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Solvent-dependent quantification of flavonoids, phenolics, and tannins from *Abutilon indicum* (L.) Sweet leaves: A comparative phytochemical assessment

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Abstract
Plant-derived therapeutic compounds are increasingly being explored by scientists due to their diverse phytochemical composition and extensive pharmacological capabilities. *Abutilon indicum* (L.) Sweet, a commonly utilized medicinal plant in traditional practices, is recognized for its substantial levels of flavonoids, phenolics, tannins, and other bioactive substances. The current research assessed the quantitative distribution of these secondary metabolites in chloroform, methanol, ethanol, and aqueous extracts of *A. indicum* leaves. The extraction utilized a Soxhlet apparatus, followed by standard colorimetric techniques: the aluminium chloride method for flavonoids, the Folin-Ciocalteu assay for phenolics, and a modified Folin-Ciocalteu procedure for tannins. The results demonstrated significant differences depending on the solvent used, with the aqueous extract containing the highest concentration of flavonoids (89.62±1.34 mg/g CE), while methanol exhibited the greatest content of phenolics (68.9±1.01 mg/g GAE) and tannins (11.03±0.88 mg/g GAE). Ethanolic extracts showed consistently high levels across all metabolite categories, while chloroform produced relatively lower quantities. These results strongly correlate with the polarity of solvents and the established solubility traits of plant polyphenols, confirming that polar solvents, especially alcohols and water, enhance extraction efficiency. The study underscores the phytochemical abundance of *A. indicum* leaves and highlights its potential as a valuable natural resource for pharmacological uses.

Keywords: *Abutilon indicum*, flavonoids, phenolics, tannins, Soxhlet extraction, solvent polarity

Introduction
Plant extracts have historically been an essential source of therapeutic agents across various cultures and traditional medical systems, including Ayurveda, Traditional Chinese Medicine, and different indigenous healthcare practices globally [1]. Their intricate chemical makeup, which includes polyphenols, alkaloids, terpenoids, flavonoids, saponins, tannins, and other secondary metabolites, supports a wide array of biological activities [2]. As natural substances, plant extracts can offer significant benefits over synthetic compounds, such as biological diversity, precision in targeting, multi-mechanistic functions, and enhanced biocompatibility [3].

Given the ongoing issues of antimicrobial resistance and the negative reactions associated with many synthetic drugs, there is an increasing global push to investigate plant-based alternatives for new drug development [4]. The scientific validation of traditional therapeutic practices, facilitated by improvements in extraction, characterization, and bioassay techniques, bolsters the legitimacy and acceptance of herbal remedies in contemporary pharmacopeias [1]. Moreover, plant extracts present intricate phytochemical interactions that may influence disease mechanisms more effectively than drugs targeting a single pathway [2]. As a result, research into the pharmacological properties of plant extracts continues to unlock new possibilities for treating metabolic, infectious, inflammatory, and degenerative diseases.

The lasting impact of medicinal plants underpins much of contemporary drug development and therapeutic advancements. There is a growing need to investigate alternative therapeutic agents with fewer side effects and improved effectiveness, driven by global health issues such as drug resistance, chronic illnesses, and complex conditions.

Traditional medicinal systems, including Ayurveda, Siddha, and Unani, have historically acknowledged the therapeutic potential of plants like *Abutilon indicum* (L.) Sweet, also referred to as Indian Mallow or Atibala, for treating a wide range of diseases and disorders [5-7]. The leaves of *A. indicum* are especially valued due to their diverse phytochemical content and various pharmacological properties.

From a phytochemical perspective, *A. indicum* leaves are abundant in bioactive secondary metabolites, including flavonoids (quercetin, luteolin), phenolic acids (caffeic acid, gallic acid), alkaloids, glycosides, tannins, steroids, saponins, and essential oils [8-10]. Flavonoids and phenolics play significant roles in providing free radical scavenging and anti-inflammatory effects, while alkaloids and glycosides have been linked to antimicrobial and antidiabetic activities [11, 12].The choice of solvents used in the extraction process significantly influences both the yield and the variety of these compounds, highlighting the importance of extraction methods for maximizing the recovery of bioactive substances. Numerous studies have connected traditional uses to scientific evidence by illustrating how these phytochemicals work either in synergy or independently to produce therapeutic outcomes. For instance, quercetin and luteolin demonstrate anti-inflammatory and antioxidant properties by inhibiting pro-inflammatory cytokines and neutralizing reactive oxygen species (ROS) [9]. Caffeic acid and gallic acid enhance antimicrobial and hepatoprotective effects by boosting antioxidant enzyme activities and diminishing pathogen virulence [5].

