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ORIGINAL ARTICLE

Quantitative and Qualitative Analysis of Phenolic and Flavonoid Content in *Moringa oleifera* Lam and *Ocimum tenuiflorum* L.

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ABSTRACT

Background: Number of secondary compounds is produced by plants as natural antioxidants. *Moringa oleifera* Lam. and *Ocimum tenuiflorum* L. are known for their wide applications in food and pharmaceutical industry. **Objective:** To compare phenolic and flavonoid content in *M. oleifera* Lam and *O. tenuiflorum* L. by quantitative and qualitative analysis. **Materials and Methods:** Phenolic and flavonoid content were studied spectrophotometrically and by paper chromatography in *M. oleifera* Lam. and *O. tenuiflorum* L. **Results:** Higher phenolic and flavonoid content were observed in Moringa leaf and flower. Ocimum flower showed higher phenolic content and low flavonoid in comparison to Moringa. Flavonoids such as biflavonyl, flavones, glycosylflavones, and kaempferol were identified by paper chromatography. Phytochemical analysis for flavonoid, tannins, saponins, alkaloids, reducing sugars, and anthraquinones were tested positive for Moringa and Ocimum leaf as well as flower. **Conclusions:** In the present study higher phenolic and flavonoid content, indicated the natural antioxidant nature of Moringa and Ocimum signifying their medicinal importance.

Key words: Antioxidants, Flavonoids, Ocimum, Phenolics, Phytochemicals, Qualitative analysis

SUMMARY

- *Moringa oleifera* Lam. and *Ocimum tenuiflorum* L. are widly grown in India and are known for their medicinal properties. Number of secondary metabolites like phenolics and flavonoids are known to be present in both the plants. The present study was conducted with an objective to qualitatively and quantitatively compare the phenolics and flavanoids in these two medicinally important plants.
- Quantitation of total phenolics and flavanoids was done by spectrophotometrically while qualitative analysis was perfomed by paper chromatography and by phytochemical tests. Our results have shown higher phenolics and flavanoid content in Moringa leaf and flower. However, higher phenolic content was absent in Ocimum flower compared to that of Moringa. Phytochemical analysis of various metabolites such as flavonoids, tanins, sapponins, alkaloids, anthraquinones revealed that both the plant extracts were rich sources of secondary metabolites and thus tested positive for the above tests. Various flavanoids and Phenolics were identified by paper chromatography based on their Rf values and significant colors. From the above study we conclude that Moringa and Ocimum are rich in natural antioxidants hence are potent source in pharmaceutical industry.

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INTRODUCTION

A wide variety of secondary metabolites such as flavonoids and flavones are known for their antioxidants and antiradical scavenging properties.[1] Most of the phenolic and flavonoid compounds includes polyphenols such as tocopherols, flavonoids, derivatives of cinnamic acid, and other organic acids.[2] Recently, these polyphenols are gaining interest as antioxidants with potential to reduce free radical induced tissue injury.[3] Phenolics and flavonoids are the active phenolic compounds isolated from higher plants.[4] Wide range of biological activities such as anti-inflammatory, antioxidant, anticancer, and antimicrobial properties, etc., are shown by these polyphenols.[4-6]

Moringa Oleifera Lam. belonging to the family Moringaceae is a widely cultivated shrub known for its medicinal and industrial uses.[7] All the aerial parts of the plant are edible, and are consumed by human beings.[8] Leaves are used as animal forage, manure, domestic cleansing agent, and a source of biopesticide.[7] Seed oil is nonsticking, good machine lubricant and used as a source in perfumery, skin and health care.[9] The plant is also known for its antioxidant nature.[9] In our recent report increased antioxidant enzyme activity in *M. oleifera* Lam. was observed.[10] Antimicrobial, antidiabetic, anti-inflammatory, and antiulcer properties are also reported in *Moringa* plant.[8,11-14] Leaves of *M. oleifera* contain essential amino acids, carotenoids, Vitamin C, and calcium.[15,16] It is hence a source of valuable nutritional supplement in food preparations.[14] *Ocimum tenuiflorum* L. belongs to the family Lamiaceae and is commonly called as Tulsi. It is known for hydroxycinnamic acids and flavonoid glycosides.[17,18]

