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RESEARCH ARTICLE

EVALUATION OF PROXIMATE PRINCIPLES AND ANTIOXIDANT ACTIVITY OF *MORINGA OLEIFERA* LAM. (DRUM STICK TREE) IN KERALA.

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Abstract

Moringa oleifera is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. It is commonly referred to as 'drumstick tree' or 'horse radish tree'. The present study investigates the nutritional and antioxidant potential of the plant. The proximate principles analyzed were carbohydrate, protein, starch, total sugar, reducing and non reducing sugar, total lipid, crude fat, fatty acid, total free amino acids, vitamins (A.C,E,B1,B2,B3) and minerals (Mg, Fe, Mn, Zn, Cu, Ag, Ni, B, Co, Li) using standard protocols. The anti nutritional factors oxalate and tannin was also estimated. The antioxidant potential of the plant was determined using DPPH radical scavenging activity, reducing power, total antioxidant activity,nitric oxide radical scavenging activity, hydrogen peroxide scavenging activity, hydroxyl radical scavenging activity and superoxide radical scavenging activity. The result reveals that *Moringa oleifera* is highly nutritious and have strong antioxidant potential.

Introduction:-

Nutraceuticals are products derived from food sources that are purported to provide extra health benefits, in addition to the basic nutritional value found in foods. Plant based nutraceuticals are plant products with nutritional and medicinal values. In other words, these are food with pharmaceutical properties (*Health Canada, 2013*).*Moringa oleifera* is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae and is native to India. *Moringa oleifera* is a single source of all the nutrients and has several traditional and therapeutic uses. It is being used as a nutritional supplement in many parts of the world. Despite the nutraceutical importance, the plant has different pharmacological activities. Moringa tree is used in treating malnutrition, especially among infants and mothers. Almost all the parts of this plant have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastro intestinal, haematological and hepato renal disorders (The wealth of India, 1962; Singh and Kumar,1999; Siddhuraju and becker,2003).

Edible plant parts with antioxidant properties are considered as plant based antioxidant nutraceuticals. Only those parts of plants that are consumed normally as human food in any parts of the world are to be used in the preparation of nutritional medicine (dietary formulation). The present study deals with the nutritional, anti nutritional and anti oxidant potential of leaves of the plant.

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Materials and Methods:-

Plant material:-

Moringa oleifera leaves were collected from Trivandrum and authenticated by Dr. G. Valsaladevi, Department of Botany, University of Kerala.

Nutritional analysis:-

The fresh leaves were collected, washed, dried and then crushed in a mortar and pestle and were subjected to various biochemical analyses. The proximate principles total carbohydrate, starch, protein, total reducing and non reducing sugar (Sadasivam and Manickam,1996), total sugar (Hodge and Hofreiter 1962),total lipid (Bligh and dyer 1959),vitamins (Vit A,B1,B2,B3,C,E) (AOAC,1994;Okwu and Josiah ,2006), total free amino acids (Moore and stein,1948), crude fat, fatty acid, anti nutritional factors oxalate (AOAC,1990) and tannin (Schanderl,1970) were determined using standard protocols. The inductively coupled mass spectrometry (ICP-MS) system Thermo Scientific ICAP Qc was used for the mineral (Mg, Fe, Mn, Zn, Cu, Ag, Ni, Co, Li) analysis.

Antioxidant analysis:-

Dried leaf powder (5g) of *Moringa oleifera* was extracted with methanol, hydro alcohol (1:1) and water using soxhlet apparatus. Each extract obtained was filtered using Whatman No.1 filter paper, dried to a semisolid mass and the yield of each extract was recorded. These extracts were screened for their antioxidant potential using various antioxidant assays. Antioxidant activity was determined using standard protocols of total antioxidant activity (Prieto et al., 1999), DPPH radical scavenging activity (Blois,1958), reducing power (Oyizu,1986)**,** nitric oxide radical scavenging activity (Ilavarasan et al., 2005), hydrogen peroxide scavenging activity (Ruch et al .,1989), hydroxyl radical scavenging activity (Halliwell et al., 1987),superoxide radical scavenging activity (Fontana et al., 2001).

Results and Discussion:-

Nutritional analysis:-

The results of nutritional analysis showed that the *Moringa oleifera* leaves were rich in various nutrients (Table 1) such as carbohydrates, proteins, free amino acids and sugars. The vitamin analysis revealed that the leaves contained appreciable quantity of vitamins. The anti nutritional factors oxalate and tannin is present in very minute quantity. *Moringa oleifera* leaves are highly nutritious and have the ability to abolish malnutrition. Mineral analysis showed that *Moringa oleifera* is rich in various mineral elements (Table 2). Mg and Fe were found in higher amounts than all the other elements checked.

