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Ayushi Sachan
Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, SVPUAT, Meerut- 250110, Uttar Pradesh, India

Shweta Anand
Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, SVPUAT, Meerut- 250110, Uttar Pradesh, India

Rachna Varma
Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, SVPUAT, Meerut- 250110, Uttar Pradesh, India

Rajiv Ranjan Kumar
ICAR-Central Institute for Research on Cattle, Meerut-250001, Uttar Pradesh, India

Ashok Kumar Mohanty
ICAR-Central Institute for Research on Cattle, Meerut-250001, Uttar Pradesh, India

Correspondence:
Dr. Rajiv Ranjan Kumar
Principal Scientist, ICAR-Central Institute for Research on Cattle, Meerut-250001, Uttar Pradesh, India
Email: dr_rajivranjan@yahoo.com

Comparative extraction efficiency of solvent systems for phytochemicals from *Moringa oleifera*, *Madhuca indica*, and *Ficus nemoloris*

Ayushi Sachan, Shweta Anand, Rachna Varma, Rajiv Ranjan Kumar, Ashok Kumar Mohanty

ABSTRACT

Background: In tropical and subtropical regions, trees and shrubs play a vital role in animal feeding systems by providing dependable nutritional resources. The health and productivity enhancing effects of plant-based feeds are largely attributed to phytochemicals, bioactive secondary metabolites with therapeutic and prophylactic properties. However, the effectiveness of these compounds is strongly influenced by the plant matrix and extraction conditions, including solvent type and method. Tree species such as *Moringa oleifera*, *Madhuca indica*, and *Ficus nemoloris* are rich sources of nutrients and bioactive compounds that improve rumen function, nutrient utilization, and milk production in cattle. Given their availability and importance in ruminant nutrition, systematic evaluation of their phytochemical profiles is essential. **Objective:** This study investigated the effectiveness of different solvents in extracting bioactive compounds from these plant species and assessed their qualitative and quantitative phytochemical composition. **Material and Methods:** Leaves of *Moringa oleifera* and *Ficus nemoloris* and flowers of *Madhuca indica* were collected, taxonomically authenticated, and processed under laboratory conditions. Plant materials were washed, shade-dried, powdered, and extracted using aqueous, ethanolic (70% ethanol), organic (TBME:hexane, 1:1), and acetonitrile solvents via maceration or Soxhlet extraction. Extracts were filtered, concentrated, dried, and stored at −20 °C, and extraction yield was calculated on a dry weight basis. Qualitative phytochemical screening was performed using standard colorimetric assays, while total flavonoid, phenolic, and tannin contents were quantified using established methods. Experiments were conducted in triplicate, and data were analyzed by one-way ANOVA ($P \leq 0.05$). **Results:** Qualitative phytochemical screening revealed marked variation in the distribution of bioactive constituents among the three plant species and solvent systems, highlighting the critical role of solvent polarity in extraction efficiency. Flavonoids were consistently present in aqueous and ethanolic extracts but absent in TBME:hexane extracts, confirming their polar nature. Terpenoids showed greater affinity for non-polar solvents, while cardiac glycosides were selectively extracted using aqueous and alcoholic solvents. Anthraquinones were not detected under the experimental conditions. Saponins were absent in TBME:hexane extracts but present in polar and semi-polar solvent systems. Alkaloids, phenols, tannins, and steroids exhibited clear solvent-dependent extractability, with ethanolic extracts generally showing stronger qualitative responses, indicating superior recovery. Quantitative analysis corroborated these findings, demonstrating significant differences in total flavonoid, phenolic, and tannin contents among solvents and plant species. Ethanolic extracts yielded the highest total flavonoid and phenolic contents, particularly in *Moringa oleifera* (101.93 mg QE/g and 97.98 mg GAE/g, respectively), while TBME:hexane extracts showed the lowest flavonoid levels. Total tannin content varied with plant matrix and solvent; *Madhuca indica* recorded the highest tannins in aqueous extracts (64.60 mg TAE/g), whereas *Ficus nemoloris* showed maximum tannins in alcoholic extracts (39.06 mg TAE/g). Although TBME:hexane was least effective for flavonoids, it selectively yielded high tannin levels, especially in *Moringa oleifera* (76.50 mg TAE/g). **Conclusion:** Phytochemical extraction was strongly influenced by solvent polarity and plant matrix. Polar solvents, especially ethanol, yielded superior recovery of flavonoids and phenolics, notably in *Moringa oleifera*, while non-polar TBME:hexane selectively enhanced tannin extraction, demonstrating solvent-specific selectivity.

