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Comparative Profiling of Phytochemical and Nutritional Constituents in Neem, Gooseberry and Tulsi Leaves

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A B S T R A C T

This study presents a comparative evaluation of the phytochemical and nutritional constituents of *Azadirachta indica* (Neem), *Phyllanthus emblica* (Gooseberry), and *Ocimum sanctum* (Tulsi) leaves using aqueous and ethanolic extracts. Physical parameters showed notable variation, with Tulsi exhibiting the highest ash content, while Gooseberry demonstrated maximum moisture content. Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids across all samples, with ethanolic extracts yielding higher concentrations of most secondary metabolites. Quantitative profiling confirmed this trend, with Tulsi showing elevated phenolic and flavonoid contents, and Gooseberry exhibiting higher alkaloid and tannin levels in ethanolic extracts. Aqueous extracts were richer in Vitamin C, iron, and magnesium, reflecting the solubility of these constituents in polar solvents. Gooseberry contained the highest Vitamin C, whereas Tulsi had the highest Vitamin E in ethanolic extracts. These findings validate the biochemical diversity of the selected medicinal plants and highlight their relevance as sources of bioactive and nutritional compounds. Although analysed separately, the distinct phytochemical and nutritional profiles suggest potential synergic benefits if formulated together. Overall, this comparative assessment supports the scientific basis for their traditional use and indicates their promise for future nutraceutical and herbal applications.

Introduction

Medicinal plants have been an integral part of traditional healthcare systems, particularly Ayurveda, which utilizes natural botanicals for their therapeutic properties. Neem (*Azadirachta indica*), Gooseberry (*Phyllanthus emblica*), and Tulsi (*Ocimum sanctum*) are renowned for their extensive use in Ayurveda due to their diverse bioactive compounds and health-promoting effects. Neem is valued for its insecticidal and antimicrobial properties (Subapriya & Nagini, 2005; Islas-Flores *et al.,* 2022), Tulsi for its strong volatile compounds and adaptogenic benefits (Pattanayak *et al.,* 2010), and Gooseberry for its rich antioxidant content and role in stabilizing and enhancing formulations (Baliga & Dsouza, 2011; Varma *et al.,* 2022). The synergistic combination of these plants presents promising potential for developing natural, eco-friendly products, including mosquito repellents, that harness their collective medicinal qualities safely and effectively (Prakash & Gupta, 2005). Rigorous scientific evaluation of their phytochemical constituents, physical characteristics, and bioactive compound quantification is essential for substantiating traditional claims and guiding future formulations.

This study aims to evaluate the bioactive potential of Neem (*Azadirachta indica*), Gooseberry (*Phyllanthus emblica*), and Tulsi (*Ocimum sanctum*). Specifically, it seeks to assess their physical properties, including ash and moisture contents, and to identify and quantify key phytochemical and biochemical constituents.

The study further aims to explore the mineral composition of these plants. Such comprehensive analysis provides a scientific basis for their traditional uses and supports the advancement of natural formulations for health and pest-control applications.

Materials and Methods

Sample Collection

Fresh leaves of Neem, Gooseberry, and Tulsi were gathered from agricultural fields located in Erode District, Tamil Nadu, India. The collected specimens were authenticated and washed thoroughly with distilled water to eliminate debris and surface contaminants.

Preparation of Plant Powder

The leaves were dried under shade for several days until a constant weight was obtained.

The completely dried samples were then pulverized using a mechanical grinder to obtain a fine leaf powder, which was stored in airtight containers until further use.

Preparation of Extracts

Extraction was performed using a Soxhlet apparatus. Approximately 50 g of the powdered plant material was subjected to continuous hot percolation with 250 ml of ethanol or distilled water at 60–80°C.

The resulting extracts were filtered, concentrated by evaporation, and kept in desiccators until they were used for subsequent analysis.

Physical Parameters

Ash Content

Approximately 1 g of sample was incinerated in a pre-weighed silica crucible until constant weight ash was obtained, then percentage ash was calculated.

Moisture Content

About 1 g of the sample was placed in a pre-weighed, preheated Petri dish and dried at 60–80°C for 2 hours. The moisture content was calculated based on the loss in weight after drying.