Although native to India, Ocimum species are widely cultivated in parts of Asia, Africa, and parts of the Mediterranean region.[19] Sweet

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basil (Shama tulsi) is known for its medicinal uses in India from ancient times.[20] *O. tenuiflorum* L. is used for treatment against a headache, constipation, kidney infections, cough, cold, and diarrhoea.[21] The medicinal properties in Tulsi are mainly due to the presence of a variety of phenolic acids such as caffeic acid, rosmarinic acid, flavonoids, and essential oils.[22] Chavicol methyl ether (estragol), linalool, eugenol, 1, 8-cineole, and methyl cinnamate are the main compounds responsible for the typical aroma in the basil plant.[23]

Considering the medicinal importance of these widely available plant species, the work was planned with an objective to qualitatively and quantitatively compare total phenolic and flavonoid content using spectrophotometric and paper chromatographic methods. Attempt was also made to identify the active metabolites in methanolic extracts of *M. oleifera* Lam. and *O. tenuiflorum* L.

MATERIALS AND METHODS

Sample collection

Fresh sample of leaves and flowers of *M. oleifera* Lam. belonging to the family Moringaceae and *O. tenuiflorum* L. (Krishna or Shama tulsi) belonging to the family Lamiaceae were collected from Chowgule College botanical garden. *M. oleifera* Lam. was authenticated by the Department of Botany, Chowgule College, Margao, Goa while, *O. tenuiflorum* L. was authenticated by Botanical Survey of India (BSI), Pune. The voucher specimens in the form of the herbarium are maintained in the Botany Department of Chowgule College (SPCC/BOT/H-438) and BSI, Pune (SS01) respectively.

Extraction of the sample

Plant material (500 g) was sun dried and homogenized overnight in 1 L of methanol. The extract was then filtered through Whatman filter paper No. 1. The filtrate was then subjected to evator rotary vaccum evaporator for evaporating the solvent.[24] The residues obtained were then dissolved in 5 mL methanol and stored at 4°C for further use. This extract was used for the analysis of phenolics and flavonoid content.

Quantitation of total phenolic and flavonoid content

Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent.[25] The plant extract (0.5 mL) was mixed with 0.5 mL of FC reagent (1:1 diluted with distilled water) and incubated for 5 min at 22°C followed by addition of 2 mL of 20% Na_2CO_3. The mixture was then incubated further at 22°C for 90 min and the absorbance was measured at 650 nm. The total phenolic content (mg/mL) was calculated using gallic acid as standard.

Total flavonoid content

The total flavonoid content (mg/mL) was determined using aluminum chloride ($AlCl_3$) method.[26,27] The assay mixture consisting of 0.5 mL of the plant extract, 0.5 mL distilled water, and 0.3 mL of 5% $NaNO_2$ was incubated for 5 min at 25°C. This was followed by addition of 0.3 mL of 10% $AlCl_3$ immediately. Two milliliters of 1 M NaOH was then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard.

Separation of flavonoids by paper chromatography

Sample preparation for flavonoids

Extraction of flavonoids for paper chromatography was based on the standard procedures.[28,29] Freshly weighed 2 g of the tissue (leaf and flower) was thoroughly homogenized in a mortar and pestle with 20 mL of 80% methanol in 1% HCl. The total volume of the extract was made to 25 mL using 80% methanol in 1% HCl. For the complete extraction of the flavonoids, the homogenate was then dark adapted for 24 h. The extract was then centrifuged at 4,000 rpm for 20 min. The supernatant was collected in a test tube and concentrated in a water bath to a final volume of 5 mL. The samples were then stored in vials at −20°C for chromatography analysis.

Separation of flavonoids by paper chromatography

Ascending paper chromatography was performed with n-butanol/acetic acid/water (4:1:5) as the solvent system.[26] Flavonoids were detected using ultraviolet transilluminator. For a better resolution of the separated flavonoids and for photography records the chromatogram was then exposed to ammonia fumes overnight.

