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ANTIOXIDANT ACTIVITY MORINGA OLEIFERA LEAVES IN DIFFERENT DRYING METHODS

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ABSTRACT

Moringa oleifera is an important source of antioxidants, tools in nutritional biochemistry that could be beneficial for human health. The leaves and flowers are used by the population with great nutritional importance. This work investigates the antioxidant activity of M. oleifera from three different dried leaves (shade drying, cabinet drying and fludized bed drying) of blanched and unblanched samples. Antioxidant components were detected in blanched and unblanched leaves of shade, cabinet and fludized bed drying methods. The radical scavenging capacity (RSC) of extracts was determined using the DPPH (1,1- diphenyl- 2- picryl hydrazyl) by spectrophotometric method and the spectrophotometric assays were recorded (517 nm) and it was noted that the ethanol extracts showed maximum antioxidant activity of blanched samples such as 65.78%, 66.09% and 71.40% respectively. The presence of polyphenols was higher in blanched moringa leaves dried in shade drying followed by cabinet drying. The fluidized bed dried leaves exhibited 1.19% of total polyphenolic content. This might be due to the processing techniques of leaves. The best RSC was obtained with blanched leaf samples. This research proves M. oleifera leaves possess higher antioxidant activity and it is used as an alternative source for nutritional supplements that support the use of the plant tissues as food sources.

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Introduction

The medicinal plants have greatest potential for benefitting people, especially those living in countries with poverty, poor health and malnutrition. According to the World Health Organization (WHO), about 80 percent of the people living in rural areas depend on medicinal plants as primary health care system [1]. From the last two decades, much importance has been given on herbal medicines, because herbal drugs constitute a major share of all official medicinal systems like ayurveda, unani, yoga, siddha, homeopathy and naturopathy. More than 70 percent of people still use non-allopathic system of medicine [12].

Antioxidants benefit the body by neutralizing and removing the free radicals from the bloodstream. Among the Green Leafy Vegetables (GLV), *Moringa oleifera* is one of the best example, which contains essential nutrients, enzymes, omega oils, minerals, antioxidants and phytochemical compounds all found in one leaf, hence it is called as "Green Gold" [2]. It is the most important nutrient rich plant of the planet.

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Moringa oleifera belongs to family moringaceae, *Moringa oleifera* is usually mentioned in literature as Moringa or Drumstick and it is called as "Nebedaye" which means never die in many African languages [3]. It is a small, fast growing ever green, delicious tree that usually grows up to 10 or 12 meter in height. It has a spreading, open crown of drooping, fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark.

Moringa leaf is an outstanding source of nutritional components especially essential amino acids, vitamins, minerals and β-carotene [4]. Apart from nutritional benefits, the ethanol extract of the leaves prevented cyclophosphamide – induced micronucleus formation and DNA damage in mice [5, 6]. reported that the leaf extract had potent antiproliferative activity and apoptosis inducing capacity on tumor (KB) cell line and it also increased the cytotoxicity of chemotherapy on pancreatic cancer cells [7].

Hence, the present study investigates the antioxidant content of the different drying methods of moringa leaves with two treatments.

Patients and methods

Chemicals

All chemicals and solvents were of analytical grade. The chemicals were purchased from THE I.L.E.& Co, Madurai.

Sample preparation from dried moringa leaves

Moringa leaves dried in different methods were powdered, and the plant material (300g) was extracted with ethanol, in Soxhlet apparatus for 10 hours.

The last traces of the solvent were removed and concentrated under vaccum by using a rotary evaporator [20]. The concentrated extract was used for the estimation of tannin, phenolic compounds, alkaloids, flavonoids and antioxidant activity. The phytochemical content of moringa leaf powder was done by adopting the procedure suggested by [8,9].

The following methods were adopted for estimation of tannins, polyphenols, alkaloids flavonoids and antioxidant activity.

Estimation of tannin content (Indigo Sulphonic Acid - Titrimetric Method (U.S.S.R.P))

Weighed accurately about 1gm of the powdered moringa leaf powder sample(W) was transferred into a 250ml glass stoppered flask and added water (100ml) was shaked for 1 hr and kept overnight. The solid material was allowed to settle and the liquid through was filtered a filter paper of 12cm diameter, discarding first 20ml of the filtrate.

10ml of filtrate was transferred to one litre conical flask, added water (750ml) and Indigo Sulphonic acid solution (25ml). Titrate with 0.1N potassium permanganate solution was shaked vigorously till a golden-yellow end point (T_2) is reached. A blank determination (T_1) was performed and necessary correction was made.

