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Estimation of functional characteristics of Moringa super greens powder

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Abstract

Antioxidant activity of the formulated Moringa Super Greens Powder containing different parts of *Moringa oleifera* was assessed. The methanolic extract of MSGP was used to determine the percentage inhibition in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, total phenolic content (TPC) and total flavonoid content (TFC). The results of TPC and TFC were compared with the *M. oleifera* plant parts (leaf, pod with pulp and flower), while the percentage inhibition was compared with standard antioxidants (both natural and synthetic). In addition, preliminary phytochemical screening of MSGP was also carried out. The results thus revealed that MSGP could be an effective alternative to synthetic antioxidants.

Keywords: Antioxidant, moringa super greens powder, *Moringa oleifera*, total phenolic content, total flavonoid content, DPPH

Introduction

Phytochemicals found in plant sources present enormous health benefits, as they operate against oxidative stress in the body by maintaining a balance between oxidants and antioxidants. Most of such phytochemical substances hold no harmful effects inside human body. Prevailing sedentary life- style and fast food practices have been the cause for oxidative stress and soaring rates of non- communicable diseases worldwide. These phytochemical components are responsible for the antioxidant property of plant sources. Antioxidants present a vital role in restraining oxidative damage that leads to various chronic diseases like cancer, atherosclerosis, neurodegenerative diseases, age-related diseases etc. Some natural enzymatic antioxidants are synthesized naturally in the body; while some are obtained through food sources.

In the recent decades, there has been a spike in the utilization of natural components present in plants as a source of natural antioxidants and therapeutic antioxidants, particularly owing to the increasing risk factors like high toxicity of some synthetic antioxidants. *Moringa oleifera* is one such important plant source that presents efficient antioxidant property due to the presence of high numbers of phytochemical substances contributing to the antioxidant property.

Various parts of the *Moringa oleifera* and their active constituents are known to possess diverse antioxidant property. The effective antioxidant activities of various solvents (methanol, acetone and water) extracts of drumstick leaves have been analyzed in different *in vitro* test systems and it was observed that methanol was the most effective solvent for the extraction of antioxidant components from the *Moringa oleifera* (Arabshahi *et al.*, 2006).

The present study evaluates the phytochemical property of the formulated Moringa Super Greens Powder (MSGP) and addresses its use as a natural antioxidant. The DPPH free radical scavenging activity, total phenolic content (TPC), and total flavonoid content (TFC) of the MSGP formulation has been analyzed. In addition, a preliminary phytochemical screening was also carried out

Materials and Methods

Formulation of Moringa Super Greens Powder (MSGP)

MSGP formulation was optimized using Linear Programming model. The objective of the present study was to use linear programming model to find the optimal combination of proposed parts of *Moringa oleifera* (leaf, pod with pulp and flower) in the design of Moringa based formulation that fulfills predefined nutritional requirements of both men and women

(moderate activity), that can be utilized as a potential food fortificant. The objective function was to minimize the cost of the formulation to be developed; while the decision variables x_1 , x_2 , and x_3 were weights of ingredients (MOLP- *Moringa oleifera* Leaf Powder, MOPP- *Moringa oleifera* Pod with pulp Powder and *Moringa oleifera* Flower Powder - MOFP, respectively) required in the formulation to meet the defined constraints.

The model recommended that a combination of 332.1100 g of MOLP and 57.3905 g of MOFP could meet the daily nutritional requirements of both men and women (moderate activity).

Qualitative screening

Sample preparation

The crude extracts was prepared by dissolving the dried powdered sample in dilute hydrochloric acid and then filtered with filter paper.

Alkaloids

Wagner's test: 2 ml of extract was treated with Wagner's reagent (iodine and potassium iodide). The formation of reddish brown precipitate indicates the presence of alkaloids (Kumar *et al.*, 2014)^[8].

Flavonoids

H₂SO₄ test: A fraction of the extract was treated with concentrated H₂SO₄ and observed for the formation of orange color that indicates the presence of flavonoids (Kumar *et al.*, 2014)^[8].