Phytochemical Screening

Aqueous and ethanolic extracts were qualitatively tested for key phytochemicals including alkaloids, flavonoids, tannins, glycosides, phenols, carbohydrates, proteins, saponins, terpenoids, amino acids, anthraquinones, lipids, reducing sugars, and vitamins using standard chemical reagents and color change observations.

Quantitative Estimations

Alkaloid Estimation

Fifty grams of dried powdered leaf samples were extracted with ethanol and water. Alkaloids were quantified using Dragendorff's reagent by forming a yellow complex measured at 435 nm.

Concentrations were calculated from standard curves and expressed as mg/g dry weight.

Flavonoid Estimation

Flavonoids were estimated by aluminum chloride colorimetric assay. Extracts (from 50 g starting material) were reacted to form a complex measured at 510 nm. Results were expressed as mg quercetin equivalents per gram.

Phenol Estimation

Phenolic content was determined using Folin-Ciocalteu reagent on extracts from 50 g powder. Blue-colored complexes were measured at 765 nm and expressed as mg gallic acid equivalents per gram.

Tannin Estimation

Tannins were quantified via reaction with Folin's reagent on extracts from 50 g samples. Absorbance was read at 650 nm and expressed as mg tannic acid equivalents per gram.

Vitamin C Estimation

Vitamin C was estimated by DNPH assay with extracts from 50 g material. Orange-red colored complexes absorbance was measured at 540 nm and expressed as mg/g.

Vitamin E Estimation

Vitamin E was quantified using oxidation and dipyridyl reaction on extracts from 50 g samples. Pink complex absorbance at 520 nm was used for calculation and expressed as mg/g.

Iron Estimation

Iron content was determined by Wong's method using potassium thiocyanate, measuring red complexes at 540 nm in extracts from 50 g powder. Results were expressed as mg/g.

Magnesium Estimation

Magnesium was estimated by titan yellow reaction forming red complexes, absorbance taken at 540 nm from extracts prepared from 50 g samples. Expressed as mg/g dry weight.

Results and Discussions

Ash and Moisture Content

The ash and moisture contents of *Azadirachta indica* (Neem), *Phyllanthus emblica* (Gooseberry), and *Ocimum sanctum* (Tulsi) leaves showed distinct variations (Table 1). Tulsi leaves exhibited the highest ash content (42%), indicating a greater proportion of inorganic and mineral constituents compared to the other samples. Neem leaves showed a moderate ash content of 36%, while Gooseberry leaves recorded the lowest value (33%).

Moisture content also differed among the three plant samples. Gooseberry leaves contained the highest moisture content (73%), suggesting higher water retention or juiciness of the leaf tissue. Neem leaves had a moisture content of 70%, whereas Tulsi leaves exhibited the lowest moisture level (63%). These findings highlight plant-specific differences in compositional characteristics that may influence their stability, processing behaviour, and suitability for formulation development.

Phytochemical Analysis

Qualitative phytochemical screening of the aqueous and ethanolic extracts of *Azadirachta indica* (Neem), *Phyllanthus emblica* (Gooseberry), and *Ocimum sanctum* (Tulsi) revealed the presence of diverse groups of bioactive compounds (Table 2). In general, ethanolic extracts exhibited a stronger and more consistent presence of secondary metabolites compared to aqueous extracts, demonstrating the higher extractability of phytochemicals in ethanol.

Alkaloids were detected in all three plants, with Tulsi showing the strongest response in the ethanolic extract, followed by Gooseberry and Neem. Flavonoids and tannins were more prominent in Gooseberry ethanolic extracts, whereas Tulsi's ethanolic extract displayed the highest intensity of phenolics. In Neem, moderate levels of saponins, terpenoids, flavonoids, and phenolics were observed in both extracts.

Saponins showed strong positivity across all ethanolic extracts, while the aqueous extracts exhibited moderate reactions.

Terpenoids were consistently present in both extract types for all plants, with comparatively stronger reactions in the ethanolic extracts of Neem and Tulsi. Proteins and amino acids were detected primarily in the aqueous extracts, especially in Neem and Gooseberry, indicating their higher water solubility.

Glycosides and cardiac glycosides appeared in low to moderate levels, mostly in ethanolic extracts. Anthraquinones were highly expressed in Neem in both extract types, while only mild reactions were observed in Gooseberry and Tulsi. Vitamin C was strongly present in Gooseberry extracts, particularly in the ethanolic extract, consistent with its known ascorbic acid richness. Reducing sugars were abundant in Gooseberry and Tulsi aqueous extracts, as indicated by the strong Benedict's test reaction.

