporated and allowed to rest for 6 minutes, subsequent to which 1 mL of 1M NaOH solution was added to the mixture. The resultant mixture was then thoroughly homogenized and permitted to stand for an additional 15 minutes. The absorbance of the mixture was quantified at 510 nm in comparison to a prepared water blank (Zou, 2004).

C. Estimation of Tannin:

The total tannin content of the plant extract was measured using the Folin-Ciocalteu technique. After dissolving about 0.1 ml of the plant extract in 7.5 ml of distilled water, 0.5 ml of Folin reagent was added to the mixture. The solution was then diluted to 10 ml with distilled water and 1 ml of 35% Na₂CO₃ was added. After giving the mixture a good shake, it was left at room temperature for half an hour. Gallic acid reference standard solutions with concentrations of 20, 40, 60, 80, and 100 µg/ml were made. A UV/Visible spectrophotometer was used to measure the absorbance of the test and standard solutions at 725 nm in relation to the blank (Harborne,1998).

D. Estimation of Alkaloid :

Add 1 ml of test extract in 5 ml of pH 4.7 phosphate buffer, and 5 milliliters of BCG solution. Shake the mixture with 4 ml of chloroform. After being collected in a 10-ml volumetric flask, the extracts were diluted with chloroform to adjust volume. When compared to a blank made as previously but without extract, the complex's absorbance in chloroform was measured at 470 nm. Using atropine as a standard material, the assay is contrasted with its equivalents (Madhu, *et al.*, 2016).

E. Estimation of Steroid :

1ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20°C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank (Madhu, *et al.*, 2016).

Result and discussion:

Table 1 to 4 presents the findings from the qualitative phytochemical screening of selected four plant leaves. The different phytochemical screening tests verified that the various solvent and its contained carbohydrates, alkaloids, phenols, tannins, steroids, cardiac glycoside, coumarins and reducing sugar. The results obtained from the quantitative analysis of extracts in four different solvent of all the selected four medicinal plants leaves showed the presence of phytochemicals from highest to least extent. The quantitative result clearly indicated (Figure: 1 to 4) that the highest amount of alkaloids are reported in methanolic extract of *Aegle marmelos* (L.) Correa leaves and least amount of alkaloids was observed in the chloroform extract of *Andrographis paniculata* (Burm.f.) Nees leaves. The highest amounts of flavonoids and phenol are reported in methanol extract of *Syzygium cumini* (L.) Skeels leaves. The least values of flavonoids are observed in chloroform extract of *Syzygium cumini* (L.) Skeels leaves. Least values of phenols are observed in chloroform extract of *Andrographis paniculata* (Burm.f.) Nees leaves. The highest amount of tannin are reported in methanolic extract of *Syzygium cumini* (L.) Skeels leaves and less amount of tannin was observed in chloroform extract of *Andrographis paniculata* (Burm.f.) Nees leaves. When the highest concentrations of steroid are reported in methanolic extract of *Caesalpinia crista* Thunb. leaves and less amount of steroid reported in chloroform extract of *Aegle marmelos* (L.) Correa leaves. When the quantitatively determined the highest concentrations result of steroids, alkaloids, phenols, tannin and steroid in methanolic extract. The lowest concentrations result of all parameter in chloroform extract. Phytochemicals are naturally occurring substances found in different plant parts. Tannins are known for their ability to heal wounds, and flavonoids which are strong antioxidants and free radical scavengers, prevent cell damage. Moreover, flavonoids have anticancer properties (Doss, A. *et al.*, 2009). One of the main classes of important polyphenolic chemicals, tannins and flavonoids have a wide range of biological activities such as anti-carcinogenic and antioxidant effects (Crozier, A.*et al.*, 2009). The main class of phytochemicals that functions as a principal reductant is comprised of polyphenolic compounds (Vasanthi, P. *et al.*, 2014). Plants with therapeutic qualities are frequently examined for tannins, alkaloids, terpenoids, and saponins before being employed pharmacologically. These natural compounds, also known as secondary metabolites, have antiviral, antioxidant, and antibacterial properties (Ndam, L. *et al.*, 2014). Most polar solvents are used to screen for polar bioactive phytochemicals including tannins and flavonoids, which have been proven to have analgesic and anti-inflammatory properties (Sani, Y. *et al.*, 2014). The phytochemical components of flavonoids and tannins have been verified by polar extracts, which also demonstrated the potency of their anti-inflammatory and anti-hyperglycemic properties (Nair, S. *et al.*, 2016).

