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Quantitative phytochemical screening of some commonly available herbs in Tamil Nadu

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Abstract
This study examines the phytochemical content of 16 medicinal herbs commonly used in Tamil Nadu, India, focusing on key bioactive compounds like alkaloids, phenols, flavonoids, saponins, terpenoids, and tannins. Aqueous and ethanol extracts were analyzed to determine compound concentrations, essential for therapeutic efficacy. Results showed significant variations in chemical composition based on plant species and solvent used. *Emblica officinalis* fruits had the highest alkaloid content (26.35 mg/g in aqueous extract), while *Andrographis paniculata* stems and leaves had the highest saponin (20.36%) and terpenoid (4.75%) levels. *Syzygium aromaticum* buds exhibited the highest flavonoid content (4.63 mg/g), and *Moringa oleifera* leaves contained the highest phenols (0.98 mg/g) and non-tannin phenolics (0.97 mg/g). *Trigonella foenum-graecum* seeds had the highest tannin content (0.34 mg/g). Ethanol extracts generally showed higher levels of bioactive compounds in plants like *Curcuma longa*, *Andrographis paniculata*, *Cinnamomum verum*, and *Emblica officinalis*. These findings highlight solvent-dependent extraction and the therapeutic potential of these herbs, supporting their traditional medicinal use and future integration into modern healthcare.

Keywords: Bioactive compounds, herbal supplementation, pharmacology

Introduction
Herbs and medicinal plants have been integral to traditional healthcare practices for centuries, particularly in India, where they play a pivotal role in managing human and animal health. In Tamil Nadu, a southern state in India, these plants are essential in therapeutic practices, particularly in poultry health management. The region is known for its rich biodiversity, with numerous plant species valued for their medicinal properties. These plants contain various bioactive compounds that offer significant health benefits. In recent years, there has been a growing interest in scientifically validating the therapeutic efficacy of these plants and their active constituents. This study focuses on the phytochemical analysis of 16 commonly used herbs in Tamil Nadu, which are recognized for their traditional medicinal applications. The primary objective is to evaluate the presence and concentration of key bioactive compounds, including alkaloids, phenols, flavonoids, saponins, and terpenoids, which contribute to the therapeutic potential of these plants. The research involves systematically collecting plant samples from different regions, processing them under controlled conditions, and performing quantitative assays to assess their chemical composition. Standardized techniques such as the estimation of alkaloids, total phenols, flavonoids, tannins, saponins, and terpenoids will be employed to quantify these compounds. The results of this study aim to provide a comprehensive understanding of the chemical profile of these herbs, thus contributing to the growing body of research on their pharmacological properties. These findings may advance the application of these plants in modern medicine, particularly in the fields of herbal supplementation, pharmacology, and nutraceuticals.

Materials and Methods
a. Collection of herbs and sample processing
Sixteen commonly available herbs in Tamil Nadu were selected for this study, with the specific parts traditionally used for medicinal purposes chosen for analysis. A detailed list of the herbs and the corresponding plant parts selected for the study is provided in Table 1.
For each herb, six samples were collected from different districts across Tamil Nadu. The collected samples were carefully cleaned to remove any extraneous matter and then assessed for moisture content following the method described by the AOAC (2012) [1].

The samples were shade-dried for a period of 72 hours to prevent degradation of sensitive compounds. After drying, the plant material was ground using a Willey mill, and the ground samples were sieved through a 1mm mesh. The processed samples were stored in airtight containers to ensure their integrity for subsequent analyses.

Table 1: List of herbs and their parts selected for the study

S. No.	Botanical name of the herb	Common name of the herb	Plant part used
1	*Allium sativum*	Garlic	Bulb
2	*Andrographis paniculata*	Nilavembu	Leaf with stem
3	*Azadirachta indica*	Neem	Leaf
4	*Cinnamomum verum*	Cinnamon	Bark
5	*Coriandrum sativum*	Coriander	Seed
6	*Curcuma longa*	Turmeric	Rhizome
7	*Emblica officinalis*	Amla	Fruit
8	*Mentha spicata*	Mentha	Leaf
9	*Moringa oleifera*	Moringa	Leaf
10	*Murraya koenigii*	Curry	Leaf
11	*Ocimum sanctum*	Tulsi	Leaf
12	*Phyllanthus niruri*	Keelanelli	Full plant with root
13	*Piper nigrum*	Black pepper	Flower bud
14	*Syzygium aromaticum*	Clove	Flower bud
15	*Trigonella foenum*	Fenugreek	Seed
16	*Zingiber officinale*	Dry ginger	Rhizome

