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INVESTIGATING PHYTOCHEMICAL DIVERSITY AND ANTIOXIDANT RICHNESS OF MORINGA OLEIFERA IN TAMIL NADU

Sanju Dahiya, Munish Garg*

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ABSTRACT
Background: *Moringa oleifera* Lam., widely known as 'The Tree of Life', is a medicinal tree native to India and extensively grown in tropical regions worldwide. In India, Tamil Nadu is the leading state, engaging an area of 20684 hectares in the production of moringa. In South India, moringa is extensively utilized as a vegetable for its exquisite taste and flavor in sambar and curry preparation. **Methodology:** Phytochemical analysis of leaves from the Tamil Nadu region and simultaneous estimation of quercetin, rutin, and gallic acid contents in moringa leaf extracts from the Tamil Nadu region via HPTLC analysis was carried out. DPPH assay was performed to determine the antioxidant potential. **Results and Discussion:** The hydroalcoholic extract obtained from the triple maceration of moringa leaves possesses high amounts of phytoconstituents such as flavonoids and polyphenols. Each gram of the extract contained 1650.401 µg of quercetin, 1136.950 µg of rutin, and 220.223 µg of gallic acid. The IC_{50} value of the extract was calculated to be 36.10 µg/ml. **Conclusion:** The extract from the leaves of the moringa plant grown in the Tamil Nadu region contains a good amount of phytoconstituents and also possesses good antioxidant activity comparable to that of standard ascorbic acid, suggesting its potential use as an antioxidant agent. The findings of the present study support the traditional use of the folklore plant for improving health.

INTRODUCTION

Moringa oleifera Lam. is a medicinal tree native to India that is extensively grown in tropical regions. The tree has attracted attention due to its high nutritional potential as a "*natural nutrient source for the tropics*". India cultivates about 2.2 million tons of moringa grown in 43,600 hectares. India is also the world's leading moringa supplier, accounting for more than 80% of global demand. The southern region of India lies in a tropical climatic zone that provides a suitable environment for the tree's natural growth. The business of exporting moringa leaves is growing in Tamil Nadu, Andhra Pradesh, Karnataka, and Odisha at an annual growth rate of 26-30%. Tamil Nadu is the leading state, engaging an area of 20684 hectares in the production of moringa. Almost all parts of the *M. oleifera* tree contain nutrients and minerals in good amounts; however, its leaves are widely consumed for their nutritional properties. A Tamil proverb, "*Murungalyai nattavan verungaiyodu povan*", mentions the importance of the tree in the region, meaning that

*Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, Haryana-124001

***For Correspondence:** **munishgarg@mdurohtak.ac.in**

the one who grows and consumes moringa becomes physically strong and will never require a walking stick in old age [1]. Previously, moringa was cultivated as the sole crop in Tamil Nadu. Still, in the early 1990s, moringa was grown as an intercrop with other crops for protection from dry winds in summer. During the summer, crop production becomes remunerative, and moringa thus gains its foothold as a summer vegetable. Practically, moringa is grown in all areas of South India, and every household has at least one to two trees in its garden for its nutritional and therapeutic properties. In South India, moringa is extensively utilized as a vegetable for its exquisite taste and flavor in sambar and curry preparation. Any meal that does not include moringa is considered incomplete among South Indians [2].

In recent decades, with the advancement of scientific research, moringa has been proven to possess antioxidant, anti-inflammatory, antifungal, and antimicrobial properties. It also protects against cardiovascular diseases, liver diseases, and DNA damage and has various therapeutic effects. A large number of phytochemicals, including quercetin, rutin, gallic acid, luteolin, flavonoids, terpenoids, glycosides, and tannins, have been identified in Moringa oleifera leaves [3]. With newer tools and technologies, the scientific research community has focused on isolating bioactive components from medicinal plants to develop new drugs [4-6]. High-performance thin-layer chromatography (HPTLC) is widely used for the phytochemical analysis of herbal extracts both quantitatively and qualitatively. It is also recognized by the United States Pharmacopoeia (USP) as an official analytical tool and offers advantages of high sensitivity, high sample throughput, and low cost of operation. Several studies have reported genetic and other variations in plants grown in different geographical regions [7,8]. Based on the significance of moringa in the diet of South Indians, a research study might be appropriate for supporting the use of folklore.

Therefore, the present study aims to analyze the diversity of phytochemicals present in *Moringa oleifera* leaf samples of a particular region in Tamil Nadu because the environmental and geographical factors influence the phytochemical composition and, subsequently, the antioxidant richness. The phytochemical and antioxidant profile database of *Moringa oleifera* from Tamil Nadu, can serve as a reference for further research and industrial applications. Thus, the research goes beyond basic antioxidant studies by integrating phytochemical diversity and antioxidant richness to provide a holistic understanding of the plant's bioactive potential. The findings may provide valuable insights for optimizing *Moringa oleifera* cultivation practices in Tamil Nadu to enhance its medicinal and nutritional benefits, offering scientific and practical contributions to agriculture and health.

MATERIALS AND METHODS

Chemicals and Reagents

All the reagents and chemicals used were of analytical grade. Ethanol (CAS No. 64-17-5 Merck), methanol (CAS No. 67-56-1 Loba Chemie), petroleum ether (CAS No. 8032-32-4 CDH), toluene (CAS No. 108-88-3 Merck), ethyl acetate (CAS No. 64-19-7 Loba Chemie), formic acid (CAS No. 64-18-6 Loba Chemie), 2,2-diphenyl-1-picrylhydrazyl (CAS No. 1898-66-4 Merck), ascorbic acid (CAS No. 50-81-7 CDH), quercetin (CAS No. 6151-25-3 CDH), rutin (CAS No. 153-18-4 Merck), and gallic acid (CAS No. 149-91-7 Merck) were used.