Phytochemical screening

Active constituents in the plant extract of *M. oleifera* Lam. and *O. tenuiflorum* L. were identified and detected by performing chemical tests. Phytochemicals such as tannins, phobatannins, saponin, terpenoids such as flavonoids and alkaloids were detected based on standard tests.

Test for tannins

About 0.1 g of dried powder plant sample was boiled in 4 mL of water in a test tube and then filtered. Few drops of 0.1% ferric chloride were added to observe brownish green or blue-black coloration indicative of the presence of tannins.[30]

Test for phlobatannins

An aqueous extract of the plant sample was boiled with 1% aqueous HCL. Deposition of red precipitate was taken as an evidence for the presence of phlobatannins.[30]

Test for saponins

Powdered plant sample (0.5 g) was boiled in 10 mL of distilled water and filtered. The filtrate (5 mL) was mixed with 2.5 mL of distilled water and shaken vigorously for stable, persistent frothing. Frothing was mixed with three drops of saturated oil and was vigorously shaken again. The emulsion formed indicated the presence of saponins.[30]

Test for flavonoids

About 3 mL of dilute ammonia was added to 2 mL aqueous filtrate of each plant extract. This was followed by addition of 1 mL concentrated sulphuric acid (H_2SO_4). Yellow coloration in each extract showed the presence of flavonoids.[31,32]

Test for terpenoids (Salkowski test)

The plant extract in a final volume of 3 mL was mixed with 1 mL of chloroform and 1 mL of conc. H_2SO_4 to observe the intense red-brown coloration indicative of the presence of terpenoids.[30]

Test for reducing sugars (Fehling's test)

About 0.2 g of powdered plant samples in 1 mL ethanol was added to 3 mL of distilled water and mixed. One milliliter of Fehling's solution A and B was taken in a test tube and heated to boiling and then poured in the aqueous ethanolic plant extract. Change in color reaction detected the presence of reducing sugars.[33]

Test for alkaloids

Powdered plant sample (0.1 g) was added to 2 mL of hexane, shaken well and filtered. This was followed by addition of 3 mL 2% HCL to the above extract. The mixture was then heated and filtered. A drop of picric acid was added to the filtrate to develop yellow precipitate indicative of the presence of alkaloids.[31,32]

Test for anthraquinones

Aqueous plant extract (2 mL) was boiled with 4 mL concentrated H_2S0_4 and shaken well. Three milliliters of chloroform was then added to the mixture. The chloroform layer was then pipetted out in another test tube containing 1 mL of diluted ammonia (1:1). Anthraquinones were detected with a change in color.[33]

Statistical analysis

All experiments were repeated thrice independently with similar results. Data shown are expressed as mean ± standard deviation with readings of three samples per tissue. Data analysis was based on one-way analysis of variance. All statistical analysis was performed using Microsoft Excel Version 2007.

RESULTS

Total flavonoid content

The results of total flavonoid content are shown in Table 1. Our results with methanolic leaf and flower extract in *M. oleifera* Lam. and *O. tenuiflorum* L. showed a significant increase in total flavonoid content (#$P < 0.001$). Total flavonoid in Ocimum leaf and flower was 4.47 mg/mL and 4.54 mg/mL and that in Moringa was 4.44 mg/mL and 4.41 mg/mL respective. The flavonoid content in Ocimum flower was 1.56% more compared to the leaf extract, whereas *M. oleifera* leaf extract showed 0.68% increase in flavonoid content. Our results indicated higher total flavonoid content in Ocimum plant extract than in Moringa plant extract.

Total phenolic content

The results of total phenolic content in Moringa and Ocimum leaf and flower extracts are shown in Table 2. Our results with the methanolic extract showed significantly (#$P < 0.001$) higher phenolic content in the leaf extract of Moringa and Ocimum than in its flower extract. We observed 60.18% increase in the phenolic content of Ocimum flower compared to that of Moringa flower extract. The phenolic content in Moringa leaf was 111% more compared to its flower extract while in Ocimum leaf we observed 26.01% increase in phenolic content compared to its flower. Total phenolic content in a leaf of *M. oleifera* Lam. and *O. tenuiflorum* L. was 2.28 and 2.18 mg/mL while flower extract of the same plants showed 1.08 and 1.73 mg/mL total phenolic content.