b. Preparation of plant extract

The procedure followed was based on Tiwari *et al*. (2011) [22]. To prepare the extracts, 10 g of herbal powder was mixed with either water or 70% ethanol and shaken at 250 rpm for 48 hours, then filtered through No. 1 filter paper. The filtrate was transferred to a pre-weighed Petri dish, dried in an incubator at 35 °C for 48 hours, and weighed. The yield of the extract was calculated as a percentage, and the dried material was stored at 5 °C for further phytochemical analysis.

1. **Aqueous extract:** 10 g of herbal powder was mixed with distilled water and kept it for shaker at 250 rpm for 48 hours, then filtered and stored at 5 °C for qualitative assays.
2. **Ethanol extract:** 10 g of herbal powder was mixed with 70% ethanol and kept it for shaker at 250 rpm for 48 hours, then filtered and stored at 5 °C for qualitative assays.

c. Quantitative determination of Phytochemicals

Phytochemical assays of the aqueous and ethanolic extracts were performed following the method of Harborne (1998) [8] at the Ethno Veterinary Herbal Research Centre for Poultry, Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal.

1. Estimation of Alkaloids

Alkaloid content was estimated using the method of Harborne (1973) [7]. To 1 g of extract, 40 ml of 10% acetic acid in ethanol was added, left it for 4 hours. The mixture was filtered and then concentrated in a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide was added drop by drop until precipitation occurred. The solution was allowed to settle, and the precipitate was collected, washed with dilute ammonium hydroxide, filtered, dried, and weighed. The alkaloid content was expressed as a percentage.

2. Estimation of Total phenol

Total phenol content was estimated using the method of (Makkar *et al*. (1993) [13]. Each extract (0.1 mg/ml) was mixed with 0.5 ml of 1:1 diluted Folin-Ciocalteu phenol reagent and 2.5 ml of 20% sodium carbonate solution. After 40 minutes, absorbance was measured at 725 nm using a UV-VIS spectrophotometer (JASCO V 730, Japan). Total phenolic content was calculated from a tannic acid standard curve (2.5 to 20 µg/ml) and expressed as milligrams per gram of extract.

3. Estimation of Non tannin phenolics

Non-tannin phenolic content was estimated using the method of Makkar *et al*. (1993) [13]. One ml of each extract was mixed with 1 ml of polyvinyl polypyrrolidone (100 mg/ml), vortexed and centrifuged at 3000 rpm for 15 minutes at 4 °C. To the supernatant, 0.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent, and 2.5 ml of 20% sodium carbonate were added and allowed to stand for 40 minutes. A set of tannic acid standard solutions (10 mg/ml) was treated similarly. Absorbance was measured at 725 nm against the blank using a UV-VIS spectrophotometer (JASCO V 730, Japan). Non-tannin phenolic content was calculated from the standard curve and expressed as milligrams per gram of extract.

4. Estimation of Tannin

Tannin content was determined using the method of Makkar *et al*. (1993) [13] by subtracting the non-tannin phenolic content from the total phenolic content.

5. Estimation of Total flavonoid

Total flavonoid content in plant extracts was determined using the aluminium chloride colorimetric method (Chang *et al*., 2002) [4]. A 0.25 ml aliquot of extract (10 mg/ml) was mixed with 0.75 ml of ethanol, 0.05 ml of 10% aluminium chloride, 0.02 ml of 1M potassium acetate, and 1.4 ml of distilled water. The reaction mixture was incubated at 37 °C for 30 minutes, and the absorbance was measured at 415 nm using a UV-VIS spectrophotometer. A standard curve was prepared using rutin (RU) dissolved in dimethyl sulfoxide (DMSO) at concentrations ranging from 10 to 100 µg/ml. The total flavonoid content was calculated from the standard curve and expressed as milligrams of rutin equivalent per gram of extract (mg RU/g).