Collectively, the phytochemical distribution highlights plant-specific variations with ethanol proving to be a more efficient solvent for extracting secondary metabolites such as alkaloids, flavonoids, tannins, phenolics, saponins, and terpenoids.

Quantitative estimation of Secondary Metabolites

The quantitative analysis of secondary metabolites (Table 3) revealed substantial variations in alkaloid, flavonoid, phenolic, and tannin contents among the aqueous and ethanolic extracts of *Azadirachta indica* (Neem), *Phyllanthus emblica* (Gooseberry), and *Ocimum sanctum* (Tulsi). Across all three plants, ethanolic extracts consistently exhibited higher concentrations of secondary metabolites than aqueous extracts, confirming ethanol as a more efficient solvent for extracting bioactive compounds.

Ethanolic extract of Tulsi recorded the highest flavonoid content (8.6 mg/g) and phenolic content (9.3 mg/g), indicating strong antioxidant potential. Gooseberry ethanolic extract showed the highest alkaloid content (8.1 mg/g) and tannin content (6.96 mg/g), reflecting its richness in nitrogenous and polyphenolic compounds. Neem exhibited moderate levels across all metabolites, with values ranging from 6.9 mg/g phenols to 7.8 mg/g flavonoids in the ethanolic extract.

Aqueous extracts showed comparatively lower metabolite levels, with alkaloids ranging from 5.13 mg/g in Gooseberry to 6.1 mg/g in Tulsi, and phenols from 3.3 mg/g in Neem to 4.1 mg/g in Tulsi. Tannin content was highest in Tulsi aqueous extract (5.8 mg/g), followed by Neem and Gooseberry. The results demonstrate notable plant-specific differences and clearly highlight that ethanol enhances the extraction efficiency of key secondary metabolites, particularly flavonoids, phenols, and alkaloids.

Vitamin Content

The levels of Vitamin C and Vitamin E (Table 4) varied significantly among the aqueous and ethanolic extracts of *Azadirachta indica* (Neem), *Phyllanthus emblica* (Gooseberry), and *Ocimum sanctum* (Tulsi). Vitamin C content was notably higher in aqueous extracts across all three plants, reflecting its water-soluble nature. Gooseberry aqueous extract recorded the highest Vitamin C concentration (7.43 mg/g), followed by Tulsi (6.5 mg/g) and Neem (6.3 mg/g). In contrast, ethanolic extracts showed comparatively lower Vitamin C levels, with values ranging from 1.5 mg/g in Tulsi to 3.7 mg/g in Gooseberry. Vitamin E, being fat-soluble, was predominantly higher in the ethanolic extracts of all samples. Tulsi ethanolic extract exhibited the highest Vitamin E concentration (9.6 mg/g), followed closely by Gooseberry (8.7 mg/g) and Neem (8.1 mg/g). Aqueous extracts showed markedly lower Vitamin E levels, ranging between 3.03 and 3.6 mg/g.

These results highlight the distinct solubility-driven extraction patterns of the two vitamins, with Vitamin C favoring aqueous extraction and Vitamin E showing enhanced recovery in ethanol-based extracts.

Mineral Content

The mineral analysis (Table 5) revealed variations in iron and magnesium levels among the aqueous and ethanolic extracts of *Azadirachta indica* (Neem), *Phyllanthus emblica* (Gooseberry), and *Ocimum sanctum* (Tulsi). In Gooseberry and Tulsi, iron content was higher in the aqueous extracts, with Gooseberry showing the highest iron level (3.3 mg/g), followed by Tulsi (3.0 mg/g). In Neem, however, iron content was slightly higher in the ethanolic extract (2.8 mg/g) compared to the aqueous extract (2.1 mg/g).

Magnesium content was generally higher in the aqueous extracts of Neem (1.7 mg/g) and Gooseberry (2.1 mg/g). Tulsi showed a marginally higher magnesium level in its ethanolic extract (1.8 mg/g) compared to the aqueous extract (1.3 mg/g). Overall, Gooseberry aqueous extract exhibited the highest levels of both iron (3.3 mg/g) and magnesium (2.1 mg/g), indicating its superior mineral composition among the samples analysed.