Keywords: *Moringa oleifera*, *Madhuca indica*, *Ficus nemoloris*, Solvent extraction, Phytochemical profiling, Extraction efficiency.

INTRODUCTION

The utilization of traditional herbs in veterinary medicine is prevalent due to their cost-effectiveness, safety, historical validation, and dependence on locally sourced materials. For generations, plants have functioned as traditional treatments for several diseases, and their varied natural ingredients have made substantial contributions to the design, research, and development of contemporary pharmaceuticals [1].

This technique has recently garnered fresh interest for its potential to improve cattle health, especially in the context of basic animal healthcare [2]. Plant-derived products have demonstrated a wide range of pharmacological actions, encompassing therapeutic (disease-curing), prophylactic (disease-preventing), and production-enhancing properties, many of which are ascribed to their bioactive secondary metabolites.

Bioactive substances obtained from plants that are produced as secondary metabolites are known as phytochemicals. Phytochemicals are becoming more and more popular in livestock management as a result of recent research showing their positive benefits on the production and health of dairy animals. They are used as production enhancers as well as preventative agents. However, their purity and structural stability play a major role in determining their capability to promote health. The plant matrix in which the phytochemical is embedded, the extraction technique and solvent, and the extraction parameters (temperature and duration) all have an impact on these [3]. Because different phytochemicals have varying solvent affinities and heat tolerances, the quality and possible uses of the recovered compounds are greatly influenced by the solvent selection. Functional qualities may be lost as a result of using incompatible solvents or being exposed to unsuitable temperatures. Additionally, the matrix in which phytochemicals are contained has a significant impact on extraction efficiency. The yield and effectiveness of phytochemical recovery are significantly influenced by characteristics such particle size, pre-treatment, matrix type, structure, and solid–liquid ratio [4]. Choosing the right extraction techniques is crucial for ensuring that phytochemicals are extracted in a way that maintains their biological characteristics and natural structure in order to produce high-quality products.

The need for alternative nutrient-rich feed sources is highlighted by the fact that livestock diets in many developing nations are mostly composed of nutrient-deficient agricultural leftovers and grasses. In tropical and subtropical areas, trees and shrubs have long been essential components of animal feeding systems, offering dependable sources of nutrition. Among these, *Moringa oleifera, Madhuca indica*, and *Ficus nemoloris* are particularly important. Native to the Indian subcontinent, *M. oleifera* is rich in essential amino acids, crude protein, and bioactive substances including tannins and saponins that promote milk yield without causing negative side effects, improve feed utilization, and lower intestinal methane emissions [5]. Mahua, or *M. indica*, is an excellent food supplement with added ethnomedicinal and economic significance due to its nutritionally rich blooms that include sugars, dietary fiber, proteins, minerals, and vitamins [6]. Although phosphorus deficit may limit its usefulness, the leaves of *F. nemoloris*, which is frequently browsed by ruminants, offers highly digestible nutrients and crude protein [7]. When added to feeding plans, these species help to increase rumen fermentation, improve nutrient delivery, and boost cow production in general. Given their availability and utility in cattle feeding, it is logical to investigate the phytochemicals present in these plants that underpin such health-promoting effects.

Phytochemical screening of plant extracts using organic or aqueous solvents has revealed a wide spectrum of bioactive compounds. Notably, polyphenols represent the largest and most ubiquitous group, associated with diverse pharmacological effects. Against this background, the present study was envisaged evaluate the suitability of different solvents (water, ethanol, and TBME:Hexane, 1:1) for preparing bioactive-enriched extracts from *Moringa oleifera*, *Madhuca indica*, and *Ficus nemoloris*, and to assess them qualitatively for major phytochemical groups and quantitatively for total flavonoids, phenolic and tannin content.

