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FREE RADICAL SCAVENGING ACTIVITIES OF EXTRACTS FROM DIFFERENT PARTS OF *MORINGA OLEIFERA* LAM. USING DPPH ASSAY.

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*Moringa oleifera (*Lam.) is one of the best known medicinal plants. The *Moringa* plant has been consumed by humans. It is one of the richest plant sources of Vitamins A, B, C, D, E and K *Moringa oleifera* has a quality of medicinal value with high nutrition value. The strong antioxidant properties of medicinal plants may improve the capability. Present investigation was mainly focused to significantly verify the antioxidant compounds in different extracts of *Moringa oleifera.* Total phenols were estimated in methanolic, ethanolic and aqueous extracts of leaf, Un-ripened pods and mature seeds in mg Gallic acid equivalent per gm dwt. were estimated by spectrophotometer. Quantitative evaluation of total flavonoid (mg QE /g DW) present in the extract of plant parts prepared in different solvents was conducted for comparative analysis. The antioxidant activities of different extracts of *M. oleifera* were estimated, DPPH scavenging activity (%), IC_{50} (mg/ml) and antioxidant capacity of plant materials were calculated. The results of this investigation revealed that phenol and flavonoid concentrations vary in different parts of the plant. Antioxidant activity was present in all the studied plant parts and had a variable correlation with TP and TF content. A number of other potential antioxidants present in (non-phenolic antioxidants) may have been responsible for its antioxidant activity along with phenols.

Keywords: Antioxidants, Flavonoids, *Moringa oleifera* and Phenols

Introduction

Moringa oleifera is a type of local medicinal Indian herb which has turn out to be familiar in the tropical and subtropical countries. This plant is locally known as Horseradish tree, Mulangay, Mlonge, Benzolive, Drumstick tree, Sajna, Kelor, Saijihan and Marango. *Moringa oleifera* division to become from Kingdom: Plantae, Division: Magnoliphyta, Class: Magnoliopsida, Order: Brassicales, Family: Moringaceae, Genus: Moringa, Species: *M.oleifera*[1].Among commoners, it has earned its name as 'the miracle tree' due to its amazing healing abilities for various ailments and even some chronic diseases.Several investigations were carried out to isolate bioactive compounds from various parts of the plant due to its various applications [2]. *Moringa oleifera is a* genus of 14 species of flowering plant in this family.

M. oleifera is one of the best known medicinal plant. The *Moringa* plant has been consumed by humans [3]. It is one of the richest plant sources of Vitamins A, B, C, D, E and K[4-8]. *Moringa oleifera* has a quality of medicinal value with high nutrition value. This plant is known to be mineral rich plant as its several parts encode a range of important minerals, and are a good source of protein, vitamin, β-carotene etc. Also, it has compelling water purifying powers and high nutritional value.

Different part of this plant such as the bark, leaves, immature pods, roots, fruit, flowers and seeds serve as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, cholesterol lowering, antihypertensive, anti-inflammatory, anantidiabetic activities. Traditionally, they serve for the treatment of different ailments in medical system. *Moringa's* seeds are considered to be antipyretic, acrid, bitter [9] and also the antimicrobial activity of this plant. The drumstick flowers, leaves, seeds and roots are used for tumors. Roots are bitter, act as a tonic to the body and lungs[10] .

Alkaloids present in *Moringa* plant acts like and can serve to treat asthma. These Alkaloids relaxes bronchioles[11]. Bronchial asthma is effectively treated using seed kernels of *Moringa oleifera,* shown in a study carried to check the efficacy and safety of these kernels with respect to asthmatic patients proved a decreased severity of asthma symptoms and also improved respiratory functions[12] .

The secondary metabolites or products extract in general exhibits a profound physiological effect on the mammalian system and thus are known as active principles of plants. Plants used as medicinally in different countries and are source of many powerful and potent drugs More than 25% of the prescribed drugs in the world are prepared from a variety of plant materials as roots, leaves, bark, stems etc.

An antioxidant is a molecule capable of slowing or preventing the oxidation of the molecules. Oxidation is a chemical reaction that causes loss of electrons or transfer of electrons from a substance to an oxidizing agent. Free radicals produce from Oxidation reactions, which start chain reactions that damage the cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, polyphenols, ascorbic acid. There are various functions of antioxidants such as; (i) Certain phytochemical have beneficial effect on heart diseases. (ii) Antioxidants such as Vitamin C and E boost our immune system. (iii) It is beneficial in prevention of cancer. (iv) Antioxidants lower the level of Low-density lipoprotein (LDL) cholesterol thus in the blood vessels preventing plaque deposition.