Materials and Methods

Collection of plant material

Dried leaves of *Abutilon indicum* (L) Sweet (Fig. 1) were gathered from Panapatti, Tamil Nadu (10.8765° N, 77.1003° E) and the collected leaves were thoroughly rinsed in running tap water, followed by distilled water. They were then shade-dried for 20 days, ground into a powder, and stored in containers for future use.

Fig 1: *Abutilon indicum* (L.) Sweet plant

Extraction procedure

Powdered plant material underwent a series of extractions using a Soxhlet extractor. This method involves extracting the active compounds present in the plant material through four different solvents. The solvents employed included chloroform, methanol, ethanol, and water. Approximately 200 g of powdered leaves were placed in a cellulose thimble and situated in the central section of a Soxhlet extractor. Around 500 mL of solvent was added to the lower flask, which was then fitted with a reflux condenser followed by 8 hours serial extraction using solvents. The extract obtained is concentrated and dried using rotor evaporator/ 0.5g of dried extract was dissolved respective positive solvent before they are subjected for quantification studies.

Total Flavonoid Content by Aluminium Chloride Colorimetric Method.

Extracts of *Abutilon indicum* leaves in chloroform, methanol, ethanol and water are utilized for the quantification of flavonoids. The flavonoid content was assessed using the aluminum chloride assay [13]. For this, 0.5 ml of the extract was introduced into a 10 mL test tube that contained 2 mL of distilled water. To every test tube, 0.15 mL of 5% NaNO2 was added. After allowing the mixture to incubate for 5 minutes, 0.15 mL of 10% AlCl3 was incorporated. After an additional minute, 1 mL of 1 M NaOH was introduced, and the total volume was adjusted to 5 mL using distilled water. After waiting for 10 minutes, the absorbance of the resulting mixture was recorded at 510 nm. Catechin served as the standard for expressing the total flavonoid content of the samples in terms of mg catechin equivalent per 100 g of sample (mg CE/100 g sample). All samples were evaluated in triplicate.

Total Phenolic Content by Folin-Ciocalteu Method

Chloroform, methanol, ethanol and water extracts of *Abutilon indicum* leaves were subjected to determine total phenolic content. The total phenolic content was determined [14] employing the Folin-Ciocalteu reagent. In this procedure, 0.5 mL of extract solution was mixed with 2.5 mL of Folin-Ciocalteu reagent diluted ten-fold. After an incubation period of 5 minutes, 2 mL of 7.5% sodium carbonate solution was incorporated, and the mixture was allowed to incubate for 30 minutes at room temperature in the dark. Absorbance was recorded at 760 nm. Gallic acid was used as a standard, with results expressed as gallic acid equivalents (mg GAE/g extract). This method establishes a strong correlation to phenolic concentration within complex plant matrices and remains one of the most dependable antioxidant profiling techniques.

Total Tannin Content by Folin-Ciocalteu Method

The total content of tannins was also carried out [15] in Chloroform, methanol, ethanol and water extracts of *Abutilon indicum* leaves. A specific quantity of the plant extract was dissolved in distilled water, and 5 mL of this solution was placed into a measuring flask. To this, 1 mL of Folin-Ciocalteu reagent was added, followed by 2 mL of a 15% sodium carbonate solution. The mixture was shaken well, allowed to sit for 5 minutes, and the final volume was brought to the mark with distilled water. The solution was then incubated at room temperature for 90 minutes, after which the absorbance was measured at 765 nm. The tannin content was determined using a gallic acid standard calibration curve and expressed as mg gallic acid equivalents (GAE) per gram of extract. This method is preferred for its ease, sensitivity, and reliability in routine tannin quantification.