Collection of plant material

Mature leaves of Moringa oleifera were collected from Aval Poondurai in the Erode district in Tamil Nadu. The leaf samples were authenticated by the National Institute of Science Communication and Policy Research (CSIR) (Authentication no. NIScPR/RHMD/Consult/2022/4215-16). A voucher specimen was kept in the Department Herbarium for future reference (MDU/PhSc/Phcog/2022/101).

The leaves were washed with water and dried under shade at room temperature. The completely dried leaves were subjected to grinding using a grinder. The leaf powder was sieved and stored in airtight containers for analyses.

Preparation and purification of moringa leaf extract (MLE)

A number of studies have been carried out by researchers to optimize the best extraction solvent and method for maximum retention of phytochemicals with good antioxidant potential [9-12].

In the present study, approximately 250 g of moringa leaf powder was subjected to triple maceration using 70% ethanol for seven days in a round bottom flask. After filtration the extract was concentrated on a rotary vaccum evaporator (IKA RV10 auto) at 45°C [9,13]. The extract was purified to remove the chlorophyll and fatty acid components of the extract. For this,

the prepared extract was redissolved in distilled water and petroleum ether was added. The mixture was stirred continuously and then poured in a separating funnel and kept undisturbed for separation of layers. Two different layers were observed in the column: the lower aqueous layer containing pure extract and the upper dark green petroleum ether layer containing chlorophyll and fatty components. Both layers were collected in separate beakers. The aqueous layer containing the purified extract was concentrated using a rotary vacuum evaporator at 45°C. The concentrate was collected in a China dish and kept in a hot air oven at 45°C until completely dry [14]. The extract was scraped off and stored in airtight containers at 4°C for further analysis. The picture flowchart of processing of *Moringa oleifera* to prepare the purified extract is depicted in figure 1.

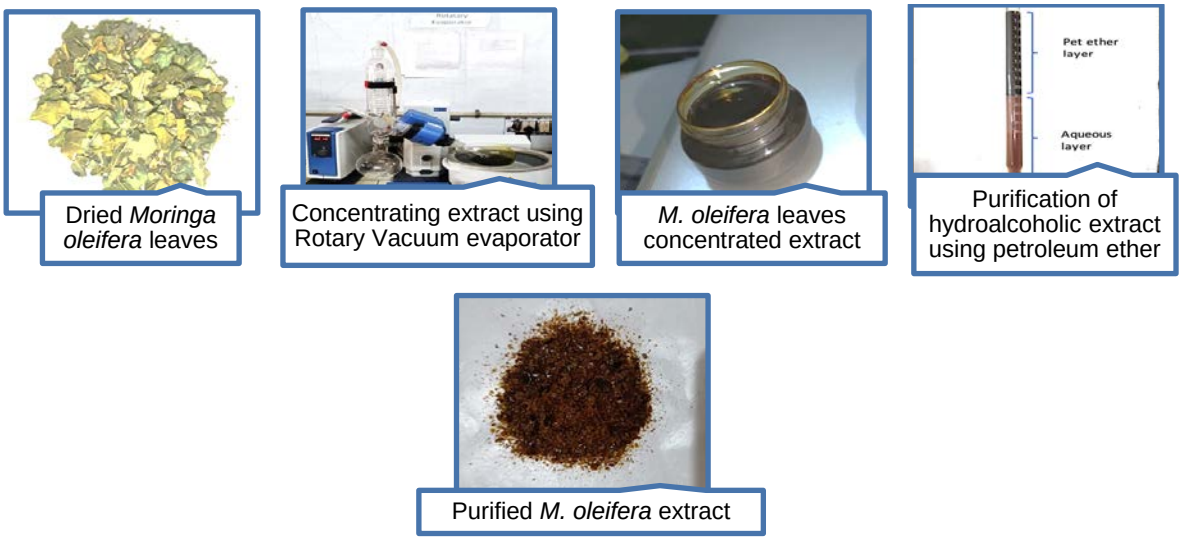

Figure 1: Processing of *Moringa oleifera* leaves to prepare purified extract

GC–MS analysis of purified extract

Gas chromatography–mass spectrometry (GC–MS) analysis of the purified extract of *M. oleifera* leaves was performed on a GC–MS instrument ("Model: QP 2010 series, Shimadzu, Tokyo, Japan") equipped with a capillary column of TR-5MS fused silica (5% diphenyl/95% dimethyl polysiloxane) 0.25 mm in diameter, 30 mm in length and 0.25 µm in film thickness. An AOC-20i+ autosampler was used to inject a 2 µl sample. The MS instrument was operated at an ionization energy of 70 eV with helium as the carrier gas. The column flow was set at 1.00 ml/min, and a linear velocity of 36.7 cm/sec was achieved by adjusting the flow control. The initial oven temperature was set at 70°C, and the ramp scan interval was 0.50 seconds to reach 310°C in 5 minutes. The total running time was 63 minutes. The identification of phytochemicals was performed by comparison with the NIST11 data. Library of National Institute of Standards and Technology (LIB) [15].

Total flavonoid content of extract

Quercetin was used as a standard for the determination of total flavonoid content. Standard solutions of different known concentrations (0.25 µg/ml, 0.5 µg/ml, 0.75 µg/ml, 1 µg/ml and 1.25 µg/ml) were prepared in methanol. 4mg extract was dissolved in 1 ml methanol to prepare the test sample. Pipetted out 1ml each of the test and standard solutions and kept in 10 ml volumetric flasks separately. Then, added 4 ml distilled water and 0.5 ml of 5% sodium nitrate solution to each flask, and the flasks were left to stand for 5 min. Then, added 2ml of 1 M NaOH solution to each flask and after 6 minutes the volume was made up to 10 ml with distilled water and incubated at room temperature for 15 min. On a UV-visible spectrophotometer (Shimadzu UV-1601) absorbance of test and standards was measured at 510 nm. Total flavonoid content of extract was determined with reference to standards by plotting a calibration curve [14].