The dried leaves of MO are a great source of polyphenol compounds, such as flavonoids and phenolic acids. Flavonoids, which are synthesized in the plant as a response to microbial infections, have a benzo-γ-pyrone ring as a common structure . Intake of flavonoids has been shown to protect against chronic diseases associated with oxidative stress, including cardiovascular disease and cancer. MO leaves are a good source of flavonoids . The main flavonoids found in MO leaves are myrecytin, quercetin and kaempferol, in concentrations of 5.8, 0.207 and 7.57 mg/g, respectively . Quercetin is found in dried *MO* leaves, at concentrations of 100 mg/100 g, as quercetin-3-*O*-β-d-glucoside (iso-quercetin or isotrifolin) Quercetin is a strong antioxidant, with multiple therapeutic properties.

The strong antioxidant properties of medicinal plants may improve the capability of plants to survive under polluted conditions. Such natural materials may provide exact advantages over synthetic ones, because they contain some essential compounds. Therefore, in present investigation it is significant to verify the antioxidant compounds in different extracts of *Moringa oleifera*

Materials and methods

Plant Materials:

Plant materials of *Moringa oleifera Lam.* were collected from Ajmer and surrounding areas. The extracts prepared from fresh materials were used for analyzing total phenols, flavonoids and antioxidant activity *in vitro*. Plant materials which were tested for determination of antioxidant activity were,

roots (R), Leaf (L), un-ripened (green colour) pods (URP) and seed (S). One gram plant material was extracted in 10 ml of 80% methanol by maceration. The solvent was then centrifuged at 14,000 rpm for 30 minutes at room temperature. The extract obtained was used for analysis.

Preparation of methanolic extracts:

The parts of the plants under investigation were roots (R), Leaf (L), un-ripened (green colored) pods (URP) and seed (S). The freshly collected plant materials were dried and coarsely owdered. The powder was defatted with petroleum ether (60-80 oc) and subsequently extracted with methanol using a soxhlet extractor. The extracts were dried under reduced pressure using a rotary vacuum evaporator. The extracts were kept in refrigerator for further use.

All chemicals used were of analytical grades, 1,1-diphenyl -2- picryl hydrazyle (DPPH) and quercitine were procured from sigma chemical co. (st., Louise, US), Gallic acid, Ascorbic acid were procured from Merck co. (Germany), Follin Ciocalteu, Aluminum chloride, Methanol, Sodium carbonate and potassium acetate were purchased from Qualigens fine chemical co. (India).

The absorbance measurements were recorded on Spectroscan-50, UV-VIS spectrophotometer (Biotech. engineering management Co. UK.)

Estimation of total phenol:

The method used to determine the total phenolic content of methanol, ethanol and aqueous extracts using the Folin Ciocalteu reagent was adapted from[13]. An aliquot of each plant extract (0.5 ml, 1:10 mg/l) or Gallic acid (phenolic standard compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and 4 ml of 1

M Na_2CO_3 solution. The mixture was kept for 30 minutes at room temperature and the absorbance was measured at 710 nm with a Systronics UV- Visible double beamSpectrophotometer. The phenol content of plant extracts was calculated by comparing the observed O.D.s of the sample at different concentrations to the standard curves of Gallic acid. Total phenol values were expressed as mg Gallic acid equivalent (GAE)/g dry weight. All samples were analyzed in triplicates.

Estimation of total Flavonoids:

The alluminum chloride method was adapted for the determination of total flavonoid content[14]. Each plant extract (0.5 ml of 1:10 mg/l) was mixed with 1.5 ml of solvent (methanol, ethanol, distilled water), 0.1 ml of 10% AlCl3, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The mixture was incubated for 30 minutes at room temperature and absorbance was measured at 415 nm. Quercetin was used to make the standard curves. The observed O.D.s of plant extracts were compared to the standard curves of Quercetin and the flavonoid content was estimated. Total flavonoid contents was expressed as mg Quercetin equivalents (QE)/g dry weight. Measurements were taken in triplicates for all Samples.