Results

The quantitative assessment of secondary metabolites across various solvent extracts of *Abutilon indicum* leaves indicated notable differences based on the polarity of the solvents utilized (Table 1, Fig. 2). Among the examined extracts, the aqueous fraction demonstrated the highest flavonoid concentration (89.62±1.34 mg/g), followed by ethanol (78.02±0.86 mg/g) and methanol (72.73±1.06 mg/g) extracts. Conversely, moderate flavonoid levels were found in chloroform (17.94±0.32 mg/g) extracts. This trend implies that flavonoids, which are polyphenolic compounds, are more soluble in polar solvents, with aqueous and alcoholic solvents yielding the highest recovery rates.

A comparable pattern was noted for phenolic compounds. The methanol extract had the highest phenolic content (68.9±1.01 mg/g), trailed by ethanol (63.23±1.52 mg/g) and aqueous extracts (19.11±0.81 mg/g), while chloroform contained a significantly low level (12.1±0.42 mg/g). The prevalence of phenolics in polar extracts further affirms their hydrophilic characteristics and preference for alcoholic solvents.

Tannins were primarily found in the methanol extract (11.03±0.88 mg/g), followed by ethanol (9.41±1.20 mg/g) and aqueous (6.43±0.89 mg/g) extracts, with chloroform revealing a relatively low level (3.01±0.23 mg/g). The results confirm that tannins, as phenolic compounds, are most effectively extracted using polar solvents, especially alcohols.

Table 1: Secondary metabolite quantification of *Abutilon indicum* leaves various extracts

	Chloroform extract	Methanol extract	Ethanol extract	Aqueous extract
Total Flavonoid (mg/g Catechin equivalents)	17.94±0.32	72.73±1.06	78.02±0.86	89.62±1.34
Total Phenolics (mg/g Gallic acid equivalents)	12.1±0.42	68.9±1.01	63.23±1.52	19.11±0.81
Total Tannin (mg/g Gallic acid equivalents)	3.01±0.23	11.03±0.88	9.41±1.2	6.43±0.89

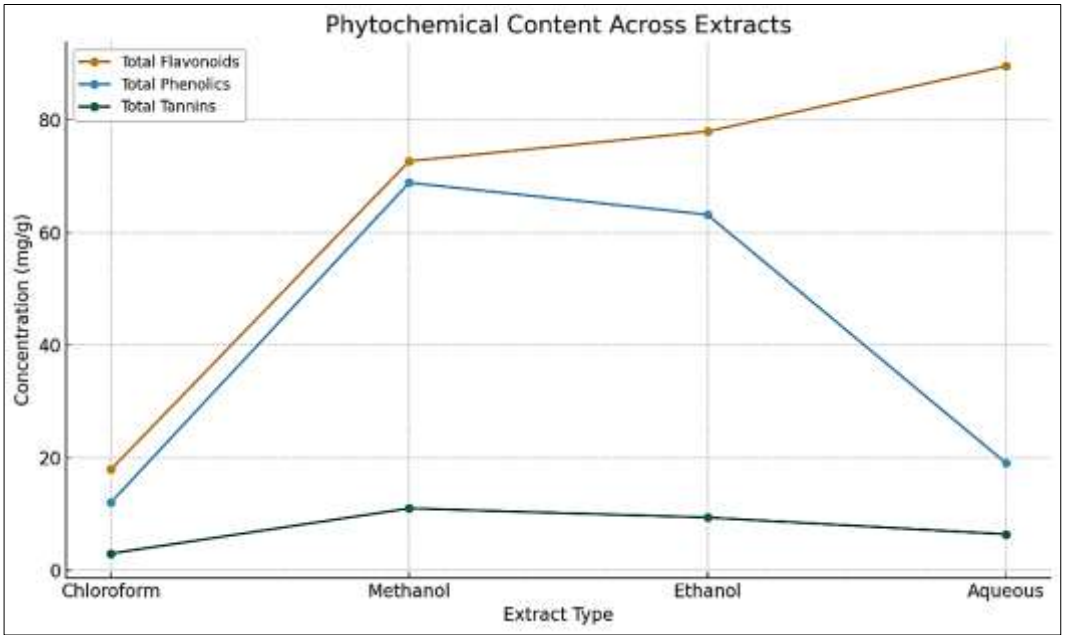

Fig 2: Secondary metabolite quantification of *Abutilon indicum* leaves various extracts