6. Estimation of Saponin

Saponin content was determined using the method of Mir *et al*. (2016) [16]. To 2 g of extract, 50 ml of petroleum ether was added, and the suspension was heated in a water bath at 55 °C for one hour with continuous stirring. The mixture was then filtered, and the residue was re-extracted with 50 ml of methanol. The combined filtrate was concentrated to 10 ml in a water bath at approximately 90 °C. The concentrate was transferred to a 250 ml separating funnel,

where acetone was added slowly and the mixture was shaken well. The aqueous layer was separated, and the purification process was repeated. The remaining solution, containing saponins, was heated in a water bath, dried in an oven, weighed and expressed as a percentage.

7. Estimation of Terpenoid
Terpenoid content was estimated as per the method of Malik *et al*. (2017). The plant extract (100 mg) (W_i) was taken and soaked in 9 ml of ethanol for 24 hours and then filtered. The filtrate was treated with 10 ml of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and dried completely (W_f). The per cent of total terpenoid was calculated using the formula (W_i-W_f / W_i × 100).

Results
1. Quantitative determination of phytochemical constituents
In the aqueous extracts of the herbal samples, saponins were the predominant active compound identified in 9 out of the 16 samples tested. In contrast, alkaloids and terpenoids were the major active principles found in the ethanol extracts of 14 out of the 16 samples. The content of alkaloids, saponins, terpenoids, total flavonoids, total phenols, non-tannin phenolics, and tannins in both aqueous and ethanol extracts of the various herbs are shown in Tables 2 and 3 respectively.

a. Quantitative analysis of Aqueous extract
In the aqueous extracts of various herbs, *E. officinalis* fruits exhibited the highest alkaloid content (26.35 mg/g of extract) with statistical significance (P<0.05). *A. paniculata* stems and leaves showed the highest levels of saponins (20.36%) and terpenoids (4.75%), also significantly different (P<0.05). *S. aromaticum* buds had the highest total flavonoid content (4.63 mg/g of extract), while *M. oleifera* leaves contained the highest total phenols (0.98 mg/g of extract) and non-tannin phenolics (0.98 mg/g of extract), all with significant differences (P<0.05). *Trigonella foenum* seeds exhibited the highest tannin content (0.34 mg/g of extract) with statistical significance (P<0.05).

b. Quantitative analysis of Ethanolic extract
Curcuma longa rhizomes, *A. paniculata* stems and leaves, *Cinnamomum verum* barks, *Ocimum sanctum* leaves, *E. officinalis* fruits, *A. indica* leaves, and *E. officinalis* fruits exhibited the highest levels of alkaloids (52.41 mg/g of extract), saponins (22.76%), terpenoids (3.02%), total flavonoids (4.47 mg/g of extract), total phenols (1.33 mg/g of extract), non-tannin phenolics (0.76 mg/g of extract), and tannins (0.72 mg/g of extract), respectively, in their ethanol extracts, all showing significant differences (P<0.05). The results clearly demonstrate variations in the content of active compounds between the aqueous and ethanol extracts of the same herbal samples.

Discussion
The quantitative analysis of phytochemicals in the aqueous and ethanolic extracts of herbal samples revealed that the active principles varied between the two extract types for the same herb. Similar observations were made by Nagajothi *et al*. (2018) [17], who reported that the phytochemical composition of *Andrographis paniculata* differed between aqueous and ethanolic extracts. Specifically, the mean concentrations of tannins, terpenoids, and saponins were higher in the aqueous extract, while alkaloids, total phenols, non-tannin phenolics, and flavonoids were more concentrated in the ethanolic extract. These findings align with previous studies indicating solvent-dependent variations in the extraction efficiency of different phytochemicals (Sultana *et al*., 2009; Sharma *et al*., 2017) [21, 20].