MATERIAL AND METHODS

Plant Materials

Three plant species *Moringa oleifera*, *Madhuca indica*, and *Ficus nemoloris* were selected for this study. According to the Plants of the World Online (POWO) database, their updated botanical names are *Moringa oleifera* Lam. (Family: Moringaceae), *Ficus neriifolia* Sm. (Family: Moraceae), and *Madhuca longifolia* (J. Koenig exL.,) A. Chev. (Family: Sapotaceae). The collected plant specimens were submitted to the Department of Biotechnology, College of Agriculture SVPUA&T, Meerut where these were examined and identified based on morphological characteristics and standard taxonomic keys. Fresh leaves of *M. oleifera* were collected from the ICAR–Indian Institute of Farming System Research, Modipuram; leaves of *F. nemoloris* were obtained from the regional campus of ICAR–Indian Veterinary Research Institute, Mukteshwar; and flowers of *M. indica* were collected from Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Durg. All plant materials were transported to the laboratory under cold conditions and immediately processed.

Chemicals, Reagents, and Laboratory Supplies

Analytical grade chemicals and reagents were procured from Sigma-Aldrich, Thermo Fisher Scientific, HiMedia, and CDH Pvt. Ltd. Glassware was purchased from Borosil Pvt. Ltd., and plasticware from Tarsons Pvt. Ltd.

Primary Processing of Plant Materials

Upon arrival in the laboratory, plant materials were washed thoroughly under running tap water to remove surface contaminants, followed by rinsing with distilled water. Only healthy and intact leaves/flowers were retained. The samples were shade-dried for 10–12 days until a constant weight was achieved. Dried samples were ground into fine powder using a grinder (FOSS CT 293 Cyclotech), packed in airtight containers, and stored at room temperature

Preparation of Extracts

Powdered plant material (10 g) was subjected to three extraction procedures: (i) aqueous extraction by maceration in 100 mL distilled water at room temperature for 72 h, (ii) alcoholic extraction by soaking in 100 mL of 70% ethanol with continuous stirring (10 rpm) for 24 h at room temperature, and (iii) organic solvent extraction using Soxhlet apparatus with 100 mL of tert-butyl methyl ether (TBME) and hexane (1:1, v/v) at 65 °C for 8–10 h and iv) with 70% acetonitrile at 82 °C for 6 h in Soxhlet extractor [8]. In each case, the extracts were filtered through Whatman No. 41 filter paper. For aqueous and alcoholic extracts, the filtrates were concentrated by rotary evaporation (Labtech, 100 rpm, 35 °C) and subsequently freeze-dried using a lyophilizer (IlShin BioBase Co., Ltd., –55 °C, –50 psi, 48 h) to obtain crystalline extracts. Organic solvent extracts were concentrated by evaporating the solvent in a hot air oven (Mac Scientific, Ambala) at 50 °C. Extraction yields were calculated as the percentage of dried extract relative to the initial dry weight of plant material (dry basis). The dried extracts were stored in Eppendorf tubes at -12°Cuntil further analysis. At the time of analysis, a 1:10 (w/v) stock solution of each extract was prepared using the corresponding solvent (aqueous, alcoholic, organic or acetonitrile) employed during extraction, and this was subsequently used for qualitative and quantitative phytochemical assays.

Phytochemical Screening of the different plants extracts for qualitative traits

Preliminary phytochemical screening of plant extracts was performed using standard qualitative assays for major bioactive groups including flavonoids, terpenoids, cardiac glycosides, anthraquinones, saponins, quinones, alkaloids, phenols, tannins and steroids as described by earlier studies [9-13]. Either change in colour or precipitate formation was recorded as positive for presence of the respective phytochemical constituents.

Evaluation of the different plants extracts for quantitative traits

Determination of Total Flavonoid Content

A modified colorimetric approach, according to [14], employing aluminum chloride was used to estimate the total flavonoid concentration in extracts.

Determination of Total Phenolic Content

The total phenolic content (TPC) of plant extracts was estimated using the Folin-Ciocalteu (F-C) colorimetric assay, following the method of [15].

Determination of Total Tannin Content

The Folin-Ciocalteu reagent was used to measure total tannins in accordance with the [16] technique. The final results were expressed as milligrams of tannic acid equivalents per gram of dried plant material (mg TAE/g, dry basis).