Total phenolic content of extract

For the preparation of the test sample, 10 mg of extract was dissolved in 5 ml methanol and ultrasonicated at 40°C for about 45 min. The mixture was subjected to centrifugation at 1000 rpm for about 10 min, and the clear supernatant obtained was collected for analysis. Gallic acid was used as a standard for the

determination of total phenolic content. Standard solutions of different known concentrations (50 µg/ml, 100 µg/ml, 150 µg/ml, 250 µg/ml, 350 µg/ml, and 500 µg/ml) were prepared in ethanol. One milliliter of each of the prepared standard solutions and test sample samples was taken in separate test tubes, and 5 ml of Folin-Ciocalteu reagent and 5 ml of 7.5% sodium carbonate solution were

The samples were then incubated for about 15 min at 25°C. 2 ml of samples were pipetted out from each test tube, and the volume was made up to 5 ml with distilled water in volumetric flasks. On a UV-visible spectrophotometer (Shimadzu UV-1601), the absorbance of the test and standards was measured at 760 nm. The total phenolic content of the extract was determined with reference to standards by plotting a calibration curve [14].

HPTLC analysis of the extract

HPTLC analysis of the purified extract of moringa leaves was performed with slight modifications to the method of Thomas et al. (2020) for quantifying quercetin, rutin, and gallic acid using a common solvent system for all three biomarkers [16].

The HPTLC method validation was performed as per the guidelines of ICH Harmonised Tripartite Q2(R1), and different parameters like linearity, range, precision, accuracy, the limit of detection (LOD), the limit of quantification (LOQ), and robustness were studied [17].

***Preparation of the solvent system and samples for analysis*:** The freshly prepared solvent system comprised toluene: ethyl acetate: methanol: formic acid (4.9:4.1:2:0.5). Standard solutions of the biomarker compounds quercetin (10 µg/µl), rutin (10 µg/µl) and gallic acid (10 µg/µl) were prepared by making suitable dilutions using methanol. The test solution of MLE was prepared in ethanol (50 µg/µl).

Sample application: The sample spots were applied on 10×10 cm precoated TLC plates using a 100 µl CAMAG syringe on a CAMAG LINOMAT 5 applicator attached to a computer system with installed Wincats software. To plot the calibration curve, 1 µl, 2 µl, 3 µl, 4 µl or 5 µl of standard solution was applied to the TLC plate. Three TLC plates were prepared, one for each standard compound. A separate TLC plate was prepared by applying four 2 µl volumes of each prepared sample.

Development: About 20 ml of the prepared solvent system was kept in a CAMAG twin-trough TLC chamber lined with filter paper for complete saturation. The TLC plates were developed by the ascending mobile phase technique.

Detection: The plates were then visualized in a CAMAG TLC visualizer at 254 nm and 366 nm under white light. They were scanned for densitometric analysis in a CAMAG TLC Scanner 3 at a 300 nm wavelength and with a deuterium lamp. The chromatograms obtained were then analyzed, and the data were recorded for further analysis.

DPPH assay of the extract

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed to determine the free radical scavenging potential of the prepared purified extract of *Moringa oleifera* leaves (MLE) by the method of Mwamatope et al. (2020) [18]. A stock solution of 0.1 mM was prepared by dissolving 3.80 mg of DPPH in 100 ml of methanol, and the solution was kept in the dark. Test samples of MLE and standard ascorbic acid (Std) were prepared in methanol at 10, 20, 40, 60, and 80 µg/ml concentrations. To each 3 ml of test sample, 1 ml of DPPH was added separately. To prepare the control 1 ml of DPPH solution was added to 3 ml of methanol. The samples were then incubated for about 30 min at room temperature. The absorbance of the samples was measured on a UV–visible spectrophotometer (Shimadzu UV-1601) at 517 nm with methanol as blank. The % radical scavenging activity (%RSA) was calculated using equation 1 [19,20].

$$\%RSA = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} \times 100 \quad (1)$$

Statistical analysis

All the experiments were carried out by taking three readings for each observation, and the mean standard deviation of the triplicates was employed to present the data [21]. The Statistical Package for the Social Sciences was used to perform the statistical analysis (IBM SPSS Statistics, USA). ANOVA was used to analyze the data for each sample, and Duncan's test was used to determine the significance level at a 5% level significance ($p < 0.05$).

RESULTS

Extraction yield

The % extraction yield of the extract obtained was determined using the formula given in equation 2.

$$\% \text{ Extraction yield} = \frac{\text{weight of extract obtained}}{\text{weight of crude drug taken}} \times 100 \qquad (2)$$

$$\% \text{ Extraction yield} = \frac{64.3}{450} \times 100 = 14.2 \text{ \%w/w}$$

GC–MS analysis of the extract

The GC–MS chromatograms obtained are shown in Figure 2. There were 46 peaks at their specific retention times, indicating the presence of 46 different compounds. The compounds were determined by comparing the data obtained with the standard NIST library. The significant peaks with their percentage area and retention times are shown in Table 1. No organophosphates or organochlorides were detected, indicating the absence of pesticidal residues in the test plant extract.