As highlighted in the current study, *E. officinalis* is known to contain various bioactive compounds, including tannins, flavonoids, saponins, terpenoids, and ascorbic acid, among others (Hassan *et al*., 2016) [9]. Chromatographic and IR spectral analyses also confirmed the presence of alkaloids such as phyllantine and phyllantidine in *E. officinalis* fruit (Khanna and Bansal, 1975) [11]. Similarly, *M. oleifera* has been shown to have a significant concentration of phenolic compounds, supporting its potential as a source of antioxidants (Ramon *et al*., 2017) [18]. In agreement with our findings, a study by Bouayed *et al*. (2007) [2] reported that *Syzygium aromaticum* (clove) stem and fruit pulps contained notably higher levels of total flavonoids (22.53 mg/100 g fresh weight) compared to other medicinal plants. Furthermore, consistent with the present study, *Andrographis paniculata* has been recognized for its major constituents, including diterpenoids, flavonoids, and polyphenols (Chao and Lin, 2010; Sharma *et al*., 2015) [19, 5]. These findings reinforce the rich phytochemical profile of these plants, supporting their therapeutic potential.

Table 2: Alkaloid (mg / g of extract), Saponin (%), Terpenoids (%), Total flavonoids (mg / g of extract), Total phenols (mg / g of extract), Non tannin phenolics (mg / g of extract) and Tannin (mg / g of extract) content (Mean*±S.E) in aqueous extract of different herbs

S. No.	Name of the herb	Alkaloid	Saponin	Terpenoid	Total flavonoid	Total Phenols	Non tannin Phenolics	Tannin
1	*A.sativum* bulbs	7.41^{l}±0.07	7.54^{f} ±0.21	1.61^{ef} ±0.03	0.53^{h} ±0.19	0.20^{i} ±0.01	0.16^{h} ±0.00	0.04^{hi}±0.01
2	*A.paniculata* stem and leaves	22.69^{e}±0.09	20.36^{a} ±0.43	4.75^{a} ±0.15	1.15^{efgh} ±0.33	0.33^{f} ±0.00	0.24^{f} ±0.00	0.09^{ef} ±0.00
3	*A.indica* leaves	24.31^{c}±0.11	2.43^{j}±0.11	1.71^{e} ±0.18	1.61^{defg} ±0.24	0.72^{b} ±0.00	0.66^{b} ±0.00	0.06^{ghi} ±0.00
4	*C.verum* barks	3.03^{m} ±0.15	3.89^{i} ±0.24	2.68^{b} ±0.02	0.82^{gh} ±0.23	0.27^{h} ±0.02	0.04^{k} ±0.01	0.24^{b} ±0.02
5	*C.sativum* seeds	14.59^{j}±0.11	6.27^{g} ±0.22	0.76^{g} ±0.02	0.91^{fh} ±0.02	0.20^{i} ±0.01	0.09^{ij} ±0.01	0.10^{de} ±0.01
6	*C.longa* rhizomes	23.44^{d}±0.12	3.83^{ij}±0.14	2.36^{c} ±0.04	2.04^{cde} ±0.14	0.21^{i} ±0.00	0.14^{i} ±0.00	0.07^{fhi}±0.00
7	*E.officinalis* fruits	26.35^{a} ±0.10	9.83^{e}±0.38	1.99^{d} ±0.05	3.44^{b} ±0.93	0.59^{d} ±0.01	0.47^{c} ±0.01	0.12^{de}±0.01
8	*M.spicata* leaves	14.74^{j} ±0.10	6.11^{g} ±0.25	1.65^{ef} ±0.01	1.41^{efgh} ±0.04	0.33^{f} ±0.01	0.22^{fg} ±0.00	0.10^{de}±0.00
9	*M. oleifera* leaves	9.73^{k} ±0.09	11.81^{d}±0.31	2.78^{b} ±0.04	2.67^{bc} ±0.12	0.97^{a} ±0.01	0.97^{a} ±0.00	0.01^{ij}±0.00
10	*M.koenigii* leaves	9.43^{k} ±0.06	6.42^{g} ±0.25	0.83^{g} ±0.01	0.44^{h} ±0.12	0.29^{g} ±0.01	0.23^{f} ±0.01	0.06^{ghi} ±0.01
11	*O.sanctum* leaves	20.71^{f} ±0.07	5.13^{h} ±0.14	0.90^{g} ±0.03	2.75^{bc} ±0.07	0.33^{f} ±0.00	0.24^{f} ±0.00	0.09^{ef} ±0.00
12	*P.niruri* leaves	15.60^{h} ±0.13	15.60^{b}±0.13	2.23^{c} ±0.02	2.51^{bcd} ±0.13	0.34^{f} ±0.01	0.13^{i} ±0.02	0.21^{cd}±0.01
13	*P.nigrum* seeds	17.02^{g} ±0.07	2.43^{j} ±0.11	2.23^{c} ±0.02	1.90^{cdef} ±0.46	0.28^{gh} ±0.01	0.21^{g} ±0.01	0.07^{fg} ±0.01
14	*S aromaticum* buds	15.29^{i}±0.15	6.42^{g} ±0.25	1.47^{f} ±0.03	4.63^{a} ±0.97	0.53^{e} ±0.01	0.33^{d} ±0.02	0.21^{c} ±0.02
15	*T. foenum* seeds	25.42^{b}±0.11	12.21^{cd}± 0.21	2.37^{c} ±0.34	2.61^{bcd} ±0.38	0.64^{c} ±0.01	0.31^{e} ±0.00	0.34^{a} ±0.01
16	*Z.officinale* dry rhizomes	14.48^{j}±0.12	12.87^{c}±0.29	0.84^{g} ±0.01	1.90^{cdefg} ±0.16	0.15^{j} ±0.01	0.03^{k} ±0.00	0.12^{d} ±0.01