Table 1:-Nutritional status of *Moringa oleifera* leaves.

Nutrients	***Moringa oleifera*(100g)**
Carbohydrate (g)	38.4±0.90
Starch (mg)	12.042 ± 0.02
Total sugar (mg)	0.8 ± 0.05
Total non reducing sugar(mg)	0.52±0.02
Total reducing sugar (mg)	0.28 ± 0.03
Protein (g)	27.1±0.725
Total lipid (g)	1.37 ± 0.04
Crude fat (mg)	5.04 ± 0.03
Fatty acids (mg)	4.03 ± 0.3
Total free amino acids (mg)	118.2 ± 0.7
Vitamin A (mg)	13.48±0.51
Vitamin C(mg)	245.13±0.46
Vitamin E(mg)	16.80±0.24
Vitamin B1(mg)	0.05±0.28
Vitamin B2(mg)	0.8±0.25
Vitamin B3(mg)	220±0.042
Oxalate (mg)	0.462±0.03
Tannin (mg)	0.30±0.03

*Data is presented as the mean ± standard deviation.

Table 2:-Mineral profiling in *Moringa oleifera.*

S.No	Minerals	(mg/Kg)
1	Mg	3689
2	Fe	360.4
3	Mn	31.3
4	Zn	24.8
5	Cu	43.1
6	Ag	1.3
7	Ni	39.2
8	Co	1.2
9	Li	1.6

Antioxidant analysis:-

Total antioxidant capacity:-

The total antioxidant capacity (TAC) was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. It evaluates both water-soluble and fat-soluble antioxidants (total antioxidant capacity). The results indicate higher total antioxidant capacity (expressed as ascorbic acid equivalent) in the methanolic extract (Table 3) of *Moringa oleifera.*

Table 3:- Results show total antioxidant activity of various extracts of *Moringa oleifera.*

EXTRACT	TAC (µg of ascorbic acid per mg of extract)
Water	21.13±0.02
Hydro alcohol	29.72±0.01
Methanol	45.61±0.03

*Data is presented as the mean ± standard deviation.

DPPH radical scavenging activity:-

The DPPH radical scavenging activity is determined based on the ability of DPPH (1, 1-diphenyl-2-picrylhydrazyl) a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. All of the assessed extracts of *Moringa oleifera* were able to reduce the stable, purple-colored radical DPPH to the yellow-colored DPPH-H form (Table 4). In the present study DPPH radical scavenging was higher in the methanolic extract of *Moringa oleifera* (IC_{50} : 160 µg/ml). The IC_{50} value of aqueous extract of *Moringa oleifera* was 400 µg/ml and that of hydro alcoholic extract was 420 µg/ml.

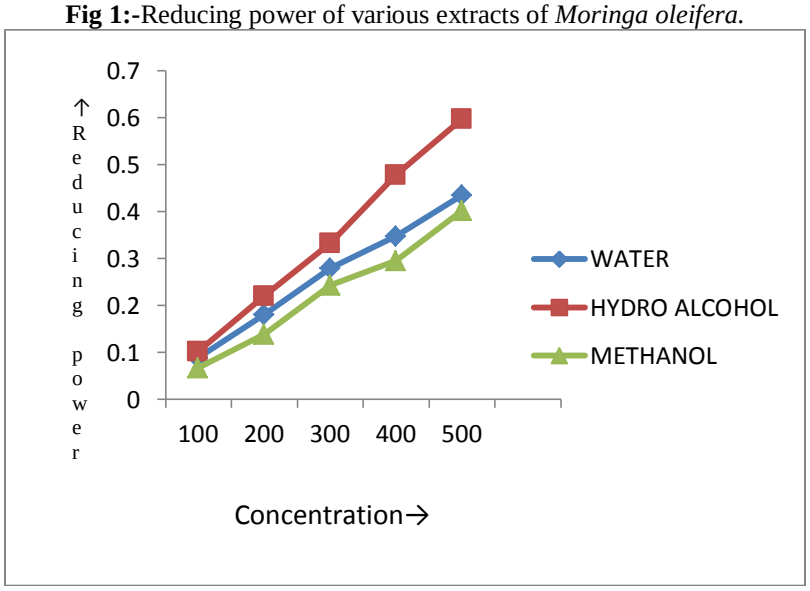
Table 4: IC_{50} values (µg/ml) of the neutralization of DPPH radical with *Moringa oleifera* extracts.