Experimental Design and Statistical analysis

Each experiment was performed in triplicate. Qualitative parameters were assessed subjectively through colorimetric assays and recorded as positive (+) or negative (-) for the presence of specific bioactive groups. For quantitative evaluation, samples were analyzed for total phenolic content, with measurements taken in duplicate and averaged across three independent replicates. Data were expressed as mean ± standard error (SE). Statistical analyses and graph plotting were carried out using Microsoft Excel, and differences between means were tested by one-way ANOVA, considering $P \leq 0.05$ as the threshold for statistical significance.

RESULTS

Phytochemical Screening of the different plants extracts for qualitative traits

Qualitative assays for the detection of major bioactive groups, including flavonoids, terpenoids, cardiac glycosides, anthraquinones, saponins, quinones, alkaloids, phenols, tannins, and steroids in plant extracts using different solvent systems are presented in Table 1 and Figure 1. Flavonoids were qualitatively absent in the extracts of all three plants when prepared using the TBME+Hexane (1:1) mixture. In contrast, other solvent systems revealed variable results, with both aqueous and alcoholic extracts showing a strong presence (++) of flavonoids, while acetonitrile extracts exhibited a weaker colorimetric sign (+). Terpenoids consistently exhibited a strong presence (++) in the extracts of all three plants when TBME+Hexane (1:1) was used as the solvent. In addition, they were also non detected (-) in the extracts of *Moringa oleifera* and *Ficus nemoloris* plants prepared with acetonitrile. However, variation was observed with aqueous and alcoholic solvents: in these cases, terpenoids were uniformly present only in *Madhuca indica,* whereas in *Moringa oleifera* they were absent from alcoholic extracts, and in *Ficus nemoloris* they were absent from aqueous extracts.

Cardiac glycosides were qualitatively detected only in the aqueous and alcoholic extracts of all three plants, whereas they were absent in extracts prepared with TBME+Hexane (1:1) and acetonitrile. Anthraquinones were not detectable in any of the plant extracts, regardless of the plant parts or solvents used. Saponin were not detected in extracts of any plant prepared using TBME+Hexane (1:1) as solvent. In rest all the extracts it was detected. Quinones were non-detectable in almost all the extracts except in organic extracts TBME+Hexane (1:1) of *Moringa oliefera*. Alkaloids were detected in all extracts of the different plant parts, except in those prepared using the organic solvent mixture TBME+Hexane (1:1). Even the alcoholic extracts of *Moringa oleifera* showed a strong presence of alkaloids; otherwise, alkaloids were uniformly detectable in extracts prepared with aqueous, alcoholic, and acetonitrile solutions. Phenols were detectable in all plant extracts, with alcoholic extracts of all plant parts showing a strong presence of phenols (++). Tannins were detected in aqueous, alcoholic, and organic solvent extracts of all three plants. However, in acetonitrile-based extracts, tannins were present in all three species. TBME+Hexane (1:1) also showed a stronger presence of tannins. Steroids were present in all plant extracts except the aqueous extracts. Organic extracts of *Madhuca indica* and *Ficus nemoloris* exhibited a strong presence of steroids (++).

Evaluation of the different plants extracts for quantitative traits

The findings on total flavonoid content (TFC), total phenolic content (TPC), and total tannin content (TTC) across three plant species and four different solvent systems are summarized in Table 2. Among the various solvents used for phytochemical extraction, the ethanolic extracts exhibited the highest TFC, whereas the lowest values were recorded in extracts prepared using the organic solvent blend of TBME+Hexane (1:1). Among the tested plant species, *Moringa oleifera* extracts showed the highest flavonoid content, while *Madhuca indica* exhibited the lowest. Flavonoid concentrations in extracts prepared with 70% acetonitrile were intermediate, whereas the TBME+Hexane (1:1) extracts consistently exhibited the lowest levels. Across all three species, the highest TPC was observed in extracts prepared with 70% ethanol. The total tannin content (TTC) varied considerably among both solvents and plant species. Among aqueous extracts, *Madhuca indica* showed the highest tannin content, while in alcoholic extracts, *Ficus nemoloris* had significantly elevated levels. TBME+Hexane (1:1) extracts showed the lowest tannin concentration in *Ficus nemoloris,* whereas *Moringa oleifera* and *Madhuca indica* exhibited comparable values. With 70% acetonitrile, tannin concentrations were relatively uniform across species, with *Moringa oleifera* showing the highest, followed by *Madhuca indica* and *Ficus nemoloris.*