Discussion

The quantitative profiling of secondary metabolites in the present work, performed on chloroform, methanol, ethanol, and aqueous leaf extracts of *Abutilon indicum*, is consistent with solvent-solute polarity principles. The highest flavonoid concentration in the aqueous fraction (89.62±1.34 mg/g CE), followed by ethanol and methanol, is plausible given that many leaf flavonoids occur as O-glycosides whose sugar moieties confer high polarity and aqueous/alcoholic solubility [16]. Mixed protic solvents, particularly hydroalcoholic systems, reduce solvent-matrix hydrogen bonding and swell cellulosic tissues, improving desorption and diffusion of glycosylated flavonoids; in several botanical systems, 50-70% ethanol or methanol maximizes total flavonoid recovery relative to neat organic solvents [16, 17]. In *A. indicum* specifically, methanolic extract of leaves frequently show substantial total flavonoids and antioxidant capacity [18], and cultivation conditions also modulate flavonoid accumulation in methanolic leaf extracts (≈ 62-107 mg catechol equivalents/100 g dry extract) [19]. Observation of an aqueous fraction outperforming alcohols for total flavonoids can therefore be rationalized by a leaf chemotype enriched in highly polar glycosides and polymeric phenolics, together with the known efficacy of water (and aqueous ethanol) for extracting such constituents. Methodological nuances also matter, the $AlCl_3$ colorimetric assay is selective for certain flavonoid subgroups (e.g., flavonols, flavones) and is sensitive to solvent, pH, and complexing counter-ions; consequently, absolute values can shift with reagent formulation even when the rank order across solvents remains stable [16, 17, 20].

Phenolic compounds were found in the highest amounts in the methanol extract (68.9±1.01 mg/g GAE), followed closely by ethanol (63.23±1.52 mg/g GAE), with much lower levels in water (19.11±0.81 mg/g GAE) and almost none in non-polar or semi-polar solvents. This finding aligns with existing research, which shows that mid-polarity solvents, such as methanol and ethanol, are most effective for extracting polyphenols using the Folin-Ciocalteu (F-C) assay [16, 21]. Alcohols are especially effective at extracting

hydroxycinnamates, flavonols, and oligomeric phenolics due to their polarity and specific interactions, whereas water often underperforms for less polar compounds unless heat or pre-treatment is used [17]. For *A. indicum*, methanolic leaf extracts consistently show high phenolic content and antioxidant activity [18], and phenolic levels in methanolic leaves vary with growing conditions, ranging from about 35 to 57 mg GAE/g dry extract [19]. It's essential to note that the F-C assay measures total reducing substances, not just phenolics; other compounds, such as sugars and ascorbic acid, can also contribute to the results. Therefore, results should be interpreted in the context of proper controls and standards [22, 23].

The distribution of tannins is highest in methanol, followed by ethanol, then water, with much lower amounts in chloroform and none in non-polar solvents. Condensed tannins (proanthocyanidins) and many hydrolyzable tannins are best extracted by alcohols or mixtures like acetone-water and alcohol-water, which break up protein-tannin and polysaccharide-tannin complexes and dissolve larger oligomers. Water alone often fails to extract these compounds unless extended heating or additives are used [16]. Thus, the results fit with the usual recommendation to use aqueous acetone or alcohol for tannin extraction, while chloroform or benzene are generally not effective for this purpose.

Conclusion

The study indicates that the efficiency of extracting secondary metabolites from the leaves of *Abutilon indicum* is greatly affected by the polarity of the solvent used. Both aqueous and alcoholic extracts exhibited the highest levels of flavonoids, phenolics, and tannins, while chloroform produced only minimal quantities. Methanol proved to be the most efficient solvent for extracting total phenolics and tannins, whereas water resulted in the highest yield of flavonoids. These findings underscore the phytochemical potential of A. indicum and support its conventional medicinal applications. Additionally, the results highlight the significance of choosing the appropriate solvent to optimize the recovery of bioactive compounds in phytopharmacological studies.