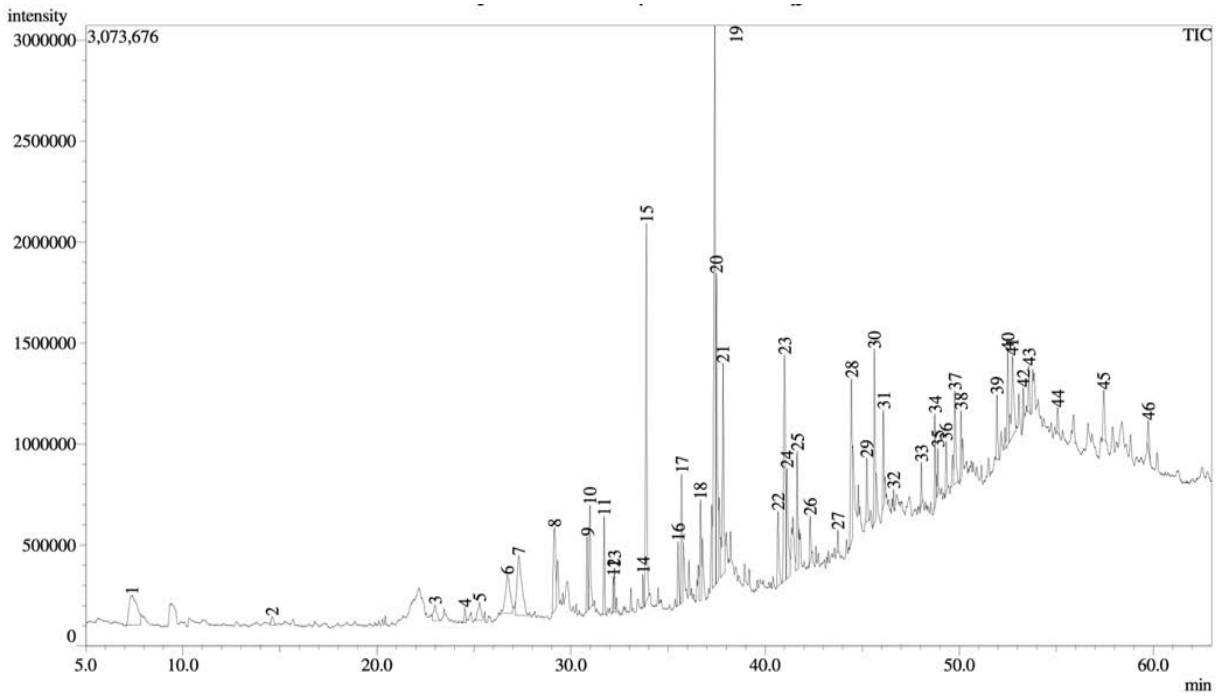

Figure 2: GC–MS chromatograms of *M. oleifera* leaf extracts

Table 1: Major GC–MS chromatogram peaks with their % area and retention time

Peak No.	Retention time (RT)	Name of the Compound	% Peak Area
1	7.075	Glycerin	3.48
2	14.586	Allyl acetate	0.31
3	22.992	Tetraethylene glycol	0.79
6	26.716	Ethyl.alpha.-d-glucopyranoside	2.64
7	27.315	2,5-Monoformal-1-rhamnitol	4.32
8	29.146	Pentaethylene glycol	2.87
14	33.708	Dibutyl phthalate	0.41
15	33.883	n-Hexadecanoic acid	7.31
17	35.693	Hexaethylene glycol	2.84
28	44.441	Erucic acid	3.93
44	55.068	gamma-Sitosterol	0.46
45	57.443	Tris(2,4-di-tert-butylphenyl) phosphate	1.24
46	59.735	Tris(2,4-di-tert-butylphenyl) phosphate	0.55

Total flavonoid content of extract

Figure 3 depicts the calibration curve; the total flavonoid content was calculated to be 16.62 mg quercetin equivalent per gram of extract.

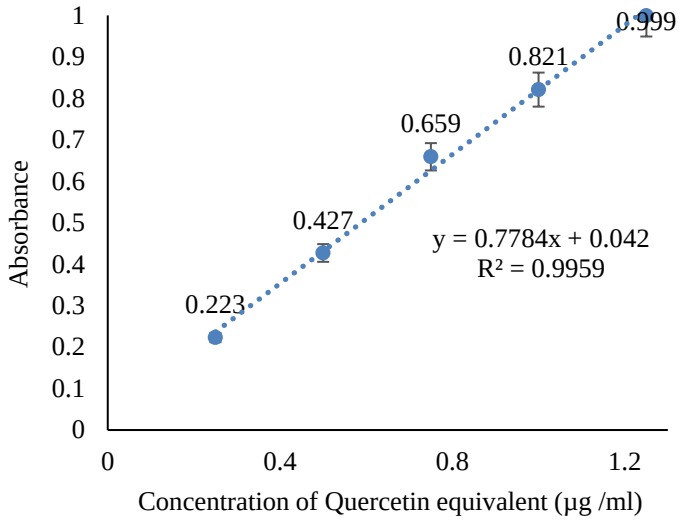

Figure 3: Calibration curve for absorbance vs. concentration of quercetin equivalent at 510 nm (after 15 min of incubation of samples at room temperature)

Total phenolic content of extract

Figure 4 depicts the calibration curve and total phenolic content of was calculated to be 152.6 mg gallic acid equivalent per gram of the extract.

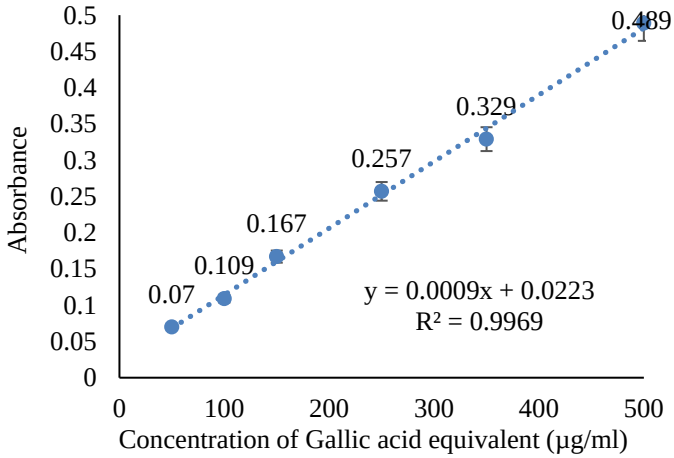

Figure 4: Calibration curve for absorbance vs. concentration of gallic acid equivalent at 760 nm (after 15 min of incubation of samples at 25°C)