DISCUSSION

The presence of flavonoids in aqueous and alcoholic extracts confirms the presence of polar flavonoids in these plants, indicating their higher affinity toward aqueous and alcoholic solvents [17]. The chemical structure of flavonoids is known to influence their solubility, as it determines their ability to form hydrogen bonds with solvents. Consequently, water and ethanol have been reported as the most commonly used solvents for flavonoid extraction through conventional extraction techniques [18]. Since their chemical structures only contain carbon and hydrogen atoms, terpenes and terpenoids are typically described as low-polarity compounds. In contrast, terpenoids have extra polar functional groups like hydroxyl, carboxyl, or even amino groups. Depending on the solvent system used, water tends to reduce their extraction efficiency because of their mostly organic nature [19].

The quantity and kind of sugar moieties bonded to the aglycone greatly influence the polarity of glycosides, which are typically thought of as rather polar molecules. When it comes to cardiac glycosides, the aglycone usually has a large steroidal shape. The polarity and solvent strength needed to effectively extract polar cardiac glycosides are absent from the 1:1 mixture of TBME and hexane. Similarly, even though acetonitrile is a polar solvent, it might not have been enough to extract measurable amounts of cardiac glycosides from the plant material. According to earlier studies, extract yields are higher in polar solvents, like water and 70% ethanol, than in nonpolar solvents [20]. The extraction of anthraquinones has been reported to be both time- and solvent-intensive, owing to their varying intrinsic polarities and solubility

Table 1: Qualitative analysis of extracts from *Moringa oleifera*, *Madhuca indica*, and *Ficus nemoloris* using different solvent system for the presence of bioactive phytochemicals

Bioactive Phytochemicals	*Moringa oleifera*				*Madhuca indica*				*Ficus nemoloris*			
	Aqueous	Alcoholic	TBME+Hexane (1:1)	Acetonitrile	Aqueous	Alcoholic	TBME+Hexane (1:1)	Acetonitrile	Aqueous	Alcoholic	TBME+Hexane (1:1)	Acetonitrile
Flavonoids	++	++	_	+	+	+	-	+	+	++	-	+
Terpenoids	+	_	++	-	+	+	++	+	-	+	++	-
Cardiac glycosides	+	++	_	-	+	+	-	-	+	+	-	-
Anthraquinones	_	_	_	-	-	-	-	-	-	-	-	-
Saponin	+	+	_	+	+	+	-	+	+	+	-	+
Quinones	_	_	+	+	-	-	-	+	-	-	-	+
Alkaloids	+	++	_	+	+	+	-	+	+	+	-	+
Phenols	+	++	+	++	+	++	+	+	+	++	+	+
Tannins	+	+	+	+	++	+	+	+	+	+	+	+
Steroids	_	+	+	+	-	+	++	+	-	+	++	+

Results are based on subjective evaluation of the perceived colorimetric assay. Symbols represent: (++) strong positive, (+) positive, and (−) negative

Table 2: Quantitative Analysis of flavonoids phenolics and tannins of *Moringa oleifera, Madhuca* indica, and *Ficus nemoloris* using different solvent system