Phytochemical tests

Our results with various phytochemical tests are shown in Table 3. The results showed that both Moringa and Ocimum are rich in tannin, saponins, flavonoids, alkaloids, anthraquinone, and reducing sugars. Terpenoids were found to be present only in Moringa leaf and flower extract and were absent in Ocimum plant extract. Phobatannins test showed negative results with both the plant extracts tested. The various test results are also shown in the Figure 1a and b.

Chromatographic separation of flavonoids

Results of paper chromatography are shown in Table 4. Flavonoids were identified based on the standard retention factor (R*f*) values. The flavonoid compounds identified in Moringa and Ocimum plant

Table 1: Total flavonoid content in *Moringa oleifera* Lam. and *Ocimum tenuiflorum* L.

Plant species	Leaf (mg/mL)	Flower (mg/mL)
Moringa oleifera Lam.	4.44±0.0045#	4.41±0.0021#
Ocimum tenuiflorum L.	4.47±0.0036#	4.54±0.0063#

The values are mean of three experiments±SD. Statistical data shows a significant difference at #$P<0.001$. SD: Standard deviation

Table 2: Total phenolic content in*Moringa oleifera* Lam. and *Ocimum tenuiflorum* L.

Plant species	Leaf (mg/mL)	Flower (mg/mL)
Moringa oleifera Lam	2.28±0.022#	1.08±0.0025#
Ocimum tenuiflorum L.	2.18±0.015#	1.73±0.0015#

The values are mean of three experiments±SD. Statistical data shows significant difference at #$P<0.001$. SD: Standard deviation

Table 3: Preliminary phytochemical analysis of two screened plant species of *Moringa oleifera* Lam. and *Ocimum tenuiflorum* L.

Phytochemical tests	*Moringa oleifera* Lam.		*Ocimum tenuiflorum* L.	
	Leaf	Flower	Leaf	Flower
Tannins	+	+	+	+
Phlobatannins	–	–	–	–
Saponin	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids (Salkowski test)	+	+	–	–
Reducing sugars (Fehlings test)	+	+	+	+
Alkaloids	+	+	+	+
Anthraquinones	+	+	+	+

+ : The presence of phytochemicals; – : The absence of phytochemicals

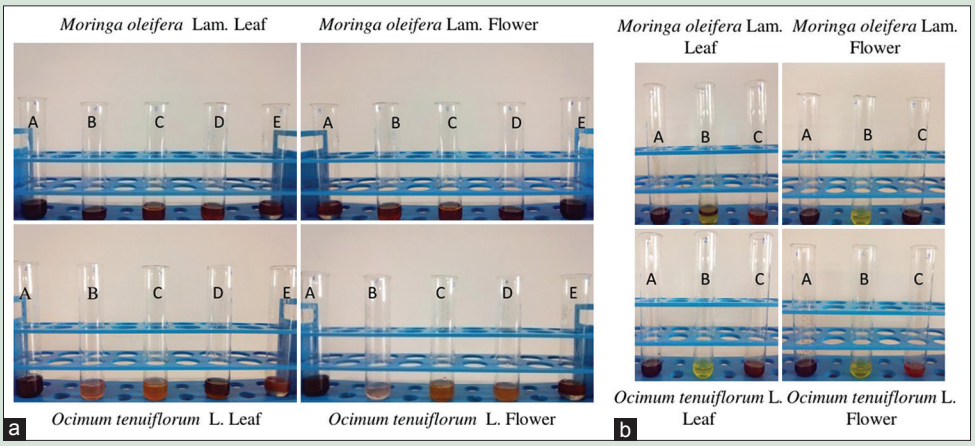

Figure 1: (a) Preliminary phytochemical analysis of two screened plant species *Moringa oleifera Lam*. and *Ocimum tenuiflorum L*. A = Test for tannins, B = test for phlobatannins, C = test for saponins, D = test for flavonoids, E = test for terpenoids. (b) Preliminary phytochemical analysis of two screened plant species *Moringa oleifera Lam*. and *Ocimum tenuiflorum L*. A = Test for reducing sugar, B = test for alkaloids, C = test for anthraquinones