Determination of the free radical scavenging activity:

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical method was used for *in vitro* determination of radical scavenging activity of the extracts [15]. In the presence of a hydrogen donatingution is reduced at 517 nm and the non-radical form

DPPH-H is formed by the reaction: DPPH° + AH→ DPPH-H + A° The remaining DPPH°, measured after a certain time, is inversely related to the radical scavenging activity of the antioxidant. The DPPH method is simple, rapid, reproducible, inexpensive and sensitive without the need for special instruments, therefore nearly all the potent natural antioxidants known to date have been shown in the DPPH assay. The methanolic solution of DPPH, freshly prepared every day, was stored in an aluminium foil covered flask and kept at 4°C in the dark. Different concentrations of each extract were mixed with the DPPH

solution in methanol (0.004 %). The mixture was incubated for 15 minutes. The absorbance was measured at 517 nm with a Systronics UV- Visible double beam Spectrophotometer. The ability to scavenge the DPPH radical (expressed as percentage inhibition of DPPH°) was calculated using the following equation:

DPPH radical scavenging activity (%) = [AC – AS / AC] x 100

Where AC517 is absorbance of the control and AE517 is the absorbance of the sample. The

degree of discoloration indicates the free radical scavenging efficiency of the substances. A standard curve was plotted using ascorbic acid as a free radical scavenger reference compound in methanol and this was compared with O.D.s of methanolic plant extracts, which

led to their estimation of free radical scavenging activity.

Determination of IC50 values:

A graph between the concentration of the extracts and the percentage inhibition of free radicals was plotted to produce a regression equation for regression analysis taking 0% inhibition. Using these regression equations, IC50 values (concentration of extracts required to scavenge 50 percent DPPH free radicals) were calculated which showed an inverse relationship between the IC50 value and the percentage scavenging potential of the sample.

Results and Discussion

Estimation of total phenols in different extracts of *M. Oleifera:*

Total phenols were estimated in methanolic, ethanolic and aqueous extracts of leaf, Un-ripened pods and mature seeds in mg Gallic acid equivalent per gm dwt. were estimated by spectrophotometer. The data obtained showed in fig.1 that in leaf extracts highest amount of total phenols were obtained in the etanolic extract (9.5 ± 0.1 mgGAE/gm dwt) whereas the lowest amount (4.12±0.07) was found in aqueous extract of leaf.

In un-ripened pods (Fig.2) the total phenols recorded in the ascending order of ethanolic extract (10.24 ± 0.12 mg GAE/gm dwt) > methanolic extract (5.7 ± 0.05 mg GAE/gm dwt) > aqueous extract (3.83 ± 0.04 mgGAE/gm dwt) .

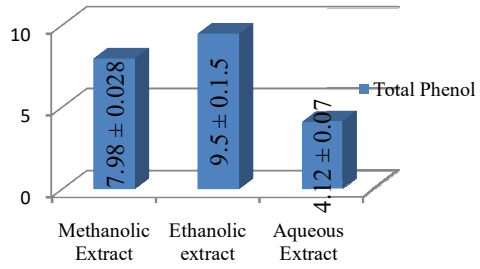

Fig .1: Total Phenol mg GAE /gm dwt. of leaf

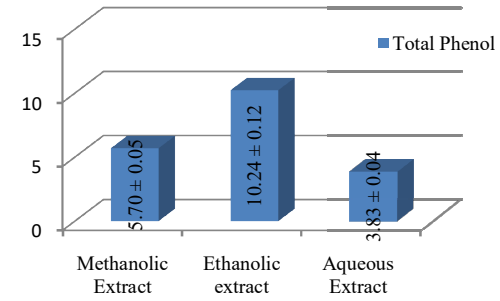

Fig. 2: Total Phenol mg GAE /gm dwt. of Un-ripened pod

In the different extract of mature seed (Fig. 3) of *M. oleifera* the highest concentration of total phenols were found in methanolic extract (8.74 ± 0.025 mg GAE/gm dwt) whereas lowest in the aqueous extract (2.5 ± 0.33 mg GAE/gm dwt).

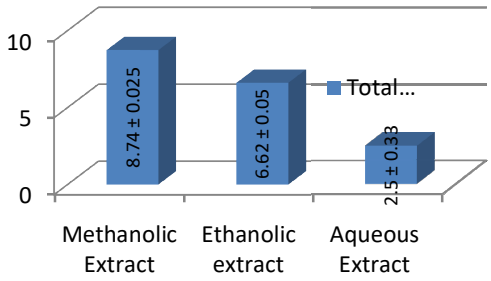

Fig. 3: Total Phenol mg GAE/gm dwt. of mature seed

Estimation of total flavonoids in different extracts of *M. Oleifera:*

Quantitative evaluation of total flavonoid (mg QE /g DW) present in the extract of plant part prepared in different solvents

were conducted for comparative analysis. The observations are shown in the Fig. 4

In the leaf of *Moringa oleifera* Lam**.** highest amount (5.12 ± 0.025 mg QE /g DW) of total flavonoid were recorded in methanolic extract whereas lowest 3.87 ± 0.014 mg QE /g DW were observed in aqueous extract. Similar concentration of flavonoid was found in the methanolic and ethanolic extracts of un-ripened pod and mature seed.