Table 1: Qualitative Phytochemical screening of *Andrographis paniculata* (Burm.f.) Nees, leaves.

Phytochemical	Water	Methanol	Ethanol	Chloroform	Acetone
Carbohydrates	+	+	-	-	-
Alkaloids	+	+	+	+	+
Tannins	+	+	+	-	+
Flavonoids	-	+	+	+	+
Cardiac Glycoside	-	+	+	+	+
phenols	-	+	+	+	+
Anthraquinone	+	+	-	-	+
Reducing sugar	+	+	+	-	+
Steroids	-	-	-	-	-
Terpenoid	-	+	+	+	+
Protein	+	+	+	+	+

Table 2: Qualitative Phytochemical screening of *Syzygium cumini* (L.) Skeels leaves.

Phytochemical	Water	Methanol	Ethanol	Chloroform	Acetone
Carbohydrates	+	+	+	-	-
Alkaloids	+	+	+	+	+
Tannins	+	+	+	-	-
Flavonoids	+	+	+	-	-
Cardiac Glycoside	-	+	-	-	-
phenols	+	+	+	-	-
Anthraquinone	+	+	+	+	-
Reducing sugar	-	+	+	-	-
Steroids	+	+	+	+	+
Terpenoid	+	+	+	+	+
Protein	+	-	-	+	-

Table 3: Qualitative Phytochemical screening of *Aegle marmelos* (L.) Correa leaves.

Phytochemical	Water	Methanol	Ethanol	Chloroform	Acetone
Carbohydrates	+	+	+	+	+
Alkaloids	+	+	+	+	+
Tannins	-	+	+	-	+
Flavonoids	+	+	+	+	+
Cardiac Glycoside	+	+	+	-	+
phenols	+	+	+	-	+
Anthraquinone	-	+	+	-	+
Reducing sugar	-	+	-	-	+
Steroids	+	+	+	-	+
Terpenoid	-	-	+	-	-
Protein	-	+	+	-	+

Table 4: Qualitative Phytochemical screening of *Caesalpinia crista* Thunb. leaves.

Phytochemical	Water	Methanol	Ethanol	Chloroform	Acetone
Carbohydrates	+	+	+	+	+
Alkaloids	+	+	+	-	+
Tannins	-	+	+	-	+
Flavonoids	+	+	+	+	+
Cardiac Glycoside	+	+	+	-	+
phenols	+	+	+	-	+
Anthraquinone	-	+	+	-	+
Reducing sugar	+	+	-	-	+
Steroids	+	+	+	+	+
Terpenoid	+	+	+	+	+
Protein	-	-	-	-	-