These findings highlight plant-specific mineral distribution patterns and demonstrate that extraction efficiency varies between aqueous and ethanolic solvents depending on the mineral and plant type.

The findings of this study highlight distinct variations in the physicochemical, phytochemical, biochemical, vitamin, and mineral compositions of *Ocimum sanctum* (Tulsi), *Phyllanthus emblica* (Gooseberry), and *Azadirachta indica* (Neem), supporting their well-established therapeutic importance in traditional and modern medicine.

Table.1 Ash and Moisture content

Source	Ash Content (%)	Moisture Content (%)
Neem leaves	36 ± 1.8	70 ± 3.2
Gooseberry leaves	33 ± 1.9	73 ± 3.8
Tulsi leaves	42 ± 2.1	63 ± 2.9

Table.2 Phytochemical screening of Solvent Extracts

S. No.	Tests	Phytochemical results observed in					
		Neem leaves		Gooseberry leaves		Tulsi leaves	
		Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
1	**Carbohydrates** Molisch's Test	++	+	++	+	++	+
2	**Alkaloids** Wagner's Test,	+	++	+	++	+	++
	Mayer's Test	-	+	+	+	-	+
3	**Saponins** Foam test	+	++	+	++	+	++
4	**Tannins** Lead acetate test	++	+	++	+	++	+
5	**Flavonoids** Acid Test	+	++	+	++	+	++
	Shinoda Test	+	+	-	+	+	+
6	**Terpenoids** Acetic Anhydride Test	+	++	++	+	+	++
7	**Aminoacids** Ninhydrin Test	++	+	+	+	-	-
8	**Protein** Million's Test	++	+	++	+	++	+
9	**Glycosides** Libermann's Test	+	+	-	-	-	-
10	**Cardiac Glycosides**	+	++	+	+	++	+
11	**Phlobotannins**	-	+	-	+	-	+
12	**Total Phenol** Ferric Chloride Test	+	++	++	+	++	+
13	Anthraquinone	++	++	-	+	-	+
14	**Lipids** Halogenation Test	+	++	+	+	+	+
15	**Reducing Sugar** Benedict's Test	+	+	++	+	++	+
16	**Cycloglycosides**	-	+	-	+	+	+
17	**Vitamin** C DNPH Test	+	+	++	++	+	+

Table.3 Alkaloids, Flavanoids, Phenol and Tannin content in various extracts

Phytocomponents (mg/g)	Neem Leaves		Gooseberry Leaves		Tulsi Leaves	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
Alkaloids	5.3 ± 0.2	7.3 ± 0.26	5.1± 0.25	8.1± 0.52	6.1± 0.25	6.6 ± 0.26
Flavonoids	4.9 ± 0.30	7.8 ± 0.25	4.6± 0.70	6.6± 0.43	5.6± 0.30	8.6 ± 0.3
Phenol	3.3 ± 0.41	6.9 ± 0.70	3.7± 0.55	7.3± 0.95	4.1± 0.40	9.3 ± 0.15
Tannin	4.5± 0.30	4.2 ± 0.3	4.0± 0.26	6.9± 0.40	5.8± 0.55	5.6 ± 0.41

Table.4 Vitamin C and Vitamin E content in Sample extracts

Vitamins (mg/g)	Neem Leaves		Gooseberry Leaves		Tulsi Leaves	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
Vitamin C	6.3 ± 0.30	2.5 ± 0.30	7.43 ± 0.32	3.7 ± 0.32	6.5 ± 0.3	1.5 ± 0,02
Vitamin E	3.2 ± 0.72	8.1 ± 0.64	3.03 ± 0.60	8.7 ± 0.60	3.6 ± 0.64	9.6 ± 0.52

Table.5 Minerals content in Sample extracts

Minerals (mg/g)	Neem Leaves		Gooseberry Leaves		Tulsi Leaves	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
Iron	2.1 ± 0.65	2.8 ± 0.45	3.3 ± 0.45	2.9 ± 0.83	3.0 ± 0.30	2.6 ± 0.56
Magnesium	1.7 ± 0.33	1.5 ± 0.51	2.1 ± 0.54	1.7 ± 0.65	1.3 ± 0.83	1.8 ± 0.69