Quantitative traits	Extract type	*Moringa oleifera*	*Madhuca indica*	*Ficus nemoloris*
TFC (mg QE/g)	Aqueous	64.60 ± 0.34^{bA}	$16.20\pm0.11^{b\,B}$	$25.03\pm0.12^{b\,B}$
	Alcoholic	101.93 ± 0.35^{aA}	$25.13\pm 0.46^{a\,C}$	57.47 ± 0.28^{aB}
	TBME+Hexane(1:1)	$32.76\pm0.11^{c\,A}$	$11.93\pm0.37^{c\,C}$	$11.66\pm0.17^{c\,C}$
	Acetonitrile	$37.54\pm0.50^{c\,A}$	$16.20\pm0.11^{b\,C}$	29.27 ± 0.18^{bB}
TPC (mg GAE/g)	Aqueous	47.50 ± 0.82^{bA}	49.63 ± 0.20^{bA}	28.60 ± 0.61^{bB}
	Alcoholic	97.98 ± 0.54^{aA}	67.05 ± 0.25^{aB}	46.99 ± 0.32^{aC}
	TBME+Hexane (1:1)	35.03 ± 0.32^{cB}	67.02 ± 0.18^{aA}	13.53 ± 0.49^{cB}
	Acetonitrile	50.27 ± 0.26^{bA}	52.02 ± 0.87^{bA}	30.28 ± 0.87^{bB}
TTC (mg TAE /g)	Aqueous	15.13 ± 0.20^{cC}	64.60 ± 0.34^{aA}	$29.48\pm0.14^{b\,B}$
	Alcoholic	$26.24\pm0.14^{c\,B}$	18.53 ± 0.06^{cC}	$39.06\pm0.12^{a\,A}$
	TBME+Hexane(1:1)	76.50 ± 0.57^{aA}	69.26 ± 0.06^{aA}	34.21 ± 0.04^{aB}
	Acetonitrile	$39.01\pm0.13^{b\,A}$	31.66 ± 0.17^{bB}	$30.36\pm0.03^{b\,B}$

Values are presented as Mean ±SE (n = 3). Means with different lowercase superscripts across extract types within each plant species (column-wise) and uppercase superscripts across plant species within each extract type for particular trait differ significantly ($p< 0.05$).