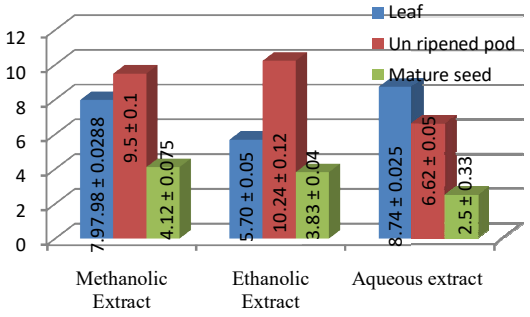

Fig. 4: Comparative analysis of total flavonoid contents (mg GAE/gm dwt.) in different extracts of *M.oleifera*

Estimation of antioxidant activity in different extracts of *M. Oleifera:*

The antioxidant activities of different extracts of *M. oleifera* were estimated, DPPH scavenging activity (%), IC_{50} (mg/ml) and antioxidant capacity of plant materials were calculated. Results are shown in Table 1.

The extracts of matured seed have shown maximum percentage (92.3 ± 0.1) of DPPH scavenging activities whereas the same was observed lowest in the extract of immature pod of *Moringa Oleifera* Lam. The poorest scavenging activity was found in the sample of leaf. Similarly the IC_{50} (29.9 ± 0.03mg/ml) and antioxidant capacity (42.5 ± 0.011mg/gm DW) was also highest in mature seed.

S. No.	parameter	Leaf	Un-ripened Pod	Mature Seed
1.	DPPH scavenging activity (%)	89.4 ± 0.1	85.96 ± 0.05	92.3 ± 0.1
2.	IC50 (mg\ml)	28.26 ± 0.017	25.4 ± 0.017	29.9 ± 0.03
3.	Antioxidant capacity (mg\g DW)	35.49 ± 0.025	35.25 ± 0.05	42.5 ± 0.011

*Data are expressed as means ± standard deviation of triplicate samples.

Table .1 Antioxidant activity of different extracts of *Moringa Oleifera* Lam.

Moringa oleifera (Moringaceae) has gained importance due to its multipurpose uses and good adaptability to both humid and dry climates. Almost all parts of the plant are economically useful. This study presents the status of diverse antioxidant potential in different extracts of *M. oleifera*. Knowledge gaps, and research and development avenues are suggested and discussed for its medicinal properties with special reference to antioxidant potential of different parts of this plant. Because of the complexity of natural phytochemicals and their different modes of action, it is inaccurate to assess the overall antioxidant potential only by a single method. Therefore, in this work they used DPPH to assess and compare the antioxidant potential of three organs of *M. oleifera*.

According to a report by one of the workers antioxidant compounds are present in diverse quantities from different *Moringa oleifera* tree leaf [16]. Antioxidative properties of phenolic acid in *Moringa oleifera* seeds arises from its great reactivity as electron or hydrogen donors from the ability to maintain, delocalize the unpaired electron (chain-breaking function) and chelate metal. *M. oleifera* is a rich mine of antioxidant[17].The antioxidant properties in the aqueous extracts of leaf, fruit and seed of *MO* is already presented by[18]. Antioxidant property of freeze dried *Moringa* leaves extracted from different procedures, gave an idea that Indian origin"s methanol and ethanol extracts of MO have the highest antioxidant activity of 65.1 and 66.8%, respectively[19]. In addition the major bioactive compounds of phenolics, like quercetin and kaempferol are attributes for antioxidant activity [20].

The results of this investigation revealed that phenol and flavonoid concentrations vary in different parts of the plant. Antioxidant activity was present in all the

studied plant parts which in line with the findings [21] and had a variable correlation with TP and TF content parallel to the studies of other workers [22-24]. A number of other potential antioxidants present in *Moringa oleifera* (non-phenolic antioxidants) may have been responsible for its antioxidant activity along with phenols. In addition, there may be interactions between different antioxidants (possible synergistic, additive and antagonistic interactions that may be observed when different natural antioxidants co-exist as reported by some workers. Reports are available on combinations of two or more phytochemicals would result in a change in the final effects of each component, create synergies in terms of antioxidant status and prevention of different *in vitro* oxidative stress and metabolic disorders[25]. Here, it is also important to understand the effects of antioxidant assay used. As far as Citrus fruits are concerned, the methods DPPH, ORAC, ABTS, FRAP are often used to assess antioxidant potential [26-29] Speed and simplicity are the main advantages of these methods, but they have their own limitations[30]. For instance, the results of these methods are affected by a number of factors, including interference materials, antioxidants and interactions, action time, pH, free radical production systems and so forth. This may be the case with this species of *Moringa* phenolic antioxidants and further research is needed in this regard to address the complexity of the issue.

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