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References

1. Shompa SA, Hasnat H, Riti SJ, Islam MM, Nur F, Alam S, *et al*. Phyto-pharmacological evaluation and characterization of the methanolic extract of the *Baccaurea motleyana* Müll. Arg. seed: promising insights into its therapeutic uses. Frontiers in Pharmacology. 2024;15.
2. Zouine N, El Ghachtouli N, El Abed S, Ibnsouda Koraichi S. A comprehensive review on medicinal plant extracts as antibacterial agents: factors, mechanism insights and future prospects. Scientific African. 2024;26:e02395.
3. Tesfaye M, Gonfa Y, Tadesse G, Temesgen T, Periyasamy S. Green synthesis of silver nanoparticles using *Vernonia amygdalina* plant extract and its antimicrobial activities. Heliyon. 2023;9(6):e17356.
4. Alaiya MA, Odeniyi MA. Utilisation of *Mangifera indica* plant extracts and parts in antimicrobial formulations and as a pharmaceutical excipient: a review. Future Journal of Pharmaceutical Sciences. 2023;9:29.
5. Rajeshwari S, Sevarkodiyone SP. Medicinal properties of *Abutilon indicum*. Open Journal of Plant Science. 2018;3(1):11-16.
6. Dharuman A, Muthusamy P, Radha R, Rubikaa G. Review on *Abutilon indicum* - an update. International Journal of Pharmacy and Pharmaceutical Research. 2023;26(4).
7. Bolleddu R, Venkatesh S, Narasimhaji CV, Hazra J. Pharmacognostical and phytochemical studies of Atibala (*Abutilon indicum* [Linn.] Sweet) fruit. Ayu. 2021;42(3):138-142.
8. Suryawanshi VS, Umate SR. A review on phytochemical constituents of *Abutilon indicum* (L.) Sweet - an important medicinal plant in Ayurveda. Plantae Scientia. 2020;3(3):15-19.
9. Sunil M, Gautam S, Hisham M, Tomy P. Phytochemical analysis and antioxidant evaluation of the ethanolic extract of the leaves of *Abutilon indicum*. Cureus. 2023;15(10):e47304.
10. Khan IM, Rahman R, Mushtaq A, Ben Ghania J. A review on usefulness and biological activities of Country Mallow (*Abutilon indicum*). International Journal of Chemical and Biochemical Sciences. 2017;11:134-137.
11. Pavithra P, Kavimani S, Pavazhaviji P, Hemalatha A, Snega R. A review on phytochemical screening and pharmacological activities of fruit of Indian mallow. Indian Journal of Pharmacy and Pharmacology. 2023;10(4):265-271.
12. Sharma A, Sharma RA, Singh H. Phytochemical and pharmacological profile of *Abutilon indicum* L. Sweet: a review. International Journal of Pharmaceutical Sciences Review and Research. 2013;20(1):120-127.
13. Seifu T, Mehari B, Atlabachew M, Chandravanshi B. Polyphenolic content and antioxidant activity of leaves of *Urtica simensis* grown in Ethiopia. Latin American Applied Research. 2010;40(1):35-40.
14. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965;16(3):144-158.
15. Amorim ELC, Nascimento JE, Souza TP, Nascimento TH. A simple and accurate procedure for the determination of tannin and flavonoid levels in herbs. Functional Ecosystems and Communities. 2008;2(SI1):88-94.
16. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-7352.
17. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, *et al*. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis. 2014;22(3):296-302.
18. Singh H, Saharia RL. Estimation of total phenolic, flavonoid content and evaluation of DPPH free radical

scavenging potential of *Abutilon indicum* L. International Journal of Pharmaceutical Sciences Review and Research. 2015;32(1):100-103.
19. Yaldiz G, Yildirim AB, Arici YK, Çamlıca M. Yield, yield features, phytochemical composition, antioxidant and antibacterial activities of *Abutilon indicum* cultivated under different fertilizers. Ciencia e Investigación Agraria. 2016;43(3):12-12.
20. Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: a critical evaluation. LWT - Food Science and Technology. 2021;150:111932.
21. Turkman N, Sari F, Velioglu YS. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chemistry. 2006;99(4):835-841.
22. Perez M, Dominguez-López I, Lamuela-Raventós RM. The chemistry behind the Folin-Ciocalteu method for the estimation of (poly)phenol content in food: total phenolic intake in a Mediterranean dietary pattern. Journal of Agricultural and Food Chemistry. 2023;71(46):17543-17553.
23. Dominguez-López I, Perez M, Lamuela-Raventós RM. Total (poly)phenol analysis by the Folin-Ciocalteu assay as an anti-inflammatory biomarker in biological samples. Critical Reviews in Food Science and Nutrition. 2024;64(27):10048-10054.