



# Comparative assessment of Phytochemicals Present in *Moringa oleifera* leaf and Branded Moringa leaf Powder

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**Abstract:** The present investigation was aimed for comparative assessment of phytochemicals present in *Moringa oleifera* leaf and branded moringa leaf powder. Natural and branded powders (carbamide forte and Himalayan organics) were taken as samples. Both methanolic & ethanolic extracts of natural and branded powders showed varied amounts of the constituents like alkaloids, flavonoids, tannins & saponins. Natural leaf powder and samples of brand 2 in ethanolic extracts have higher amounts of alkaloids. In case of flavonoids, Natural sample in methanolic extract had higher amount as compared to the other extracts. All the samples lack terpenoids. Variations in phytochemical constituents among natural and branded samples indicate that some alteration may happened during processing and preservation. Branded leaf powders must be labeled with the exact amounts of phytochemicals present, as this will help consumers to be aware about their constituents and may use them as herbal supplement/remedies. Therapeutic role of plant is due to phytochemicals present in them. Any alteration either by addition of toxins or increase in concentration of any constituent may show negative impact or neutral role in curing certain diseases. Hence present work justifies, constituents of natural and branded powder of moringa varies during the process of manufacturing, processing and preserving.

**Index Terms** - *Moringa oleifera*, phytochemicals, Thin Layer Chromatography (TLC), qualitative, quantitative, therapeutic.

## I. INTRODUCTION:

*Moringa oleifera* is referred as 'Drum stick Tree' and belongs to the family Moringaceae and it is considered as native plant of India, Pakistan and Africa (Valdez-solana, et al,2015). Plants have been an important source of medicine from time immemorial. Even today, WHO estimates that about 80% of people still rely primarily on traditional remedies such as herbs for their medicines (Ekor, 2014). Moringa is a plant with great medicinal values. This tree has beneficial properties from roots to the leaves. Various phytochemicals are present in leaves of *Moringa oleifera* which includes alkaloids, flavonoids, tannins, saponin and steroids which makes the plant a natural source of phytochemical compounds of great medicinal and commercial value. Among the 13 cultivars of moringa (*M. arborea*, *M. rivae*, *M. oleifera*, *M. longituba*, *M. stenopetala*, *M. concanensis*, *M. borziana*, *M. pygmaea*, *M. ruspoliana*, *M. drouhardii*, *M. hildebrandtii*, *M. ovaiifolia* and *M. peregrine*) (Mahmood et al.,2010) *Moringa oleifera* is the most studied species and most used species because of its phytochemical and pharmacological properties were related to human health. (Valdez-solana, et al,2015). In order to contribute to the growing body of knowledge on this subject, present study analysed the phytochemical constituents of natural and alcoholic extract of *Moringa oleifera* leaf meal.

These phytochemicals are major class of plant secondary metabolites. Moringa is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yogurt, 15 times more potassium than bananas and 25 times more than spinach (Rockwood, et al.2013) The leaves are good source of vitamins, proteins, minerals and amino acids. Antioxidant, antimicrobial, anti-cancer and anti-inflammatory are some of biological activities exhibited by this medicinal plant.

*Moringa oleifera* has great importance in human health as well as in animals, it can cure more than 300 diseases. Many children who do not get breast milk from their mother show malnutrition symptoms. In this situation an agent i.e galactagogue is given to women to promote secretion of milk. The galactagogue is made up of phytosterols like beta-sitosterol, campesterol, stigmasterol which can increase estrogen production. Younger than 3 years children malnutrition may be treated by these compounds. During pregnancy women's need for iron and calcium increases which can be fulfilled by 6 spoonsful of moringa leaf powder. (Valdez-solana, et al,2015)

Natural moringa is very beneficial but it is not available Worldwide. So many people use branded moringa powder, as it is easily available in the market. To know whether the phytochemicals present in natural leaves of moringa were also present in the branded moringa leaf powder, two branded moringa powders (Carbamide forte organic moringa leaf powder and Himalayan organics moringa powder) were taken.

## II. METHODOLOGY

Collection of plant material:

Fresh leaves of *Moringa oleifera* were collected from the local area of Patna in the months of August 2022. Collected leaves were thoroughly washed with the tap water and they were air dried for 24 hours and then oven dried below 60 degrees. Leaves were then grinded into fine powder by using Grinder. The powdered *Moringa* leaves were stored in a clean and tightly closed container for extract preparation.