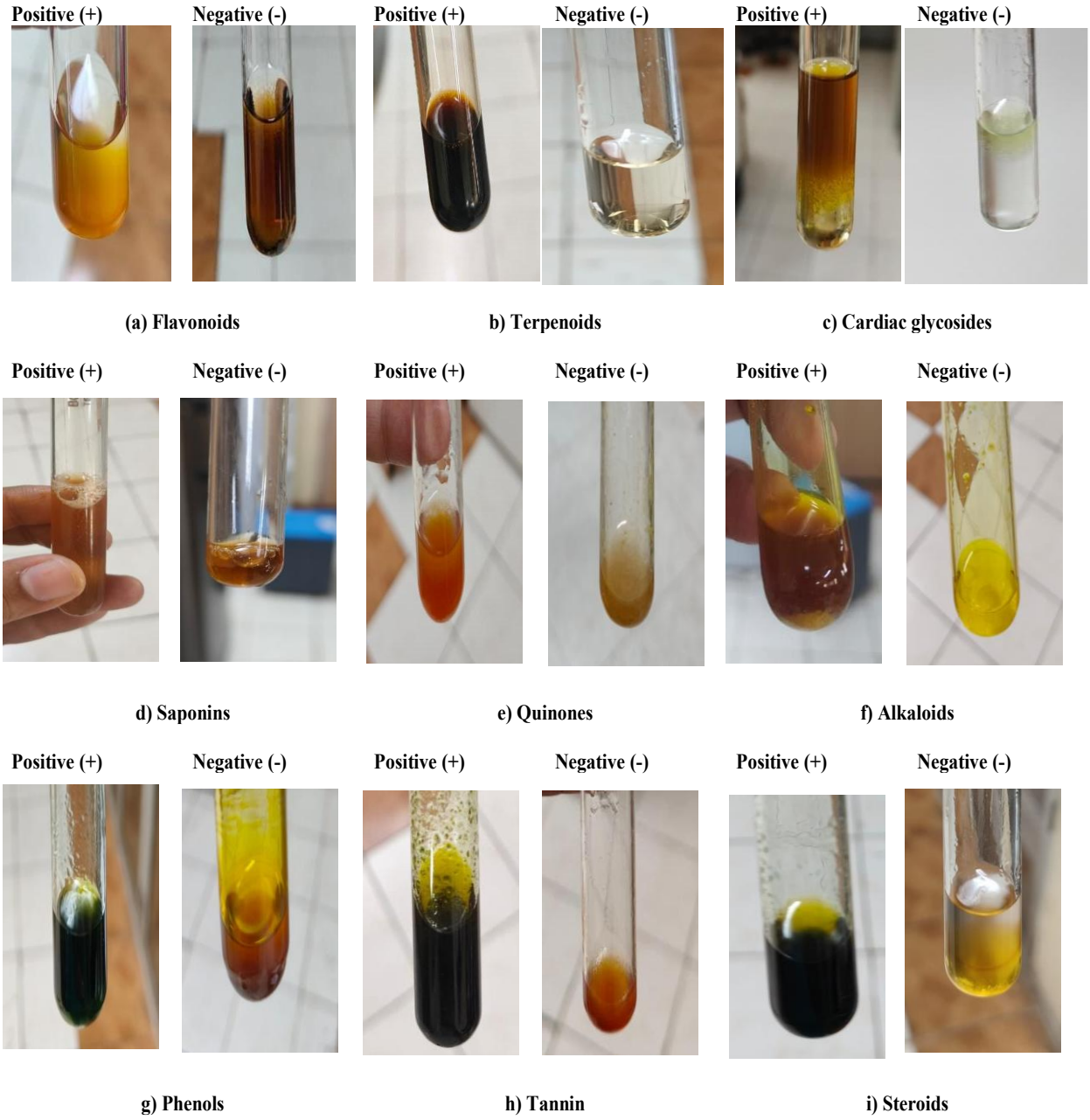

Figure 1: Qualitative analysis of extracts for the presence of bioactive phytochemicals by colorimetric assay

characteristics. Factors such as the nature and type of solvent, the sample-to-solvent ratio (feed: solvent), extraction temperature, and duration are known to influence their qualitative presence in plant extracts [21]. The present findings may be attributed to the fact that anthraquinones are practically insoluble in water, and therefore mixtures of aqueous and organic solvents may only occasionally facilitate more effective extraction, which was not evident under the conditions of the present experiment. Saponins are amphiphilic compounds, containing both hydrophilic (water-soluble) and lipophilic (fat-soluble) moieties. The dissolution of their hydrophilic sugar components in water reduces surface tension, thereby promoting the formation of stable foam. In the present study, all three solvent extraction systems included water as a key component except for the fourth system, tert-butyl methyl ether (TBME) and hexane (1:1) which facilitated the extraction of the water-soluble fraction of saponins and resulted in a positive outcome in the froth test. The absence of detectable quinones in the extracts may be attributed to the fact that many quinones are bound to macromolecules through ester or other covalent linkages. Effective extraction of such bound forms typically requires alkaline hydrolysis to cleave these bonds. In the present study, no such pre-treatment was employed, which likely accounts for the non-detection of quinones in the colorimetric assay. Alkaloids are nitrogen-containing organic compounds, mostly with complex cyclic structures, in which the nitrogen atom is incorporated within the ring. They generally exhibit alkaline properties and form salts upon combining with acids. Owing to their alkaline nature, alkaloids are usually present in plants in salt form, making them extractable with water or acidic water. Alcoholic extraction further facilitates the dissolution of both free and salt alkaloids [22]. Free or non-ionized alkaloids are typically lipophilic (fat-soluble) and dissolve well in polar organic solvents such as acetonitrile, whereas in organic solvents of lower polarity they remain non-extractable due to poor solubility. Among the various methods, extraction of total alkaloids with alcohol is highly recommended for its maximum efficiency and economic viability, whereas extraction with organic solvents generally requires additional considerations.

For the extraction of total polyphenols from plants, aqueous solutions of organic solvents such as ethanol have been reported to be more effective than water alone [23]. This can be attributed to the fact that phenolic compounds possess a broad range of solubilities in mixed solvent systems and are often more soluble in solvents of lower polarity than water. Therefore, the differences observed in extraction efficiencies among the solvent systems can primarily be explained by their varying polarities and affinities for the phenolic compounds present. The efficiency of different solvents for phenols extraction was cited by [24] as (ordered from highest to lowest): methanol > water > ethanol > acetone > chloroform > hexane. Tannin compounds are readily extractable with water or alcohol, as both hydrolysable and condensed tannins are highly soluble in these solvents but generally insoluble in organic solvents. Acetonitrile, being an aprotic solvent, does not readily donate hydrogen bonds. Since tannins are large, complex molecules with multiple hydroxyl groups, solvents capable of forming hydrogen bonds (such as water or alcohol) are more effective at dissolving them through interactions with these functional groups [25]. The absence of steroids in aqueous extracts is expected, ascribed to their low polarity [26]. In alcoholic solvents, steroids were detectable due to initial dehydration and denaturation of proteins associated with steroids, which renders the proteins insoluble and facilitates the dissolution of many steroids. Organic solvents such as TBME+Hexane, being lipophilic, readily dissolved steroids, while acetonitrile, with its ability to extract both polar and non-polar substances, was also effective in extracting them. This observation aligns with the findings of [27], who reported elevated flavonoid concentrations in ethanolic extracts, supporting the effectiveness of ethanol as a solvent for bioactive compound extraction. However, aqueous extracts of *Moringa oleifera* have greater concentrations of flavonoids and phenolics than ethanolic extracts was reported by [28] discovered that. Variations in plant origin, drying conditions, or extraction procedures could be the cause of these disparities. These results are supported by the fact that the highest phytochemical concentrations were obtained from *Moringa oleifera* ethanolic extracts in this investigation.