HPTLC analysis of the extract

The HPTLC analysis confirmed the presence of quercetin, rutin, and gallic acid in the extract. Figure 5 shows photographs of the developed HPTLC plates at different wavelengths. Figure 6 shows the densitometric scans of the plates at 254 nm. The scans show sharp peaks for quercetin at an R_f of 0.59 ± 0.02, rutin at an R_f of 0.10 ± 0.03 and gallic acid at an R_f of 0.38 ± 0.03. The calibration curves of standard concentrations of the marker compounds were plotted to determine their concentrations in moringa leaf extract. Figure 7, 8, 9 shows the calibration curves for standards quercetin, rutin and gallic acid respectively. Densitometric scanning of MLE at 254 nm revealed eight sharp peaks. The concentrations of the compounds of interest were determined by comparing the peak areas of MLE with those of the standard biomarkers. It has been estimated that 1 gm of MLE contains 1650.401 µg of quercetin, 1136.950 µg of rutin, and 220.223 µg of gallic acid.

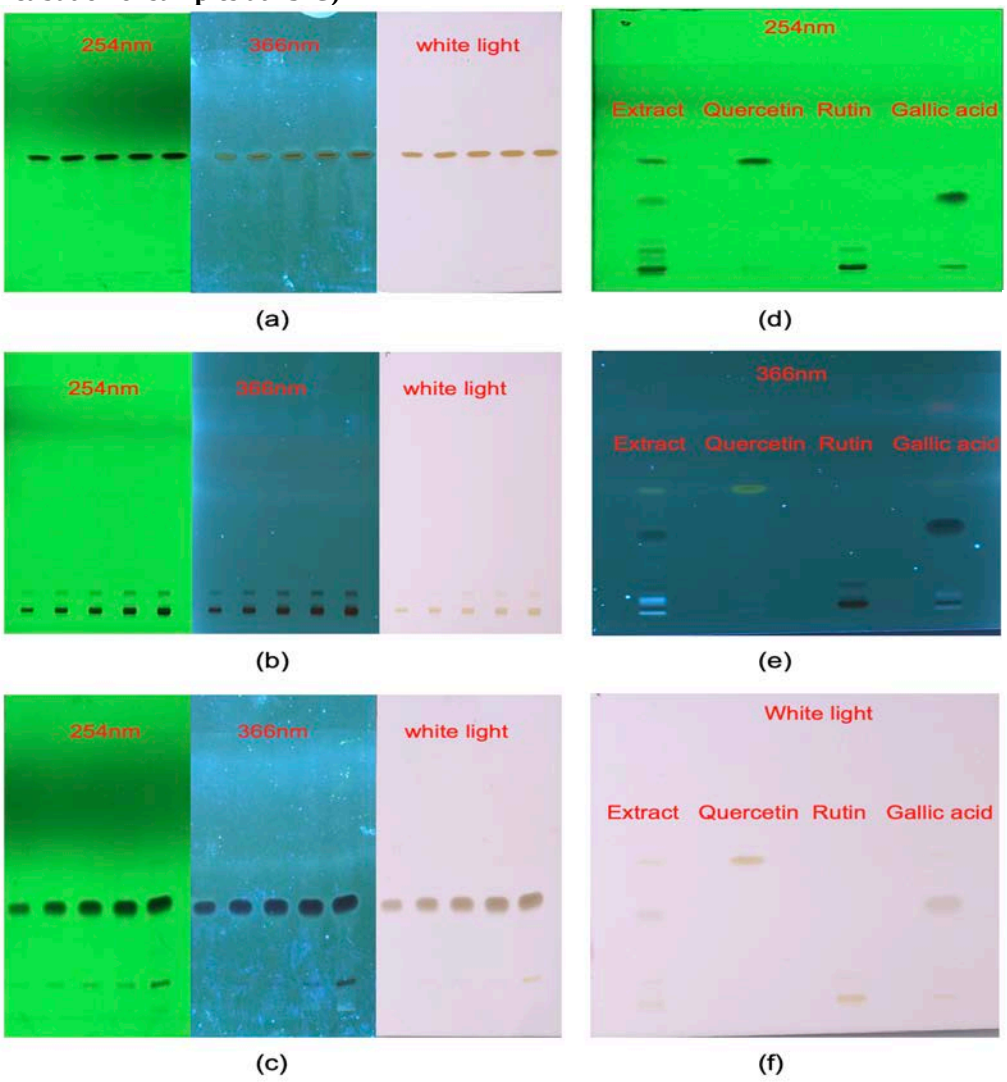

Figure 5: Photo documentation of developed HPTLC plates visualized on CAMAG® TLC Visualizer 3 and Wincats software (version 4.0) at different wavelengths: (a) standard quercetin (1-5 µg), (b) standard rutin (1-5 µg), (c) standard gallic acid (1-5 µg), (d) at 254 nm, (e) at 366 nm, and (f) under white light