Methanolic/Ethanolic extract preparation:

10g of each sample (powdered moringa leaves and 2 branded *Moringa* powder) were weighed and kept in different percolators. 200 ml of methanol/ethanol were added to each sample and kept for 24-48 hours. Percolated extracts were filtered and filtrates were collected for further testing.

For Thin Layer Chromatography extracts were concentrated with the help of an incubator.

## III. QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

Qualitative analysis of phytochemicals present in the methanolic/ethanolic extracts of *Moringa* leaf were carried out as per method of Harborne,1998.

Test for alkaloids

Wager's test was performed to detect alkaloids. 1% of HCL was prepared and sample extracts were added in different test tubes and were heated for 15-20 mins with gentle shake then left to cool. Then a few drops of Wager's reagent were added, creamy brown appearance indicate the presence of alkaloids.

Test for flavonoids

Alkaline reagent test was performed to detect flavonoids. 2-3 drops of sodium hydroxide were added to 2 ml of extract initially deep yellow colour appeared and then few drops of dilute HCL added to it which became colourless indicate presence of flavonoids.

Test for tannins

10 ml of bromine water added to 0.5 ml of each extract then de-colouration of bromine water indicates presence of tannin.

### Test of saponins

5 ml of distilled water mixed with each extract in the test tube and mixed vigorously then frothing was mixed with a few drops of olive oil, again mixed vigorously. Appearance of foam indicate the presence of saponin.

### Test for terpenoids

Chloroform added to each extract and evaporated on the water bath then boiled with sulfuric acid, appearance of grey colour indicate the presence of terpenoids.

## IV. QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

This analysis was carried to determine the amounts of phytochemicals present in the methanolic/ethanolic extract of the *Moringa oleifera* leaf powders.

### Thin Layer Chromatography (TLC)

The TLC plate was prepared by silica gel and calcium sulphate. Different solvent phase was used to perform TLC. After that it was observed under ultraviolet light and the Rf values were calculated.

$$R_f = \frac{\text{Distance covered by solute}}{\text{Distance covered by solvents}}$$

## V. RESULT AND DISCUSSION

### Comparative analysis of natural moringa powder in methanolic/ethanolic extract:

The comparative study of methanolic and ethanolic extracts of natural powder showed that the alkaloids were high in ethanolic extract as compared to sample of methanolic extract. Flavonoids were more in the sample of methanolic extract than ethanolic extract whereas tannins were equal in both the extracts. While saponins were more in ethanolic extract. The results are tabulated below in Table 1.

### Comparative analysis of natural and branded sample in methanolic extracts:

The comparative study of natural powder and 2 branded samples in methanolic extract showed that alkaloids were approximately equally high in natural and brand 1 as compared to brand 2. In case of flavonoids, a higher amount was present in natural leaves followed by brand 1 and brand 2. In both cases brand 2 showed presence of lesser amounts. Presence of tannins and saponins in natural and brand 2 showed approximately equal amounts and brand 1 showed comparatively higher amounts of alkaloids and flavonoids. All the extracts showed complete negative results for terpenoids. The results are tabulated below in Table 2.

### Comparative analysis of natural and branded sample in ethanolic extracts:

The comparative study of extracts of natural leaf powder and branded samples in ethanol showed that presence of alkaloid was approximately equal in natural and brand 2 as compared to brand 1. In case of flavonoids, it was high in natural leaf powder followed by brand 2 and brand 1 showed negative result. In case of tannins, it was high in brand 1 as compared to natural and brand 2 which showed approximately equal amounts. However, in the case of saponins, it was high in brand 2 as compared to natural leaf powder which showed less amount followed by brand 1. All the extracts showed complete negative result for terpenoids. The results are tabulated below in Table 3.

## Quantification of Alkaloid by TLC method:

Methanol: Ammonium Hydroxide (200:3) was the solvent used. TLC of methanolic/ ethanolic extracts of natural sample and 2 branded samples showed 6 bands of Rf value of 0.542, 0.533, 0.309, 0.973, 0.88 & 0.951 respectively. Natural powder in ethanolic extract showed highest Rf value of 0.973 and brand 2 in methanolic extract showed lowest Rf value of 0.309 which means that natural powder in ethanolic extract was more polar than other extracts and brand 2 in methanolic extract was less polar than other extracts. Natural powder in ethanolic extract runs faster and brand 2 in methanolic extract runs slower than other extracts. The results are tabulated below in Table 4.