Plate.1 Phytochemical screening of Aqueous Extracts

Neem Leaves (*Azadirachta indica*) | Gooseberry leaves (*Phyllanthus emblica*) | Tulsi leaves (*Ocimum sanctum*)

Plate.2 Phytochemical screening of Ethanolic Extracts

Neem Leaves (*Azadirachta indica*) | Gooseberry leaves (*Phyllanthus emblica*) | Tulsi leaves (*Ocimum sanctum*)

The higher ash content observed in Tulsi leaves indicates a greater level of inorganic constituents, consistent with earlier reports suggesting Tulsi's rich mineral profile (Pattanayak *et al.,* 2010). Gooseberry exhibited the highest moisture content, which may enhance the extraction efficiency of hydrophilic compounds, in agreement with previous studies documenting its high water content and polysaccharide-rich nature (Khan, 2009, Baliga & Dsouza, 2011). The phytochemical results demonstrated a higher yield of secondary metabolites in ethanolic extracts, reaffirming ethanol's superior solvent capacity for alkaloids, flavonoids, phenolics, tannins, and terpenoids (Harborne, 1998, Trease & Evans, 2002; Sofowora, 1993).

Tulsi's elevated flavonoid and phenolic contents support its recognized antioxidant potency attributed to constituents such as rosmarinic acid and eugenol (Pattanayak *et al.,* 2010, Jamshidi & Cohen, 2017). Gooseberry's high alkaloid and tannin levels align with its documented antimicrobial and astringent properties (Khan, 2009, Ghosal *et al.,* 1996). Neem's broad but moderate presence of various phytochemicals reinforces its therapeutic versatility reported in earlier pharmacological studies (Biswas *et al.,* 2002, Kharwar *et al.,* 2020). Vitamin estimations reflected characteristic solubility patterns: Vitamin C, being water-soluble, was present in higher concentrations in aqueous extracts, particularly in Gooseberry, which is widely recognized as a potent natural source of ascorbic acid (Scartezzini *et al.,* 2000, Levine *et al.,* 1995). Vitamin E, a lipid-soluble vitamin, showed maximum levels in ethanolic extracts, confirming ethanol's effectiveness in extracting lipophilic components (Brigelius-Flohé & Traber, 1999).

Mineral analysis revealed that aqueous extracts generally contained higher levels of iron and magnesium, consistent with earlier findings that these essential minerals exhibit greater solubility in polar solvents (Gupta *et al.,* 2005, Kabata-Pendias, 2010; Marschner, 2012). Gooseberry's superior mineral profile further supports its nutritional importance. Although the analyses in this study were conducted on each plant extract separately, the distinct biochemical and phytochemical characteristics of Neem, Gooseberry, and Tulsi indicate the possible benefits of synergic effects if they are formulated together. Tulsi contributes high phenolic and flavonoid content, Gooseberry offers substantial Vitamin C, alkaloids, and minerals, while Neem provides a balanced range of bioactive compounds. When combined, these constituents may complement one another and potentially enhance overall antioxidant, antimicrobial, or nutraceutical properties compared to their individual effects. These findings reinforce their continued relevance in herbal formulations, dietary supplements, and traditional health practices. Further studies should explore purification, quantitative profiling of specific bioactive molecules, and validation of biological activities to support their wider nutritive and pharmacological applications.

This study comprehensively evaluated the physical, phytochemical, biochemical, vitamin, and mineral composition of Neem, Gooseberry, and Tulsi leaves. The findings showed that ethanolic extracts contained higher levels of key bioactive compounds such as alkaloids, flavonoids, phenols, and tannins, whereas aqueous extracts were richer in carbohydrates, proteins, vitamins, and minerals.

The variations in phytochemical composition among the plants highlight their diverse therapeutic potentials, with Tulsi displaying strong antioxidant characteristics and Gooseberry exhibiting notable antimicrobial properties. These results support the long-established traditional uses of these plants and emphasize their value as natural sources of nutritionally and pharmacologically important compounds. Further studies may explore formulation strategies, synergic interactions, and biological efficacy to enhance their application in herbal and nutraceutical preparations.

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Author Contributions

R. Rasu: Investigation, formal analysis, writing—original draft. S. Blessina: Validation, methodology, writing—reviewing. N. Sangeetha:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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