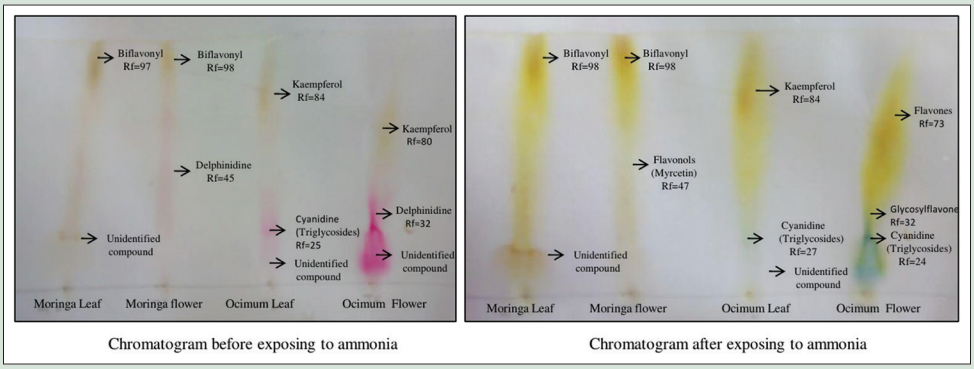

Figure 2: Paper chromatogram of *Moringa oleifera Lam*. and *Ocimum tenuiflorum L*. showing seperation of flavonoids with and without ammonia

Table 4: R*f* values of flavonoid compounds showing respective coloration with and without ammonia in *Moringa oleifera* Lam. and *Ocimum tenuiflorum* L.

Plant species	Spot number	R*f*×100	Spot color with ammonia	Compound	Spot number	R*f*×100	Spot color without ammonia	Compound
Moringa oleifera Lam.								
Leaf	1	22	Brown	-	1	20	Brown	-
	2	98	Brown	Biflavonyl (kayaflavone)	2	97	Brown	Biflavonyl
Flower	1	47	Yellow	Flavonols (myrcetin)	1	45	Light pink	Delphinidine
	2	98	Brown	Biflavonyl	2	98	Brown	Biflavonyl
Ocimum tenuiflorum L.								
Leaf	1	12	Light blue	-	1	12	Purple	-
	2	27	Purple	Cyanidin 3-(2a glucosylglucoside-5-glucoside) (triglycosides)	2	25	Pink	Cyanidine 3-rhamnosylglucoside-5-glucoside (triglycosides)
	3	84	Brown	Kaempferol	3	84	Brown	Kaempferol
Flower	1	12	Blue	-	1	32	Pink	Delphinidine
	2	24	Purple	Cyanidine 3-rhamnosylglucoside-5-glucoside (triglycosides)	2	80	Brown	Kaempferol
	3	32	Yellowish green	Glycosylflavones				
	4	73	Brown	Flavones				

extract included Flavonols (myrcetin) (R*f*-47), flavones (R*f*-73), biflavonyl (kayaflavone) (R*f*-98), kaempferol (R*f*-84), delphinidin (R*f*-45), triglycosides (R*f*-27), and glycosylflavones with (R*f*-32). Figure 2 shows the chromatographic separation of flavonoids with and without ammonia.