Saponins

Froth test: 2 g of the powdered sample was boiled with 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. Three drops of olive oil was added to the froth and then shaken vigorously. The formation of emulsion indicates the presence of saponins (Kagbo *et al.*, 2009)^[7].

Phenols

Ferric chloride test (Brayer's test): The extract was treated with three to four drops of ferric chloride solution. The formation of bluish-black color indicates the presence of phenols (Prashant *et al.*, 2011)^[10].

Tannins

Ferric chloride test: Few drops of 0.1% ferric chloride was added to the extract and observed for brownish green or a blue-black coloration for a positive result (Trease *et al.*, 2002)^[11].

Steroids

Salkowski test: 2 ml of aqueous extract was added to 2 ml of chloroform and 2 ml of H₂SO₄ followed by vigorous shaking. The formation of red chloroform layer and greenish-yellow fluorescence in the acid layer indicates the presence of steroids (Harsha *et al.*, 2013)^[6].

Terpenoids: 3 ml of the extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulfuric acid was added and heated for 2 min. The formation of greyish color indicates the presence of terpenoids (Pradeep *et al.*, 2014)^[9].

Anthraquinone

Borntrager's test (for free anthracene derivatives): 5 ml of chloroform was added to 0.5 g of powdered sample, then shaken for 5 min and filtered. To the filtrate, equal volume of 10% ammonia solution was added. A pink, red, or violet color in the aqueous layer after shaking indicates the presence of free anthraquinone (Sofowora, 1993)^[12].

Anthocyanin

2 ml of aqueous extract was mixed with 2 ml of 2N HCl and ammonia. The appearance of pink-red which turns to blue-violet indicates the presence of anthocyanin (Harsha *et al.*, 2013)^[13].

Sample preparation for phytochemical assays

The sample preparation was as per the method given by Nambiar *et al.* (2013)^[15] with slight modifications. All sample extracts were prepared in methanol solvent. Dissolve 1 g of dried sample in 50 ml solvent (methanol). Shake it for 30 min in magnetic shaker or water-shaker bath. And add 20 ml of solvent to the Supernatant. Again shake it for 30 min. Centrifuge and separate supernatant. Make volume upto 50 ml with the help of the solvent. The extract was then stored at refrigeration temperature. The above extracts were used for the following assays.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The free radical scavenging activity of the *Moringa* extracts and the standard solution (ascorbic acid) were determined by the method as given by Alhakmani *et al.* (2013)^[11]. The assay mixture containing 2 ml of 1.0 mmol/l DPPH radical solution prepared in methanol and 1 ml of standard solution of different concentrations (10-500 µg/ml). The solution was rapidly mixed and incubated in dark at 37° C for 20 minutes. The decrease in absorbance of each solution was measured at 517 nm using UV/Vis-spectrophotometer. Ascorbic acid was used as positive control whereas DPPH radical solution with 1 ml ethanol was taken as blank.

Calculation was done using the formula

$$\% \text{Inhibition} = \frac{(\text{Optical Density of Control}) - (\text{Optical Density of Sample})}{(\text{Optical Density of Control})} \times 100$$

Mean ± SD was calculated for each sample. The resultant values were expressed as percentage radical inhibiting property.

Total Phenolic Contents (TPC)

The Folin-Ciocalteu micro method of Wangcharoen *et al.* (2013)^[16] was used. 20 µl of the extract solution were diluted with deionised water to 4.8 ml and 300 µl Folin-Ciocalteu reagent were added and shaken. After 8 min, 900 µl of 20% sodium carbonate were added with mixing. The mixture was allowed to stand at 40°C for 30 min. before the absorbance at 765 nm was read. Gallic acid (0-600 µg/ml) was used as standard and the results for TPC were reported as mg gallic acid equivalent per gram (dried leaves) sample.