Calculation of Rf value: -

Rf = distance covered by solute / distance covered by solvent Sn M =

$$12.2/22.6 = 0.542 \quad S1 M = 12/22.6 = 0.533$$

$$S2 M = 7/22.6 = 0.309 \quad Sn E = 22/22.6 = 0.973$$

$$S1 E = 20/22.6 = 0.88 \quad S2 E = 21.5/22.6 = 0.951$$

## Quantification of Flavonoids by TLC methods:

Ethyl acetate: Acetic acid: Formic acid: water (100:11:11:27) were used as solvent phase. TLC of methanolic/ethanolic extracts showed 5 bands of Rf values of 0.929, 0.84, 0.530, 0.831 and 0.526 respectively. Natural powder in methanolic extract showed highest Rf value of 0.929 and brand 1 in ethanolic extract showed lowest Rf value of 0 which indicates absence of flavonoid and sample of natural powder in methanolic extract was polar and runs faster than other extracts. The results are tabulated below in Table 5.

Calculation of Rf value: -

Rf = distance covered by solute/ distance covered by solvent

$$Sn M = 21/22.6 = 0.929$$

$$Sn E = 18.8/22.6 = 0.831$$

$$S1 M = 19/22.6 = 0.84$$

$$S1 E = 0/22.6 = 0$$

$$S2 M = 12/22.6 = 0.530$$

$$S2 E = 11.9/22.6 = 0.526$$

**Table 1:** Comparative analysis of natural moringa powder in methanolic/ethanolic extracts

PHYTOCHEMICALS	Sn M	Sn E
Alkaloids	++	+++
Flavonoids	+++	++
Tannins	+	+
Saponins	+	++
Terpenoids	-	-

**Table 2:** Comparative analysis of natural and branded samples in methanolic extracts

PHYTOCHEMICALS	Sn M	S1 M	S2 M
Alkaloids	++	++	+
Flavonoids	+++	++	+
Tannins	+	++	+
Saponins	+	++	+
Terpenoids	-	-	-

**Table 3:** Comparative analysis of natural and branded samples in ethanolic extracts

PHYTOCHEMICALS	Sn E	S1 E	S2 E
Alkaloids	+++	++	+++
Flavonoids	++	-	+
Tannins	+	++	+
Saponins	++	+	+++
Terpenoids	-	-	-

**Table 4:** Rf value calculated for methanolic/ethanolic extracts for quantification of Alkaloid

SAMPLES	DISTANCE COVERED BY SOLUTE	DISTANCE COVERED BY SOLVENT	Rf VALUE
Sn M	12.2	22.6	0.542
S1 M	12	22.6	0.533
S2 M	7	22.6	0.309
Sn E	22	22.6	0.973
S1 E	20	22.6	0.88
S2 E	21.5	22.6	0.951

**Table 5:** Rf virtue calculation of methanolic/ethanolic extracts for quantification of flavonoid

SAMPLES	DISTANCE COVERED BY SOLUTE	DISTANCE COVERED BY SOLVENT	Rf VALUE
Sn M	21	22.6	0.929
S1 M	19	22.6	0.84
S2 M	12	22.6	0.531
Sn E	18.8	22.6	0.831
S1 E	0	22.6	0
S2 E	11.9	22.6	0.526

Note; +++ = very much, ++ = much, + = little, - = nil

Sn = Sample of natural powder

S1 = Brand 1 (Carbamide Forte)

S2 = Brand 2 (Himalayan Organics)