*****Mean of six observations.
Means bearing different superscripts within column differ significantly (P < 0.05)

Table 3: Alkaloid (mg / g of extract), Saponin (%), Terpenoids (%), Total flavonoids (mg / g of extract), Total phenols (mg / g of extract), Non tannin phenolics (mg / g of extract) and Tannin (mg / g of extract) content (Mean*±S.E) in ethanol extract of different herbs

S.No	Name of the herb	Alkaloid	Saponin	Terpenoid	Total flavonoid	Total Phenols	Non tannin Phenolics	Tannin
1	*Allium sativum* bulbs	7.43^{m} ±0.16	9.54^{e} ±0.10	1.90^{e} ±0.03	0.16^{g} ±0.02	0.63^{h} ±0.01	0.43^{ij}±0.00	0.21^{efg}±0.00
2	*Andrographis paniculata* stem and leaves	38.56^{b}±0.12	22.76^{a} ±0.54	0.86^{h} ±0.02	1.39^{f} ±0.16	0.64^{h} ±0.01	0.44^{ij}±0.00	0.21^{ef} ±0.00
3	*Azadirachta indica* leaves	25.48^{f} ±0.11	3.47^{g} ±0.10	0.93^{gh}±0.01	2.57^{cd}±0.21	0.81^{c} ±0.00	0.76^{a} ±0.00	0.05^{ij}±0.00
4	*Cinnamomum verum* barks	6.59^{n} ±0.25	7.87^{f} ±0.35	3.02^{ab}±0.03	2.23^{de} ±0.05	0.77^{d} ±0.01	0.50^{gh} ±0.02	0.26^{d} ±0.01
5	*Coriandrum sativum* seeds	20.42^{h} ±0.10	7.83^{f} ±0.08	0.96^{gh} ±0.01	0.34^{g} ±0.05	0.68^{f} ±0.01	0.28^{l} ±0.01	0.40^{bc}±0.01
6	*Curcuma longa* rhizomes	52.41^{a}±0.14	2.33^{h} ±0.09	1.77^{f} ±0.03	2.39^{cde} ±0.14	0.80^{c} ±0.02	0.69^{b}±0.00	0.11^{h} ±0.00
7	*Emblica officinalis* fruits	21.40^{g} ±0.12	12.66^{d}±0.41	2.09^{d} ±0.09	3.51^{b} ±0.06	1.33^{a} ±0.02	0.60^{e} ±0.01	0.72^{a} ±0.02
8	*Mentha spicata* leaves	12.48^{k}±0.15	7.88^{f} ±0.06	2.30^{abc} ±0.06	3.31^{b} ±0.21	0.81^{c} ±0.00	0.40^{k} ±0.00	0.40^{b} ±0.00
9	*Moringa oleifera* leaves	11.63^{l}±0.12	14.26^{c}±0.27	2.98^{ab} ±0.01	4.41^{a} ±0.16	0.72^{d} ±0.00	0.54^{f} ±0.00	0.19^{fg} ±0.00
10	*Murraya koenigii* leaves	11.42^{l}±0.13	7.83^{f} ±0.08	0.98^{gh}±0.01	1.35^{f} ±0.03	0.79^{d} ±0.01	0.67^{cd}±0.01	0.12^{h} ±0.01
11	*Ocimum sanctum* leaves	33.31^{c}±0.11	7.33^{f} ±0.19	1.72^{f} ±0.03	4.47^{a} ±0.17	0.88^{b}±0.00	0.63^{de}±0.00	0.25^{de}±0.00
12	*Phyllanthus niruri* leaves	19.47^{i}±0.12	19.47^{b}±0.12	2.90^{b}±0.04	2.61^{cd} ±0.12	0.80^{c} ±0.00	0.45^{ij}±0.00	0.35^{cd}±0.00
13	*Piper nigrum* seeds	38.27^{b}±0.07	3.47^{g} ±0.10	2.90^{b}±0.04	2.74^{c} ±0.14	0.72^{e} ±0.01	0.51^{gh} ±0.01	0.20^{fg}±0.01