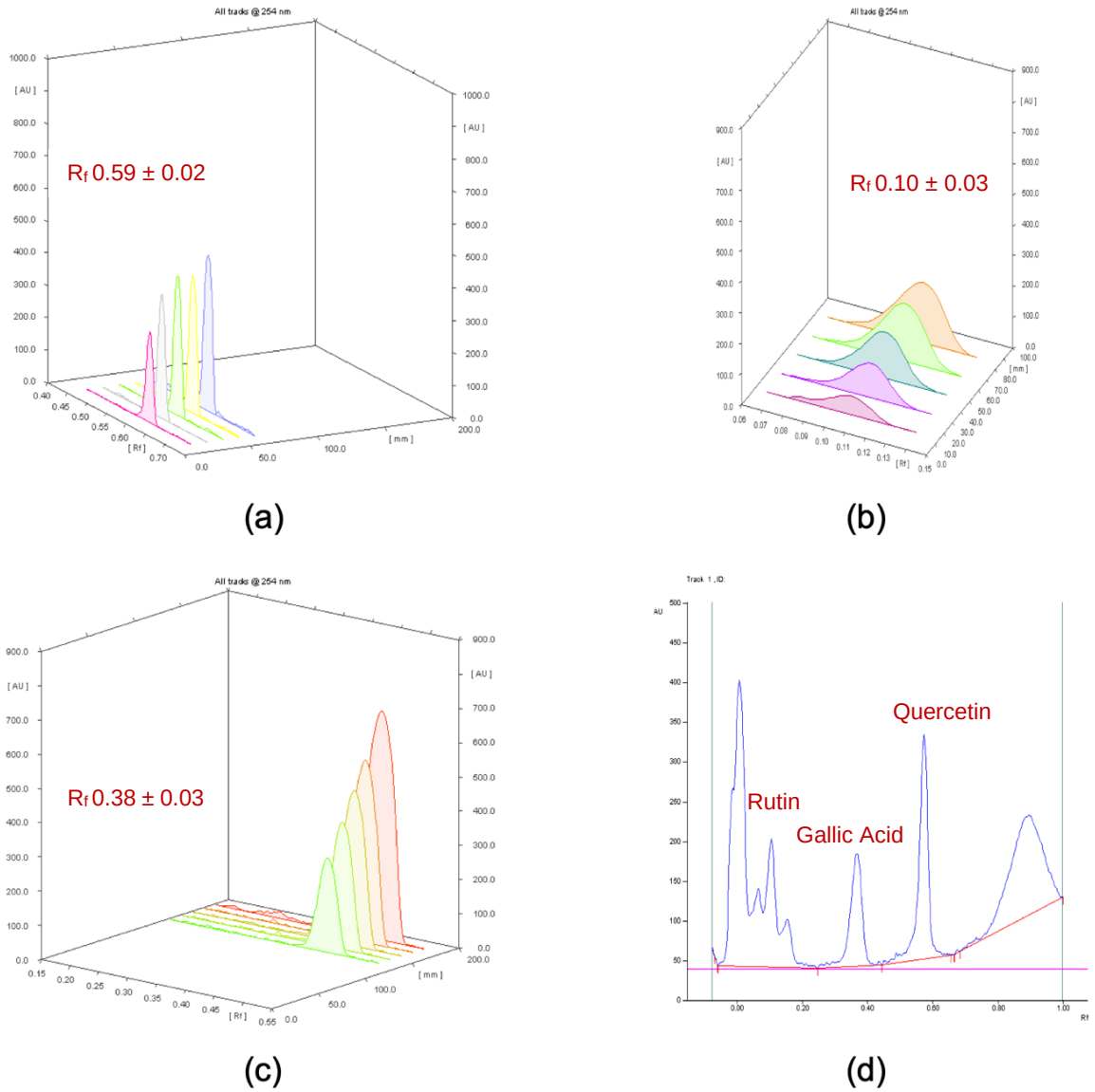

Figure 6: HPTLC Densitometric scans of developed HPTLC plate spots at 254 nm scanned on CAMAG® TLC Scanner 3 and Wincats software (version 4.0). (a) Standard quercetin (1-5 µg), (b) standard rutin (1-5 µg), (c) standard gallic acid (1-5 µg), and (d) *Moringa oleifera* leaf extract (MLE)

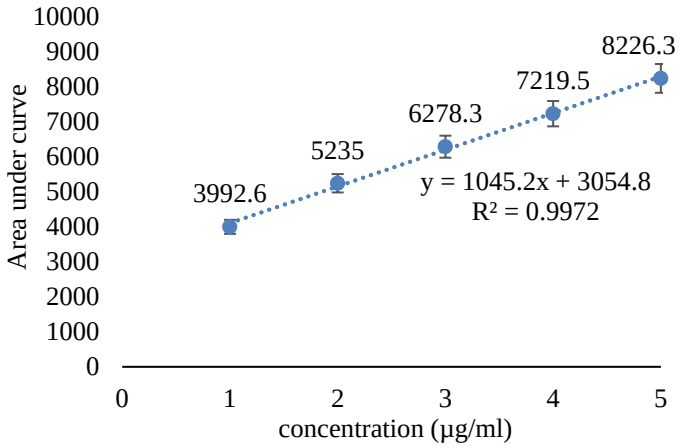

Figure 7: Calibration curve for quercetin standard with concentrations 1-5 µg/ml at 254 nm

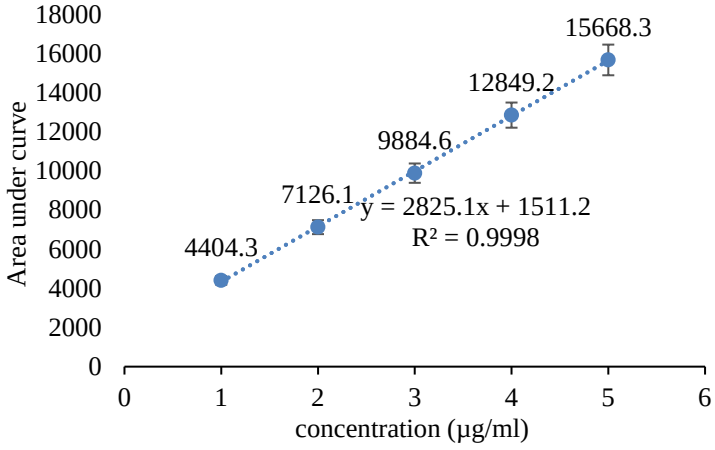

Figure 8: Calibration curve for rutin standard with concentrations 1-5 µg/ml at 254 nm