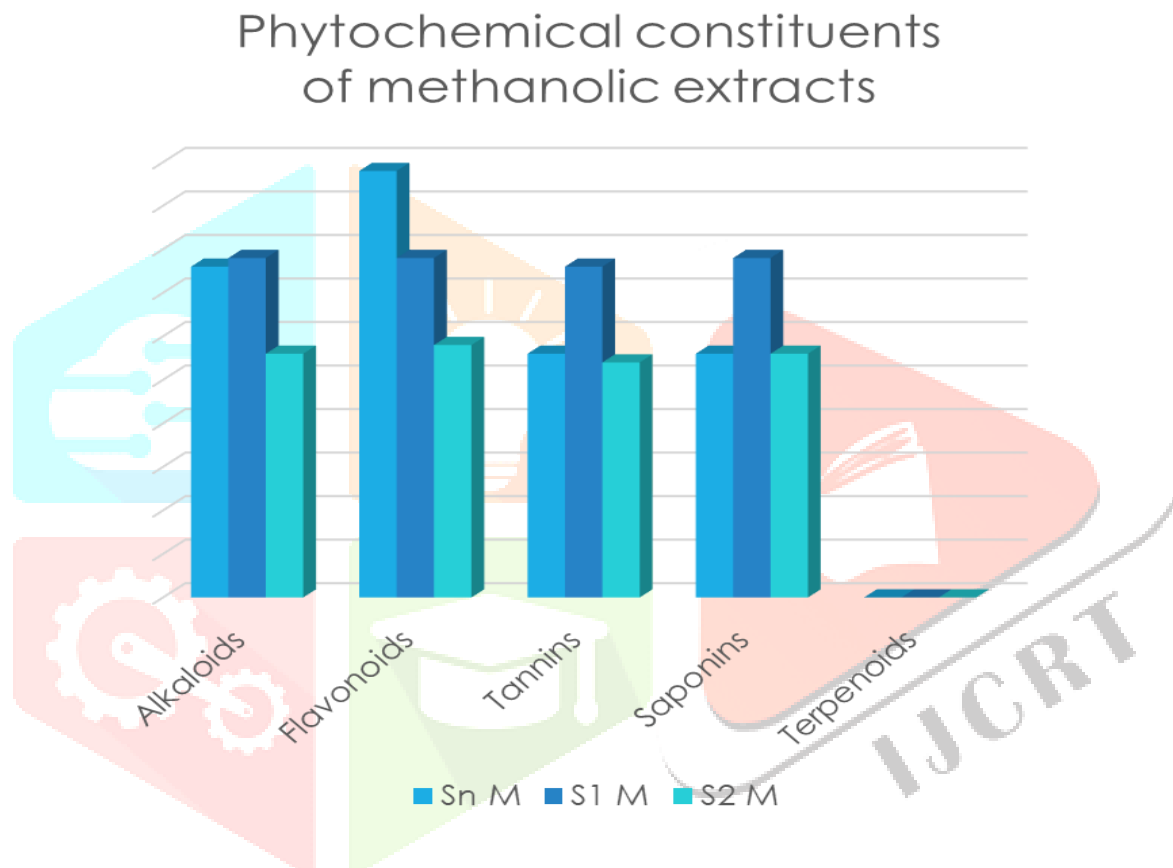
Sn M = Sample of natural powder in methanolic extract