14	*Syzygium aromaticum* buds	32.25^{d}±0.07	7.83^{f} ±0.08	1.82^{ef} ±0.03	2.60^{cd}±0.14	0.72^{e} ±0.01	0.67^{c} ±0.01	0.04^{j} ±0.00
15	*Trigonella foenum* seeds	28.56^{e} ±0.08	14.25^{c}±0.30	3.00^{ab}±0.05	2.08^{e} ±0.10	0.68^{f} ±0.00	0.49^{gh} ±0.00	0.19^{fg} ±0.01
16	*Zingiber officinale* dry rhizomes	17.62^{j} ±0.11	13.01^{d} ±0.54	0.96^{gh} ±0.01	1.67^{f} ±0.09	0.67^{g} ±0.00	0.60^{e} ±0.01	0.06^{ij} ±0.01

*Mean of six observations. Means bearing different superscripts within column differ significantly (P < 0.05)

Conclusion

In the quantitative analysis of aqueous extracts, *E. officinalis* fruits exhibited the highest alkaloid content (26.35 mg/g of extract), significantly higher (P<0.05) compared to other samples. *A. paniculata* stems and leaves contained the highest saponin (20.36%) and terpenoid (4.75%) content, both showing significant differences (P<0.05). *S. aromaticum* buds had the highest total flavonoid content (4.63 mg/g of extract), while *M. oleifera* leaves exhibited the highest levels of total phenols (0.98 mg/g) and non-tannin phenolics (0.97 mg/g), both significantly higher (P<0.05). *Trigonella foenum* seeds demonstrated the highest tannin content (0.34 mg/g), also significantly higher (P<0.05). In the ethanol extracts, *C. longa* rhizomes, *A. paniculata* stems and leaves, *C. verum* barks, *O. sanctum* leaves, *E. officinalis* fruits, *A. indica* leaves, and *E. officinalis* fruits exhibited significantly higher (P<0.05) levels of alkaloids (52.41 mg/g), saponins (22.76%), terpenoids (3.02%), total flavonoids (4.47 mg/g), total phenols (1.33 mg/g), non-tannin phenolics (0.76 mg/g), and tannins (0.72 mg/g), respectively, compared to other herbs. These findings are consistent with previous studies that highlight the variation in phytochemical content based on the solvent used for extraction (Mackeen *et al*., 2000; Mandal *et al*., 2010) [12, 15]. Such variations underscore the solvent-dependent extraction of bioactive compounds, which has important implications for their therapeutic applications (Chandra *et al*., 2011; Kaur *et al*., 2019) [3, 2].

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