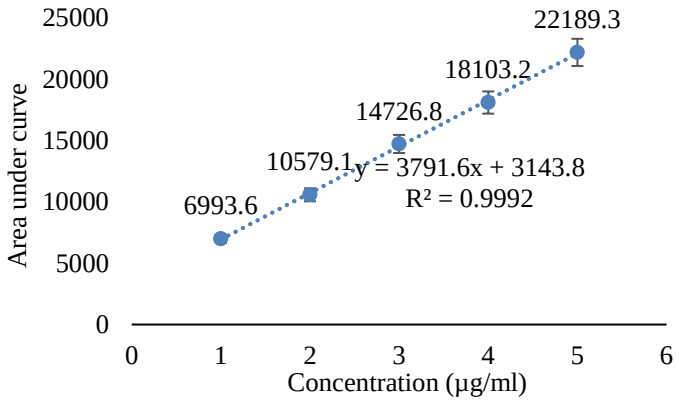

Figure 9: Calibration curve for gallic acid standard with concentrations 1-5 µg/ml at 254 nm

HPTLC method validation parameters: The HPTLC method was validated, and the different parameters studied are described in Table 2. The regression data of all the standards showed a linear relation over the concentration ranges of 2-10 µg/spot. Precision studies were carried out by studying variation at different time intervals and concentrations for inter-day and intra-day variations. The LOD and LOQ for all three standards under study were 2 µg and 4 µg, respectively. The instrument under study showed precision and repeatability values under an acceptable range.

DPPH assay of the extract

The antioxidant activity of *Moringa oleifera* leaf extract (MLE) was observed to increase in a dose-dependent manner. Table 3 represents the responses as %RSA and IC_{50} of MLE and Std at different doses, i.e., 10, 20, 40, 60, and 80 µg/ml concentrations. At 10 µg/ml, the %RSA of MLE was 24.61%, which increased to 86.92% at 80 µg/ml. The IC_{50} value for MLE was calculated to be 36.10 ± 1.54 µg/ml. The %RSA for Std was 43.46% at 10 µg/ml and 96.15% at 80 µg/ml. The IC_{50} value of Std was calculated to be 8.190 ± 1.18 µg/ml. Figure 10 shows the dose-response curves for the antioxidant activities of MLE and Std. The obtained data show that MLE possesses strong free radical scavenging activity. The regression analysis of the curve was performed, and the correlation coefficient was noted.

Table 2: HPTLC method validation parameters for quercetin, rutin, and gallic acid standards

Parameter	Quercetin standard	Rutin standard	Gallic acid standard
Linearity range (µg/spot)	2-10	2-10	2-10
Intra-day precision (%RSD, n=3)	1.12 ± 0.14	0.78 ± 0.20	1.26 ± 0.16
Inter-day precision (%RSD, n=3)	0.84 ± 0.08	0.66 ± 0.24	1.02 ± 0.12
Correlation coefficient (R^2 ± SD)	0.9972	0.9998	0.9992
Regression equation	y = 1045.2x + 3054.8	y = 2825.1x + 1511.2	y = 3791.6x + 3143.8
R_f	0.59 ± 0.02	0.10 ± 0.03	0.38 ± 0.03
LOD (µg)	2	2	2
LOQ (µg)	4	4	4
Instrumental precision (%CV, n=6)	1.28	1.14	1.06
Repeatability (%CV, n=6)	0.94	1.08	1.12

Table 3: % RSA of MLE and Std at different concentrations

Concentration (µg/ml)	%RSA of MLE	% RSA of Std
10	24.61 ± 0.017	43.46 ± 0.023
20	37.50 ± 0.034	60.38 ± 0.009
40	55.38 ± 0.026	82.88 ± 0.014
60	71.34 ± 0.019	93.46 ±0.030
80	86.92 ± 0.034	96.15 ± 0.018

Values are expressed as the means ± standard deviation (n=3)

DISCUSSION

Herbal extracts are employed as alternative therapies for various ailments because of their validated pharmacological properties, affordability, and lower toxicity than synthetic substitutes [22]. The *Moringa oleifera* plant is widely utilized in South India as a dietary supplement because of its nutritional and therapeutic properties. In India, Tamil Nadu is the leading producer and exporter of moringa. Despite numerous studies on the plant's therapeutic activities, inconsistencies and gaps in the regional variation of phytoconstituents remain. The therapeutic potential of moringa is primarily attributed to several phytochemicals present in it, such as flavonoids, polyphenols, and many more.

The present study used GC-MS analysis to determine the number of phytoconstituents in the *M. oleifera* leaf extracts. The

total flavonoid and phenolic content were also determined. The major peaks in the GC-MS chromatogram confirm the presence of compounds like n-hexadecanoic acid and gamma-sitosterol, which have significant biological activities. Bharath et al. 2021 evaluated hexadecenoic acid's anticancer potential and confirmed its significant antioxidant potential [23]. Researchers have extensively studied gamma-sitosterol to possess significant antidiabetic and anticancer activities [24]. It was observed that the moringa leaves of the Tamil Nadu region are rich in various phytoconstituents that exert synergistic effects to produce beneficial therapeutic effects. The synergistic effect of various flavonoids and polyphenols to boost antioxidant activity is well documented. The synergistic effect may be because of one or more mechanisms like the protection of one phytochemical by the other from oxidative agents; one antioxidant oxidizes to become a free radical, and the other captures it to regenerate its antioxidant capacity, binding at different orientations on the membranes to facilitate synergistic interactions, etc. [25].