EXTRACT	IC_{50} (µg/ml)
Water	400
Hydro alcohol	420
Methanol	160

Reducing power:-

Various concentrations (100-1000 µl) of leaf extracts of *Moringa oleifera* were found to have significant reducing power. All the extracts exhibited a concentration dependent increase in reducing power. (Fig: 1). Methanolic extract of *Moringa oleifera* showed superior reducing activity. The reducing power of the extracts increased with increasing concentration, which suggests that the electron donating ability of the extracts is concentration dependent.

Fig 1:-Reducing power of various extracts of *Moringa oleifera.*



Nitric oxide radical scavenging activity:-

The methanolic extract of *Moringa oleifera* effectively reduced the generation of nitric oxide from sodium nitroprusside. *Moringa oleifera* methanolic extract showed nitric oxide scavenging activity at the concentration of 318 µg/ml (IC_{50}) (Table 5). The result shows that methanol extract of *Moringa oleifera* is a superior nitric oxide radical scavenger. Water (IC_{50} :1501µg/ml) and hydroalcoholic (IC_{50} :1458µg/ml) extracts also shows a significant nitric oxide radical scavenging activity.

Table 5:-Nitric oxide radical scavenging acivity of (IC_{50}) *Moringa oleifera* extracts.

EXTRACT	IC_{50} (µg/ml)
Water	1501
Hydro alcohol	1458
Methanol	318

Hydrogen peroxide scavenging activity:-

The results showed all the extracts had potent H_2O_2 scavenging activity which may be due to the antioxidant compounds. As the antioxidant components present in the extracts are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O. Methanolic extract (IC_{50} :593 µg/ml) of *Moringa oleifera* exhibits greater scavenging activity compared to all other extracts (Table 6). The IC_{50} value of aqueous extract of *Moringa oleifera* was 868 µg/ml and that of hydro alcoholic extract was 1101µg/ml.

Table 6:-Hydrogen peroxide scavenging activity of (IC_{50}) *Moringa oleifera* extracts.

EXTRACT	IC_{50} (µg/ml)
Water	868
Hydro alcohol	1101
Methanol	593

Hydroxyl radical scavenging activity:-

In the present study the methanolic extract of *Moringa oleifera* showed potent hydroxyl radical scavenging activity. The IC_{50} value of methanolic extract of *Moringa oleifera* was 2063µg/ml (Table 7). All the extracts showed a significant hydroxyl radical scavenging activity. The IC_{50} value of aqueous extract of *Moringa oleifera* was 3540 µg/ml and that of hydro alcoholic extract was 2775 µg/ml.

Table 7:-Hydroxyl radical scavenging activity (IC_{50}) of *Moringa oleifera* extracts.

EXTRACT	IC_{50} (µg/ml)
Water	3540

Hydro alcohol	2775
Methanol	2063

Superoxide radical scavenging activity:-
The result of superoxide radical (SO) scavenging activity obtained for the extracts of *Moringa oleifera* showed dose dependent free radical scavenging activity and the percentage of inhibition was shown in [Table 8]. In the present study, methanolic extract of *Moringa oleifera* was found to be a superior scavenger of superoxide radicals (IC_{50} :151 µg/ml). The IC_{50} value of aqueous extract of *Moringa oleifera* was 180 µg/ml and that of hydro alcoholic extract was 220 µg/ml.

Table 8:-Superoxide anion (O_2.-) radical-scavenging activity (IC $_{50}$) of *Moringa oleifera* extracts.

Extract	**IC_{50}(µg/ml)**
Water	180
Hydro alcohol	220
Methanol	151

Conclusion:-
The present study evaluated the nutritional and antioxidant potential of leaves of the plant. The nutritional analysis revealed that *Moringa oleifera* is rich in carbohydrates, proteins, free amino acids and vitamins. Anti nutritional factors were found in very minute quantity. *Moringa oleifera* is rich in various mineral elements and could be utilized to treat a number of diseases that are mainly caused due to the deficiency of these minerals. Since the plant is rich in nutrients and anti nutrients were found in very minute quantity suggests their utility for consumption. Antioxidant assays confirmed the antioxidant potential of the plant. Methanolic extract of *Moringa oleifera* shows strong antioxidant activity than the other two extracts. The present study confirms the nutraceutical potential of the plant.

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