Total Flavonoid Content (TFC)

The total flavonoids were measured using the Aluminium Chloride colorimetric method as given by Mehra *et al.* (2015)^[14] and the results were expressed in terms of mg quercetin equivalents (QE)/g of sample. Quercetin was used as a

standard for the calibration curve (20-120 µg). The sample extract (250 µl) was added to 4.5 ml distilled water, followed by 5% NaNO₂ (0.03 ml). After an incubation of 5 minutes, AlCl₃ (0.03 ml, 10%) was added at 25 °C. At the sixth minute, the reaction mixture was treated with 2 ml of 1M NaOH. The reaction mixture was then diluted to 10 ml using distilled water and absorbance was measured at 510 nm.

Result and Discussion

Phytochemical analysis of MSGP

Table 1: Qualitative screening of phytochemicals

Phytochemical	Presence/Absence
Alkaloids	+
Flavonoids	+
Phenols	+
Terpenoids	+
Antraquinone	+
Saponins	+
Tannins	+
Steroids	+
Anthocyanin	+
Quinone	+

+ indicates presence of the specific phytochemical

Qualitative screening for the above mentioned phytochemicals were carried out for the MSGP sample. The screening indicated the presence of all the tested phytochemicals. Phytochemicals, in general, present an extremely diverse range of biochemical and pharmacological properties. Some of its role includes anti-inflammatory agent, antioxidant, antiparasitic, antispasmodic, antipyretic, antimutagenic, anti-allergic, anti-edematous, anticancer activities, therapeutic effect on rheumatoid arthritis and improved cognitive functions (Godwin., 2018) [5].

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

Table 2: DPPH free radical scavenging assay (Mean±S.E) @

Phytochemical property	DPPH (%)
MSGP	85.602±1.040
MOLP	79.12±0.458
MOPP	27.682±0.831
MOFP	58.42±0.970
Ascorbic acid	91.670±1.611
Butylated Hydroxy Toulene	81.670±1.011

@Average of six trials

The scavenging effect of methanolic extract of MSGP formulation on DPPH radical was compared with two standards: Ascorbic acid (natural antioxidant) and Butylated Hydroxy Toulene (BHT) (synthetic antioxidant). From table 2, the mean ± S.E value of percentage inhibition of methanolic extract of MSGP, MOLP, MOPP and MOFP were found to be 85.66±2.547, 79.12±0.459, 27.682±0.831 and 58.42±0.970, respectively. The mean ± S.E values of percentage inhibition of ascorbic acid and Butylated Hydroxyl Toluene were found to be 91.370±01.611 and 81.670±1.011, respectively.

The order of the DPPH free radical scavenging activity (high to low) of samples is: MSGP> MOLP>MOFP >MOPP. The

results provide us the evidence that such natural plant based sources could be a great alternative to conventional antioxidant.