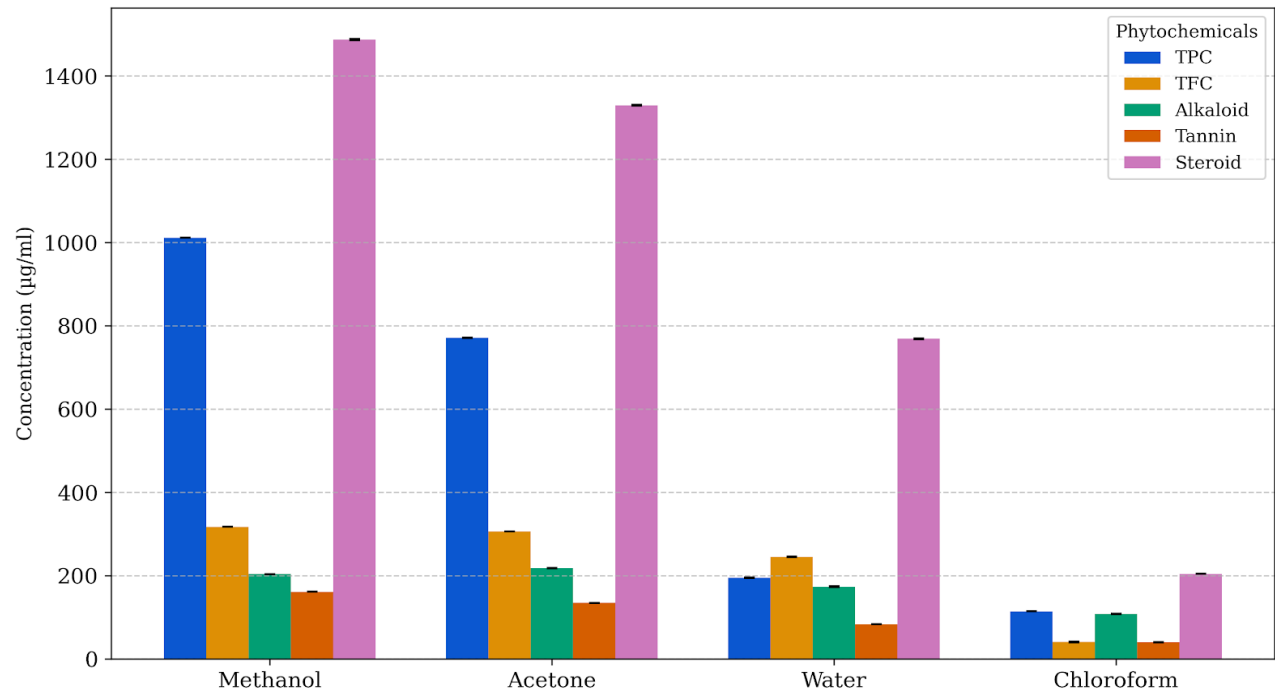


Figure 1: Quantitative screening of *Andrographis paniculata* (Burm.f.) Nees, leaves.