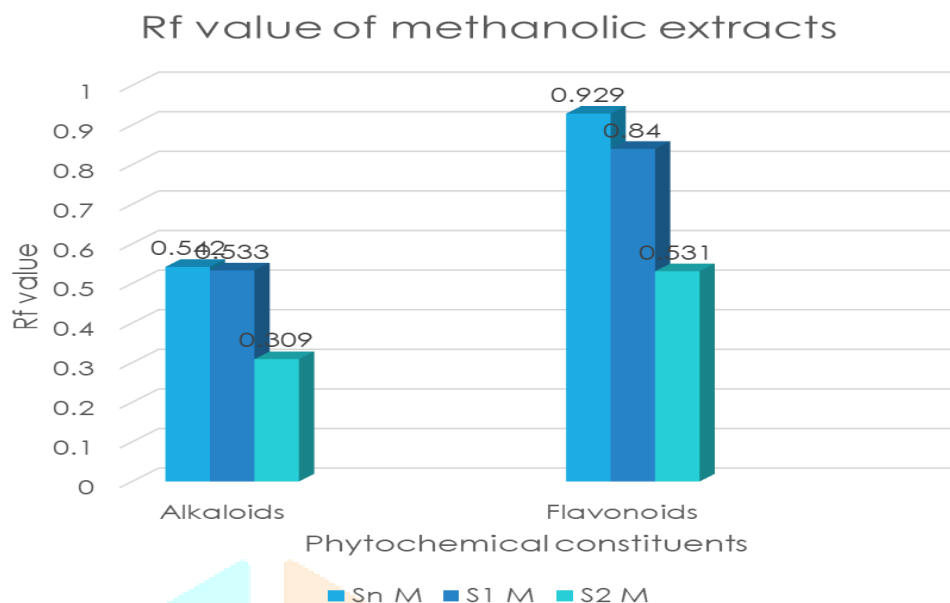
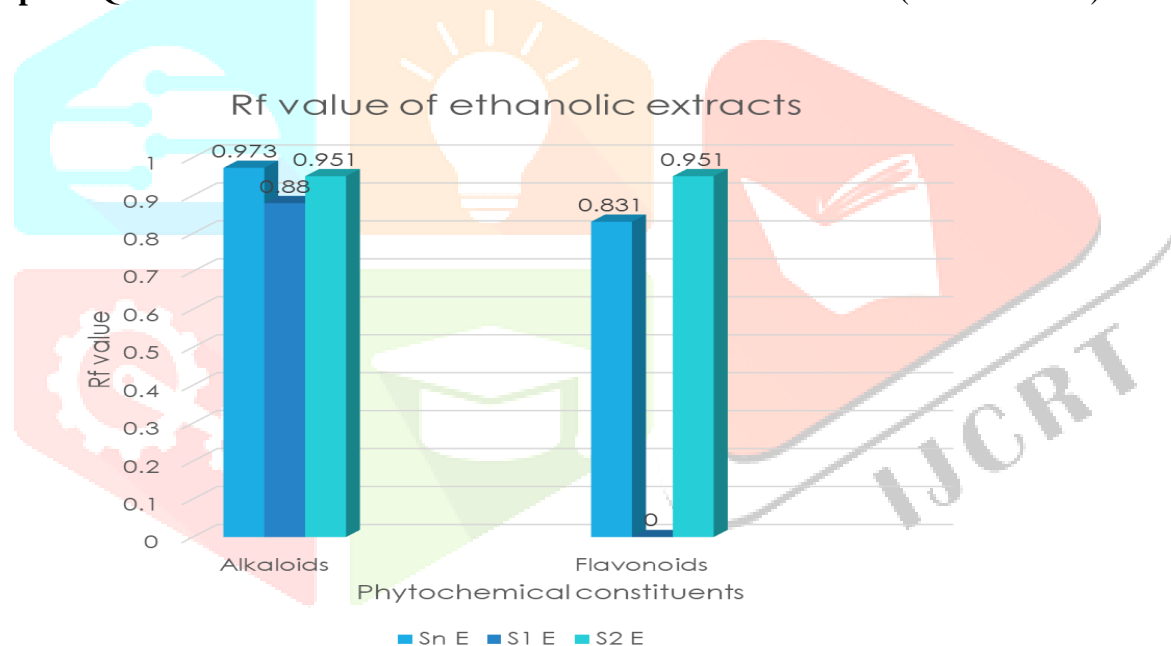
S1 M = Brand 1 in methanolic extract      S2 M = Brand 2 in methanolic extract

Sn E = Sample of natural powder in ethanolic      S1 E = Brand 1 in ethanolic extract

S2 E = Brand 2 in ethanolic extract

**Graph 1:** Comparative analysis of phytochemicals present in natural and branded samples in ethanolic extract (based on biochemical test)



**Graph 2:** Quantification of alkaloids and flavonoids in methanolic extract (based on TLC)**Graph 3:** Quantification of alkaloids and flavonoids in ethanolic extract (based on TLC)

## CONCLUSION

Methanolic/ethanolic extracts of natural and branded samples confirmed the presence of alkaloids, flavonoids, tannins, and saponins except terpenoids. Natural leaf powder and samples of brand 2 in ethanolic extracts had approximately higher amounts of alkaloid than others. In case of flavonoids, Natural samples in methanolic extract had higher amounts as compared to the other extracts and showed complete absence in brand 1.

In the case of tannins, brand 1 in methanolic/ethanolic showed higher amount. However, in the case of saponins, brand 2 showed higher amount in ethanolic extract.

Variations in phytochemical constituents among natural and branded samples indicate that some alteration may happened during processing and preservation. Branded leaf powders must be labeled with the exact amounts of their phytochemical constituents present in it, as this will help consumers to be aware about their composition and may use them as herbal supplements/remedies. Therapeutic role of plants is due to natural presence of one or more phytochemicals. Any alteration, addition of toxins or increase in concentration of the constituents may show either negative impact or may have a neutral role in curing

certain diseases. Hence the present work justifies, that constituents of natural and branded powder of moringa varies during the process of manufacturing, processing and preservation. Due to the short duration of this project, a limited number of phytochemicals were compared. Quantification of these phytochemicals have to be done in near future. Herbal remedies are thought to be harmless but proper documentation is required before consuming them as medicine or herbal supplements.

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