Calculation:

$$\text{Quantity of Total Tannins (\%)} = \frac{T_2\text{-}T_1 \text{ X Actual Normality X Strength X } 1000}{W \text{ X } 0.1}$$

Estimation of total polyphenol (Folin- Ciocalteu method)

An aliquot of methanolic extract 5ml was mixed with 0.2 ml of Folin Ciocalteu reagent and 2ml distilled water in to a 10ml test tube. The mixture was warmed and vortexed for 20 sec and kept aside for 10 minutes and 1ml of 20% solution sodium carbonate was added. The mixture was incubated in a water bath at 40^0C for 30 minutes. After cooling to room temperature, the absorbance was taken at 670nm using UV-spectrophotometer. Gallic acid solution was used as standard. The total polyphenol was calculated by using the formula,

$$\text{Total amount of polyphenol} = \frac{\text{O.D of Test solution}}{\text{O.D of standard solution}} \times 100$$

Estimation of total alkaloids (titre method)

A sample of 5gm of methanolic extract was treated with 50ml of methanol- chloroform (2:1) and filtered. The extract is mixed with of 100ml 0.8% of sodium sulphate. The upper chloroform layer was separated, dried and the residue was dissolved in 15ml of 2N sulphuric acid. Then the solution was heated for 2hrs and made basic with 10ml of 4N sodium hydroxide. The alkaline layer was extracted with 30ml of benzene in 3 aliquots and collected the benzene layer and evaporated to dryness until it formed a residue. The residue was dissolved with 5ml of methanol. The samples were titrated with a solution of 0.067 % bromo phenol blue and 10 % phenol in absolute methanol. A blank is performed without extract.

$$\% \text{ of Alkaloids} = \frac{(\text{Assay- blank}) \times \text{Eq. Wt. factor} \times \text{strength of phenol}}{\text{Weight taken}} \times 100$$

Weight taken

Estimation of total flavonoid (colorimetric method)

An aliquot of 5ml methanolic extract was taken in a test tube containing 4ml of distilled water. 0.5ml of 5% sodium nitrate solution was added to the test tube. After 5 minutes, 0.5ml of 10% aluminum chloride was added and stayed for 6 minutes before addition of 2ml of 1.0M NaoH and adjust the total volume to 10ml with distilled water and then vortexed for 10 second. The absorbance was measured at 510nm. Rutin solution was used as standard. The total flavonoid content was calculated by using the formula.

$$\% \text{ of Flavonoid} = \frac{\text{O.D of Test solution}}{\text{O.D of standard solution}} \times 100$$

Estimation of Antioxidant activity (Free radical scavenging activity by DPPH method)

The DPPH (1,1- diphenyl- 2- picryl hydrazyl) radical scavenging activity was measured by spectrophotometric method. The methanolic and ethanolic solution of extracts (100mcg/ml) was mixed with 400µl of DPPH ethanol solution at a ratio of 1:3. The mixture was kept in dark at room temp for 90 min. The absorbance of the resulting solution was measured at 517nm. The capability of scavenging DPPH radical was calculated by the following equation. Analysis of all samples was run in triplicate. Ascorbic acid was used as standard (100mcg/ml).

Abs – Absorbance.

Control abs = 0.640

Percentage of scavenging activity = (1- abs of sample / abs of control) × 100

Statistical analysis

The obtained data were expressed as mean, standard Deviation, Factorial Randomized Block Design and Dunnets test.

The data collected from biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Newman-Keuls multiple range test (NKMRT). Values were considered statistically significant at $p<0.01$.

Results and conclusions

From the results, it was observed that the presence of tannins, polyphenols, alkaloids and flavonoids was increased to various extent, depending on the type of drying methods. The presence of the tannins, polyphenols, alkaloids and flavonoids was high in the blanched samples when compared to unblanched samples.

Tannin and poly phenol content of M. Oleifera leaves extracts

The tannin content of M. Oleifera leaves was determined by Indigo Sulphonic Acid - Titrimetric Method. The composition varied from 2.97 to 3.30 percent. From the above table, it was observed that shade dried blanched sample exhibited higher tannin content

of 3.30 percent followed by fluidized bed dried samples. [10] studied the total tannins content of different medicinal plants, they established that moringa leaves had the highest content of tannins among the other medicinal plants studied.