The extraction efficiency of phenolic compounds was greatly impacted by the polarity of the solvents used; polar solvents such as aqueous ethanol and acetonitrile performed better than non-polar solvents like TBME+Hexane. These findings were corroborate with [29] by emphasizing that hexane's low polarity makes it useless for extracting phenolic compounds. TBME (also referred to as MTBE), while more efficient than hexane, tends to extract lipophilic substances like lipids, sterols, fatty acids, and esters. Hexane, being a purely non-polar solvent, primarily extracts neutral lipids and apolar compounds [30]. The ethanolic extracts of plant leaves often contain higher phenolic contents than their aqueous counterparts as noted by [31]. However, aqueous extracts tend to co-extract highly polar non-bioactive compounds such as sugars and proteins, which may inflate the extract yield but reduce the concentration of targeted phytochemicals [32]. While 70% ethanol is commonly used due to its balance of polarity and efficiency, A 50% acetone-water mixture extracted greater quantities of phenolics and flavonoids than 70% ethanol, suggesting that solvent choice should be plant-specific as reported by [31]. Enhanced extraction with increased water content in solvent mixtures has been observed in other studies as well.

Tannin content in plants typically comprises two major classes: hydrolyzable tannins, simple phenolics with ester linkages, and condensed tannins, flavonoid polymers with varying degrees of condensation [32]. In this study, TTC was influenced by both solvent characteristics and plant species. Remarkably, when compared to other solvents, the TBME+Hexane (1:1) mixture showed better tannin extraction. This could be because of its mixed polar and non-polar characteristics, which improve extraction efficiency by increasing solvent penetration, decreasing viscosity, and speeding up the diffusion of bioactive chemicals [33]. The utilization of *Madhuca indica*'s flowers, which are high in fermentable sugars, may be the cause of the increased tannin content in aqueous extracts of the plant. By changing the solubility or mobility of the tannin molecules, these sugars may make it easier to extract tannins in aqueous environments.

CONCLUSION

This study confirms that phytochemical composition and extraction efficiency in *Moringa oleifera*, *Madhuca indica*, and *Ficus nemoloris* are strongly influenced by the solvent system used. Qualitative screening showed the presence of several bioactive compounds, including flavonoids, phenols, tannins, alkaloids, terpenoids, saponins, and steroids, while anthraquinones and quinones were largely undetected under the applied conditions. Quantitative analysis revealed that 70% ethanol was the most effective solvent for extracting total flavonoids, phenolics, and tannins across all species, with Moringa oleifera exhibiting the highest concentrations of these compounds. Non-polar solvents such as TBME+Hexane (1:1) were least effective for polar phytochemicals but efficiently extracted lipophilic constituents. Overall, the results highlight the importance of solvent polarity in phytochemical extraction and support the use of aqueous ethanolic solvents for maximizing bioactive yield in plant-based studies.

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Author contributions

Ayushi Sachan: Writing Original draft, Methodology, Investigation and Formal analysis. Shweta Anand: Conceptualization, Supervision; Rachna Varma: Review & editing; Ashok Kumar Mohanty: Funding acquisition, Project administration; Rajiv Ranjan Kumar: Data curation, Writing - review & editing and Resources.

Conflict of interest

The authors declared no conflict of interest.

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ORCID ID

Rajiv Ranjan Kumar: https://orcid.org/0000-0001-5495-3825

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