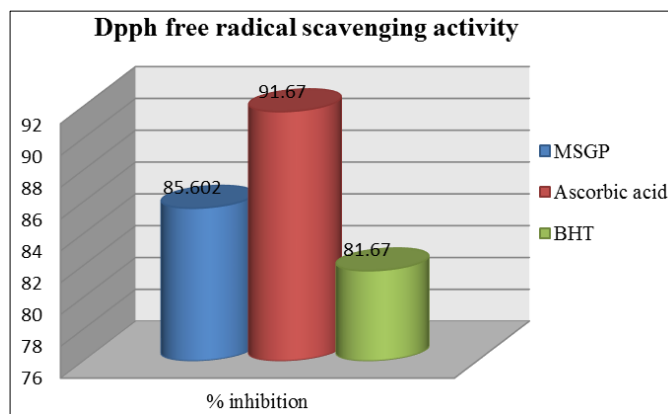


Fig 1: Comparison of scavenging activity of MSGP and standard

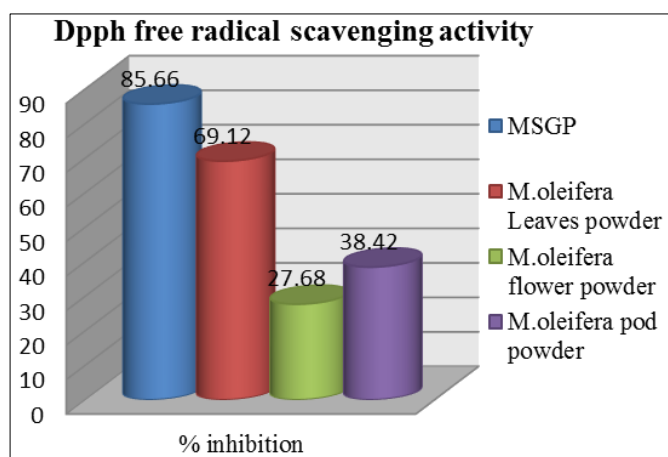


Fig 2: Comparison of scavenging activity of MSGP, MOLP, MOPP and MOFP

Total Flavonoid Content (TFC)

Table 3: Total Flavonoid Content of MSGP (Mean±S.E) @

Sample	TFC (mg Quercetin equivalents (QE)/g sample)
MSGP	84.904±4.13
MOLP	80.394±1.246
MOPP	7.140±0.210
MOFP	69.749±1.164

@ Average of six trials

TFC was determined using the calibration curve plotted between concentration (µg/ml) vs. absorbance (OD). The calibration curve for quercetin (20-120µg/ml) as standard is given below (figure 4). The linear equation for the calibration curve for quercetin was y = 0.010x - 0.063, R² = 0.969. The coefficient of determinants (r²) was higher than 0.969, which indicates that the data is closest to the line of best fit. From table 3, mean ± S.E values of TFC of MSGP, MOLP, MOPP and MOFP were found to be 84.904±10.22, 80.394±1.246, 7.140±0.210 and 69.749±1.164, respectively. From the results, the order of total flavonoid content (from high to low) was as follows: MSGP>MOLP>MOPP>MOFP. The TFC of MSGP and MOLP were closer while MOFP had lower TPC values.

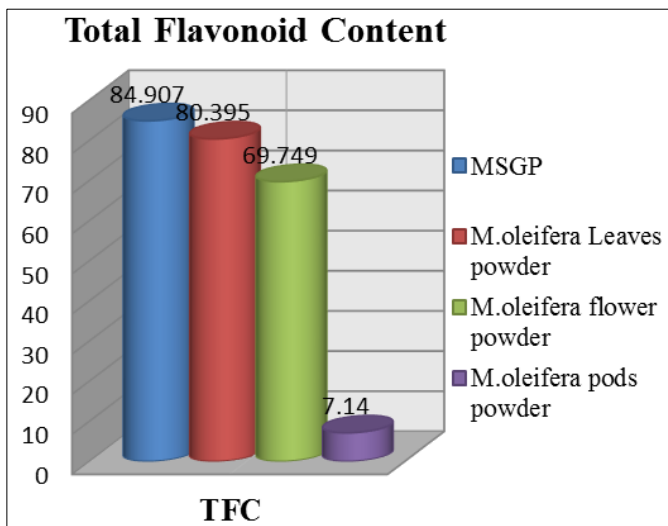


Fig 3: Comparison of Total Flavonoid Content of MSGP, MOLP, MOPP and MOFP

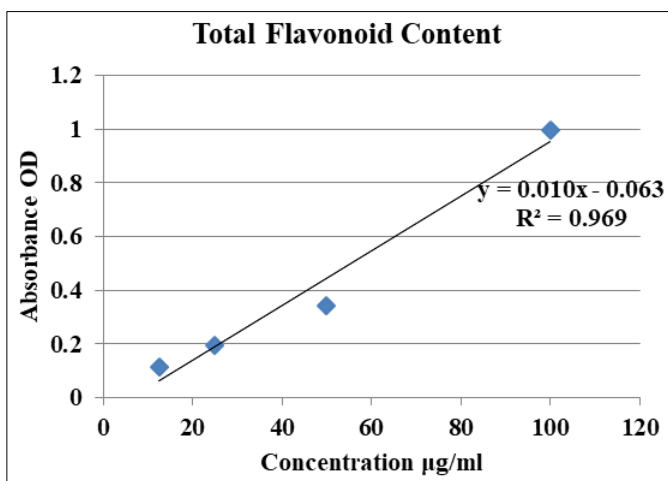


Fig 4: Calibration curve for Total Flavonoid Content (Aluminium Chloride colorimetric method)