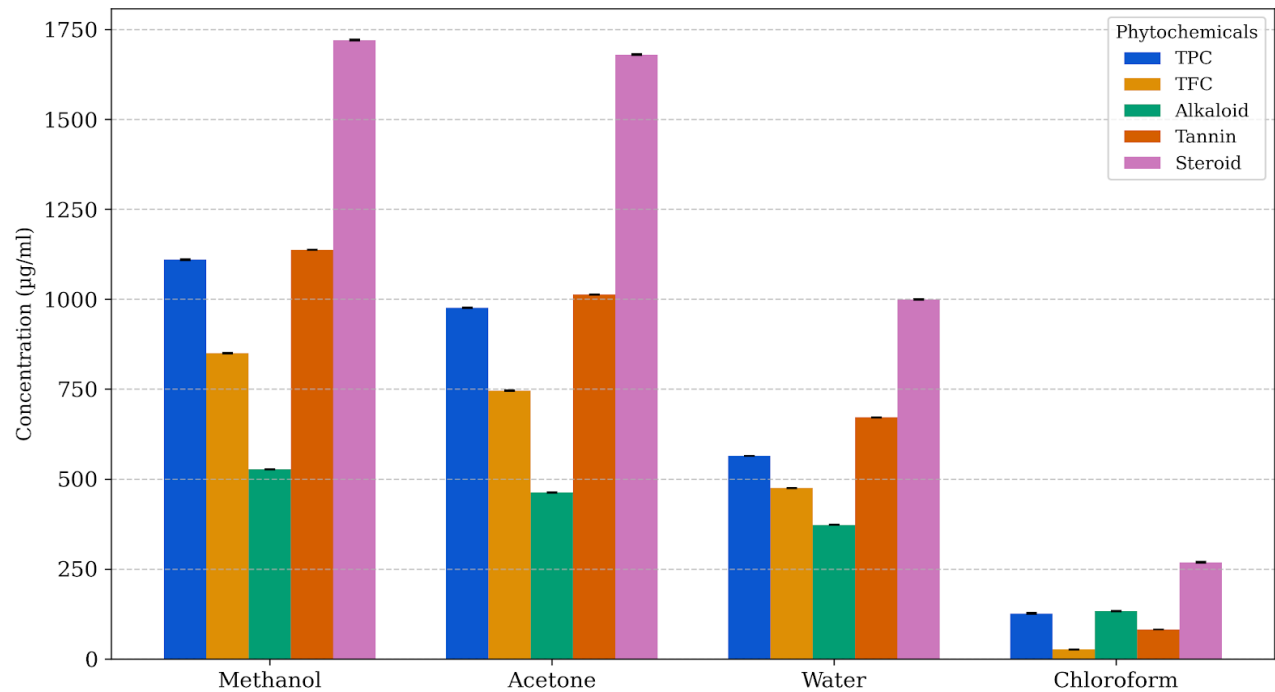


Figure 2: Quantitative screening of *Syzygium cumini* (L.) Skeels leaves.

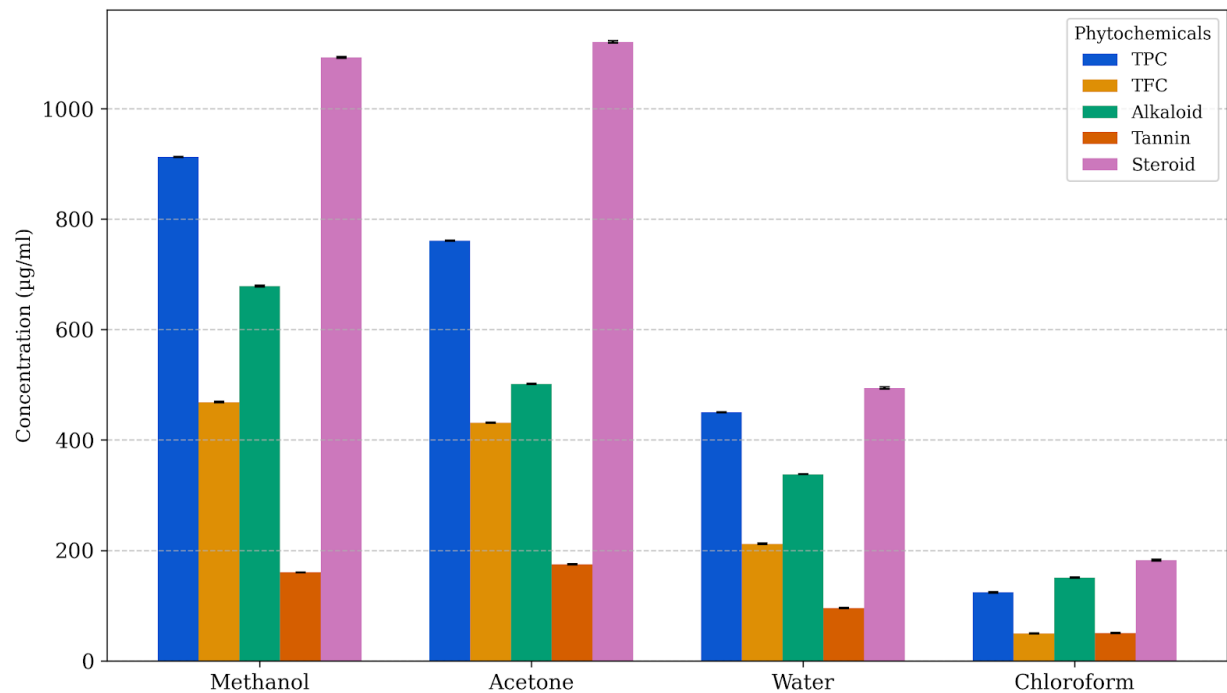


Figure 3: Quantitative screening of *Aegle marmelos* (L.) Correa leaves.