DISCUSSION

High antioxidant activity is reported from various medicinal plants.[34-36] Phenolics and flavonoids are the common antioxidants known in plants.[10,16] The present work was carried out to compare the phenolics and flavonoid content in medicinal plants such as *M. oleifera* Lam. and *O. tenuiflorum* L. The study showed more phenolic content in leaves of Moringa and Ocimum. However, flavonoid content was more in leaf and flower extracts of Ocimum. Various reports also exist that indicate *M. oleifera* as a rich source of phenolic compounds.[37] Similarly, genus Ocimum is also known to be rich in phenolic compounds and thus is widely used in traditional systems of medicine.[18] Phenolics and flavonoids have at least one hydroxyl ion substituted with aromatic ring and can form chelate complexes with the metal ions thereby getting easily oxidized and are the means for donating electrons to scavenge free radicals.[16,38] Higher phenolic content in *M. oleifera* Lam. is also correlated with increased antioxidant activity.[39] Good amount of total flavonoid and phenolic content has been reported in methanolic crude extracts of *Thymus vulgaris*.[40] The linear correlation between phenolic content and antioxidant activity has also been reported.[41,42] In our earlier report in Moringa, we have also shown the correlation of increased antioxidant activity with increasing phenolic and flavonoid content.[10]

Variation in antioxidant properties in relation to leaf position is seen in Ocimum.[43] Similar observation is shown by no of reports.[39] In the present study, chromatographic separation of flavonoids in extracts of Moringa and Ocimum (leaf and flower) showed the presence of flavones, flavonols, biflavonyl, kaempferol, delphinidin, triglycosides and glycosylflavones. Flavonoids like quercetin and kaempferol are reported in ethanolic leaf extracts of *M. oleifera*.[16] Reports are available that show the presence of flavones, flavonols, flavonoid aglycone, tannins, polyphenols, etc., in Ocimum species.[44] Myrecetin and kaempferol are the major flavonoid observed in *Cyprus rotundus*.[45] Leaves of *Cistus salvifolius* showed the presence of kaempferol, quercetin and Myricetin.[46] Flavonoids such as luteoline, kaempferol and quercetin are found in the bark and leaves of *Terminalia arjuna*. All these flavonoids are known for their strong antioxidant properties.[47] Antioxidant activity of *M. oleifera* plant extract is correlated with the presence of flavonoid such as kaempferol.[48] Our results are thus in accordance to the above findings.

Phytochemicals are the chemical constituents in plants with distinct physiological action on the human body.[49] Alkaloids, flavonoids,

phenolics, terpenoids, and essential oils are some of the important bioactive phytochemicals.[11] It is observed that the geographic location of the plant and the solvent system used in the extraction process may act as determining a factor for the distribution of these phytochemicals.[50] A number of reports are available that shows the presence of phytochemicals such as quercetin, glycosides, rutin, kaempferol glycosides, tannins, and chlorogenic acids.[51-53] Major phytochemicals reported in *M. oleifera* are quercetin, glycosides, rutin, kaempferol glycosides and chlorogenic acids.[54] Rosmarinic acid is the predominant phytochemical reported in the flower and leaf extracts of *Ocimum basilicum*.[55] In the present study with Moringa and Ocimum, we report the presence of flavonoids, phenolics and antioxidants. Like earlier reports, we also hypothesize that antioxidant nature of *M. oleifera* Lam. and *O. tenuiflorum* L. may be due to the increase in phenolic and flavonoid content. However, further quantitation and identification of the chemical structure of these phytochemicals need to be undertaken by high-performance liquid chromatography, gas chromatography mass spectrometry, nuclear magnetic resonance spectroscopy. Identification of active constituent in these medicinal plants is thus highly significant in food and pharmaceutical industry because the natural antioxidants are less harmful than synthetic antioxidants. However, physicochemical evaluation of the drug is a very important parameter to study the quality of the plant material for future research applications.

CONCLUSIONS

From the present study, we conclude that the methanolic leaf and flower extract of *M. oleifera* Lam. and *O. tenuiflorum* L. exhibit high antioxidant and phytochemical potential. The plant extracts contains large amounts of flavonoids, phenolics, and tannins. The study showed that both the plants are a source of significant natural antioxidants and may be useful in protection against oxidative stresses. *O. tenuiflorum* L. shows more antioxidant activity in comparison to *M. oleifera* Lam. Thus, there exist a strong correlation between the increase in phenolics and flavonoid content with increased antioxidant enzyme activity in both the plant species studied.

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Conflicts of interest

There are no conflicts of interest.

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