Total Phenolic Content (TPC)

Table 4: Total Phenolic Content of MSGP (Mean±S.E) @

Sample	TPC (mg Gallic Acid Equivalent (GAE) per g sample extract)
MSGP	177.61±5.778
MOLP	142.372±1.412
MOPP	53.058±0.364
MOFP	109.559±0.890

@ Average of six trials

TPC was determined using the calibration curve plotted between concentration (µg/ml) vs. absorbance (OD). The calibration curve for gallic acid (0-600 µg/ml) as standard is given below (figure 6). The linear equation for the calibration curve for quercetin was $y = 0.003x + 0.033$, $R^2 = 0.999$. The coefficient of determinants (r^2) was higher than 0.999, which indicates that the data is closest to the line of best fit. From table 4, the mean ± SE values of TPC for MSGP, MOLP, MOPP and MOFP samples were recorded to be 177.61±5.778, 142.372±1.412, 53.058±0.364, and 109.559±0.890, respectively. The total phenolic content (TPC) of samples can be ranked as (high to low): MSGP>MOLP> MOFP> MOPP. The extract of MSGP studied presented appreciable amount of total phenolic content on

comparison with MOLP, MOPP and MOFP.

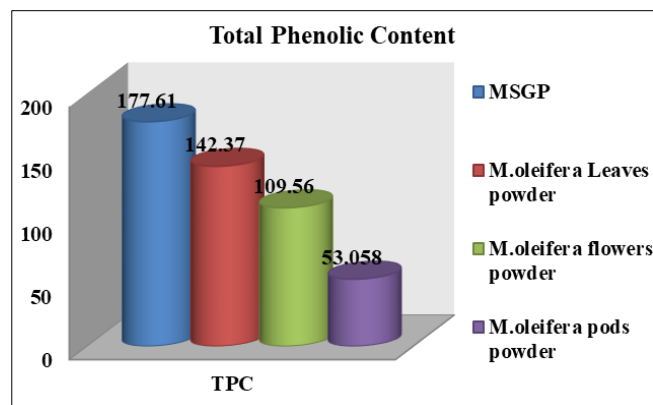


Fig 5: Comparison of Total Phenolic Content of MSGP, MOLP, MOPP and MOFP

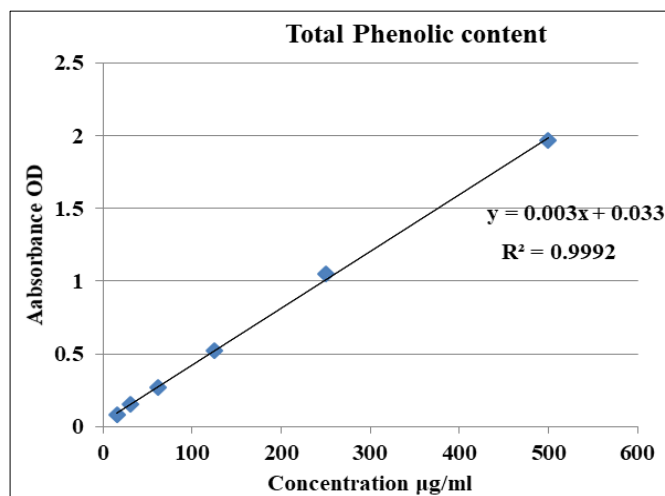


Fig 6: Calibration curve for Total Phenolic Content (Folin-Ciocalteu method)

Conclusion

Plants are rich source of phytochemicals, that have proven therapeutic and biochemical roles in many conditions. Antioxidant property is one such important biological role offered by these phytochemical substances. MSGP formulation was found to contain good amount of DPPH radical scavenging activity, phenolic content and flavonoid content. The results of this study showed that the MSGP formulation possess potent antioxidant property, thus acting as a natural source of antioxidants. The results of phytochemical analysis shows that MSGP formulation has potent antioxidant effects and may provide significant health benefit as well as supplementary sources for synthetic antioxidants.

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