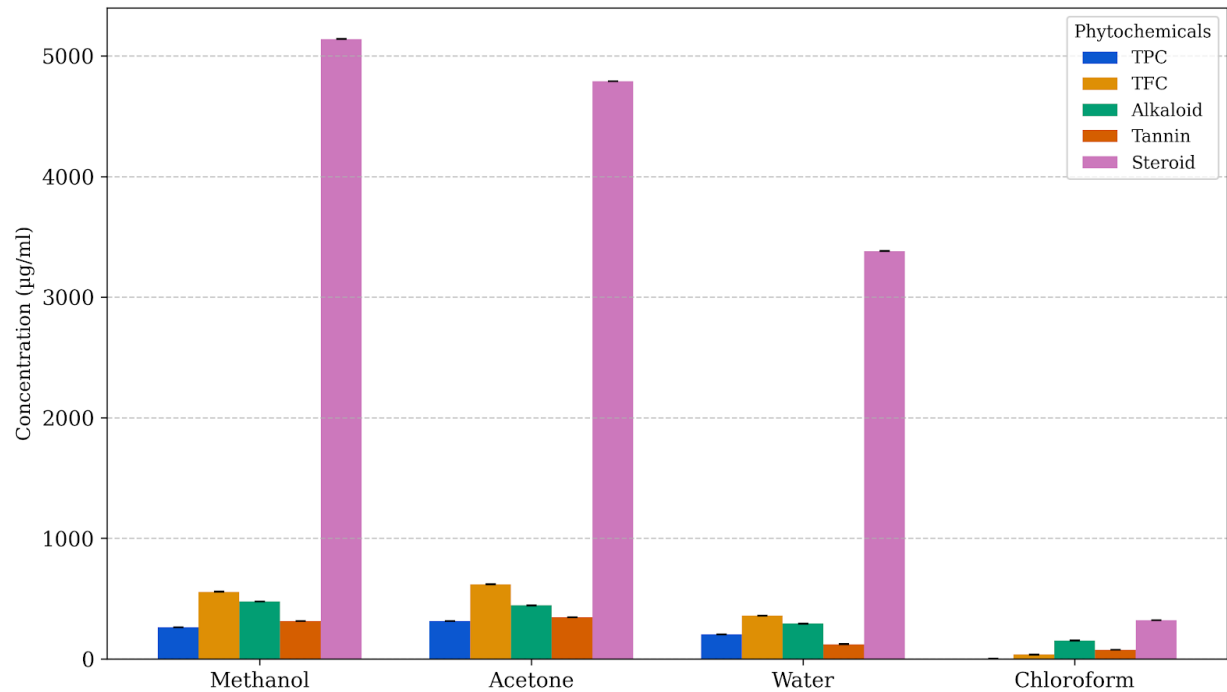


Figure 4: Quantitative screening of *Caesalpinia crista* Thunb. leaves.

Conclusion :

Medicinal plants and phytochemicals hold significant value in contemporary contexts, particularly in developing nations where resources are constrained. The consistent consumption of herbal medicines that incorporate these phytochemicals may yield advantages for various health ailments. The results demonstrated the presence of pharmacologically relevant constituents within the plants under investigation. An extensive array of evidence accrued from prior research has corroborated the bioactive potential of the identified phytochemicals. Numerous studies have confirmed that the incorporation of these phytochemicals endows the examined plants with both therapeutic and physiological attributes, facilitating the management of a wide range of health issues. Hence, extracts obtained from these botanical sources can be considered as promising candidates for the formulation of beneficial pharmacological compounds. The utilization of traditional medicine is strongly advocated for these botanical entities, and it is additionally recommended that further inquiries be undertaken to isolate, purify, and characterize the active components that contribute to the therapeutic properties of these plants. Moreover, additional research endeavors are encouraged to clarify the potential mechanisms of action related to these extracts.

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