The polyphenol content was determined by Folin- ciocalteau assay. The presence of polyphenols was higher in blanched moringa leaves dried in shade drying followed by cabinet drying. The fluidized bed dried leaves exhibited 1.19% of total polyphenolic content. This might be due to the processing techniques of leaves. Dietary phenolic compounds have generally been considered as non- nutrients but their strong antioxidant property has medicinal and nutritional interest. Plant phenols are powerful antioxidants and their activity is related to their chemical structure. Recently [11] reported that the aqueous extract of M. oleifera leaves are rich in polyphenols and had DPPH radical scavenging activity.

Drying methods	Tannins (%)	Total polyphenols (%)	Total alkaloids (%)	Total flavonoids (%)	Antioxidant activity (%)
Shade drying					
Blanched	3.30	1.36	1.46	1.60	65.78
Unblanched	3.09	1.19	1.34	1.20	57.96
Cabinet drying					
Blanched	3.12	1.36	1.53	1.20	66.09
Unblanched	3.05	1.19	1.34	1.00	60.93
Fluidized bed drying					
Blanched	3.16	1.19	1.46	1.20	71.40
Unblanched	2.97	1.02	1.31	1.00	62.81

[13] reported that total phenolic contents were increased in green beans (114%) and spinach (101%) after boiling for 5 minutes. [17] reported that most of the phenolic compounds trapped in fibre of green leafy vegetables are actually more available on cooking than the raw. According to [17], blanching of green leafy vegetables in boiled water for 5 minutes increased the polyphenols. The results of the present study are in agreement with the above findings.

The higher the quantity of phenols predicts the higher antioxidant property. Because, in the case of phenolic compounds, the ability of the phenolics act as antioxidants depends on the redox properties of their phenolic hydroxyl groups, that allow them to act as reducing agents, hydrogen- denoting antioxidants, oxygen quenchers [21]. [14] reported there is an excellent correlation between antioxidant activity with total poly phenolic content.

Flavonoids and alkaloids content of M. Oleifera leaves extracts

Total alkaloids content was showed 1.53% in cabinet drying of blanched samples which is higher when compared to other drying methods studied. The shade and fluidized dried samples were shown 1.46% total alkaloids.

The total flavonoid contents were successfully analyzed, the result showed that the shade dried moringa leaves sample showed highest flavonoids content of 1.60% in blanched samples followed by cabinet and fluidized bed dried blanched moringa leaves respectively. The total flavonoid content of the blanched samples was higher than the unblanched samples. This indicates possible release of flavonoids during cooking. [10] studied the total flavonoid content in moringa oleifera leaves and expressed as catechin

equivalents, they reported that the sun dried moringa leaves had 0.44% of flavonoids. The plant flavonoids in general are highly effective free radical scavengers and antioxidant properties.

The non-enzymatic antioxidants like phenols, flavonoids and tannins act by one or more of the mechanisms like reducing activity, free radical scavenging, and potential complexing of pro- oxidant metals and quenching of singlet oxygen.

Antioxidant activity og M. Oleifera leaves

From the table, it was noted that the ethanol extracts showed maximum antioxidant activity of blanched samples such as 65.78%, 66.09% and 71.40% respectively. The unblanched samples had less free radical scavenging activity when compared to blanched samples. The results proved that blanching enhanced the free radical activity. [15] found that antioxidant capacity of cooked artichokes, enormously increased after cooking, particularly after steaming the antioxidant activity has increased up to 15 fold. The increase in antioxidant profile is probably due to matrix softening and increased extract of compounds. Singleton and Rossi [18] reported that the antioxidant activity of leaf extracts is as a result of phenolic compounds. The antioxidant activity of phenolic compounds is mainly due to their redox properties, quenching singlet and triplet oxygen or decomposing peroxides. Recent studies have shown that many polyphenols contribute significantly to the antioxidant activity and act as highly effective free radical scavengers due to their redox properties that allow them to act as reducing agents [16].

[19] studied the preliminary phytochemical screening and antioxidant activity of five different extracts like methanol, ethanol, petroleum ether, n-hexane, chloroform, ascorbic acid and butylated hydroxyl toluene of moringa oleifera leaf, the leaf exhibited significant DPPH free radical scavenging activity with the ethanol and methanol having of 62.09 and 68.32 respectively of dried moringa leaves powder.

The data obtained in the present study suggests that the extract of moringa leaves have potential antioxidant.

Conclusion

Conclusively, the M. Oleifera leaves extract shows high antioxidant activity and leaves have high medicinal value. Various parts of this plant such as leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepato protective, antibacterial and anti-fungal activities and are being employed for the treatment of different ailments in the indigenous system of medicine.

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