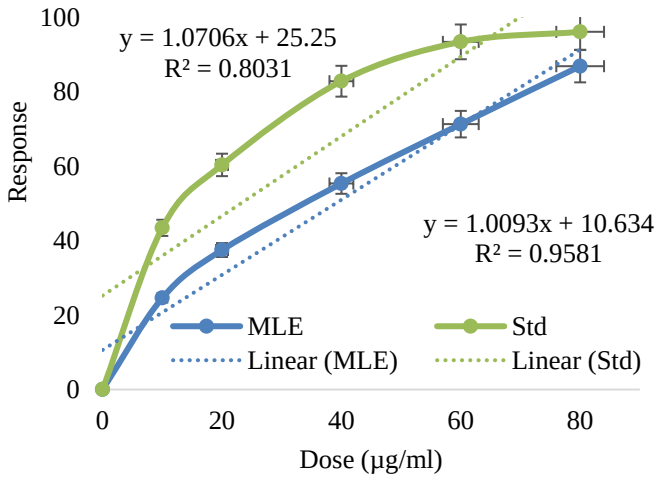

Figure 10: Dose-response curve for the antioxidant activity of MLE and Std at 517 nm (after 30 min of incubation of samples at room temperature)

HPTLC analysis determined the amount of significant phytoconstituents of specific importance. The method employed for simultaneous analysis of phytoconstituents quercetin, rutin, and gallic acid was rapid and cost-effective because of the requirement of very little solvent. The R_f values for the standards were cross-validated with previous research studies [16]. The amount of quercetin, rutin, and gallic acid needed to promote therapeutic effects was significantly high. The DPPH assay showed good antioxidant activity in the leaf extracts. Large amounts of polyphenols and other bioactive compounds may collectively be responsible for good antioxidant potential and other pharmacological effects. Quercetin belongs to the class of flavonoids and is present in abundant quantities in fruits and vegetables. It acts by different mechanisms, such as eliminating reactive oxygen species, regulating signal transduction pathways, and affecting glutathione and other enzyme activities [26]. Rutin, a polyphenolic compound, is an analog of quercetin. It is structurally known as quercetin-3-rhamnosyl glucoside. It has been reported to possess high antioxidant potential and to inhibit lipid peroxidation [27]. However, the poor water solubility of rutin prevents its wide use in pharmaceuticals. It has been encapsulated with β-cyclodextrin to enhance its solubility. Enzymatic and chemical modification studies have also been carried out [28]. Gallic acid occurs as a polyphenolic plant metabolite in most herbal plants. It has been reported to possess various therapeutic activities, such as anti-inflammatory, antimicrobial, and antiproliferative effects, mainly attributed to its high antioxidant activity. It is hydrophilic and has good absorption potential [29].

The quantity of phytoconstituents in the extract depends on the solvent and technique employed for extraction. Fachriyaha et al. (2020) found 70% ethanol to be the most suitable solvent for the maximum extraction of flavonoids and phenolics from *M. oleifera* leaves [7]. Vongsak et al. (2013) conducted research using different solvents (water, 50% ethanol, 70% ethanol) and extraction methods (squeezing, decoction, maceration, percolation and soxhlet extraction) for maximum polyphenols and flavonoid extraction from *Moringa* oleifera leaves. They concluded that for the production of pharmaceutical and nutraceutical products, maceration with 70% ethanol as menstruum is best for extraction of phytochemicals with good antioxidant potential from *M. oleifera* leaves [11].

Gómez-Martínez et al. (2020) studied the phytochemical composition of different parts of *Moringa oleifera*. The authors reported that the phytochemicals present in the ethanolic extract of leaves and petioles are responsible for the good antioxidant activity of the plant [10]. Einafshar et al. (2024) studied the antioxidant activities of natural antioxidants (7-tocopherol) and synthetic antioxidants(BHT). Comparing to their results, it can be observed that the IC_{50} value of MLE (36.5 ± 1.54 µg/ml) in the present study were higher than that of α-tocopherol (32.50 ± 2.15 µg/ml) and significantly higher than BHT (7.45 ± 0.35 µg/ml) [30]. Sudha et al. (2020) also confirmed the presence of

various phytochemicals in *M. oleifera* leaves grown in the Tamil Nadu region [1]. The number of phytoconstituents in the moringa grown in the Tamil Nadu region was more significant than in the transported study by Thomas et al. (2020) [16]. However, from the results of the present study, it is observed that various phytoconstituent leaves dominate the moringa tree cultivated in the Tamil Nadu region. This may be because suitable soil and weather conditions favor the production of a better variety of moringa. The results also strongly support the use of folklore in South India.

While the study offers valuable insights, several limitations should be considered; its limited geographical scope and an overlook of seasonal changes in phytochemical compositions restrict the results' generalizability. Also, the study's primary emphasis on antioxidant activity might overshadow other potential therapeutic or nutritional benefits of *Moringa oleifera*. The study supports future researchers' studies on the bioavailability and pharmacokinetics through in-vivo studies. Addressing these limitations in future research would enhance the comprehensiveness and applicability of the findings.

CONCLUSION

This study comprehensively analyzes Moringa oleifera samples, specifically from Tamil Nadu, capturing unique geographical influences on phytochemical diversity. The results obtained by different analyses show that *Moringa oleifera* leaves of the Tamil Nadu region contain reasonably good amounts of phytoconstituents, especially quercetin, rutin, and gallic acid. A sustainable and cost-effective method requiring minimal solvent for simultaneous estimating phytochemicals has been employed. An in vitro antioxidant assay revealed that moringa leaves possess strong antioxidant activity.

The results suggest that high amounts of phytoconstituents favor the high consumption of *Moringa oleifera* as a staple food in South India, especially in the Tamil Nadu region. The analysis of phytoconstituents in plants grown in different regions becomes of particular importance in present times as nowadays artificial environments like polyhouses are constructed to manipulate the surroundings and grow the plants anywhere. However, this takes a toll on the quality and quantity of phytoconstituents responsible for the therapeutic potential of herbals. Creating a localized phytochemical profile offers a reference point for developing standardization protocols for *Moringa oleifera*-based nutraceuticals and therapeutics. This research could also contribute to developing natural antioxidant agents and promoting *Moringa oleifera* locally and globally.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Sanju Dahiya conceptualized, performed the experimentation and drafted the manuscript. Munish Garg conceptualized and revised the draft manuscript. Both authors read and approved the final manuscript.

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