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INFLUENCE OF PHENOLIC COMPOUNDS ON ANTIOXIDANT CAPACITY OF LEAVES EXTRACTS OF *MORINGA OLEIFERA* FROM TAMANRASSET REGION

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ABSTRACT

In the present research the crude extract and all fractions of *Moringa oleifra* leaves grown in Tamanrasset, were examined for its antioxidant activities by using three different assays such as DPPH scavenging activity, superoxide anion scavenging activity and phosphomolybdenum capacity.The quantitative estimation of total phenolics, flavonoids and tannins content additionally was calculated by spectrophotometric methods.The results showed that the extracts contained total phenolic compound at concentration of 32.985±0.07 to 309.418 ± 0.71 (mg GAE/g EW) and higher total flavonoids contents of 5.192 ± 0.17 to 44.695 ± 0.23 (mg QE/g EW), for total tannins contents of 3.864 ±0.07 to 15.148 ± 0.66 (mg/g). It also this experiment found out that each extract confirmed a good antioxidant. Consequently, it can be concluded that the phenolic compounds content may be answerable for the good activities of Moringa oleifra leaves.

Keywords: Antioxidant Capacity, Moringa oleifera, leaves, phenolic compounds.

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1. INTRODUCTION

Plant life is the most important natural source of phenolic compounds that is considered one of the largest groups of plant metabolites located in various parts such as leaves, flowers, seeds, roots and fruits. More of 8000 phenolic structures have mentioned and they are extensively dispersed in the plant kingdom [1]. Phenolic compounds an essential a part of the human diet [2] and has a great role in many biological and preventive activities of various diseases as shuch Hypertension,Cholera, Asthma, Diabetes, Cancer and rheumatic diseases[3-] [4] [5].

These biologically active compounds are used as medicines, food additives, dyes, pesticides, cosmetics, perfumes and fine chemical substances [6].

Moringa Oleifera is one of the important aromatic and medicinal species from the Moringaceae family usually called ‘sahajan’ in Hindi, Horse radish in English, Rawag in Arabian. Is a small, fast-growing, evergreen or deciduous tree, Up to 12 m high.Distributed in tropical and subtropical countries. It has an excellent range of medicinal uses and high nutritional value. Preceding research have mentioned that *Moringa Oleifera* acquires diverse biological activities including, antioxidant, anti-inflammatory, anticancer, hepatoprotective activity ,cardiovascular activity and antifertility activity[7].

All plant parts of *Moringa oleifera* are traditionally used for diverse purposes, however leaves are commonly the most used. Particularly, they are used in human and animal nutrition and in the traditional medicine.*Moringa oleifera* leaves are wealthy in protein, mineral, beta-carotene and antioxidant compounds. Leaves are added to food preparations as integrators of the diet. In traditional medicine, these leaves are used to treat several ailments which include fever, malaria, typhoid, arthritis, parasitic illnesses, and cuts, illnesses of the skin, swellings, and genito-urinary ailments [8].

Our work aims to determine the total content of phenolic, flavonoid and tannin compounds of leaves of *Moringa Oleifera* and study the correlation between them and the antioxidant capacity.

2. EXPERIMENTAL METHODS

2.1. Plant Materials

Moringa oleifera fresh leaves were collected from Tamanrasset, Algeria between November and January period. The plant was identified and authenticated by Prof. AIDOUD Amor (Department of agronomy, Faculty of nature and life, University of Ouargla (Algeria)).

2.2. Extraction

Extraction of the leaves part was executed with special solvents primarily based on the variation in the polarity. *Moringa oleifera* leaves had been cleaned, washed and dried in the shade for days and then grinded to powder. The powdered leaves material was macerated at room temperature with MeOH–H_2O (70:30, v/v) for 48 h, two times. The methanolic plant extract solution was filtered via filter paper then was evaporated till dryness, recovered with distilled water and partitioned successively by using three solvents represented in chloroform,ethyl acetat and n-butanol.The extracts were concentrated after which re-dissolved with minimal of methanol and saved in frozen at 4C°. The result of the procedure was represented respectively: Moringa crude extract (MCE), Moringa chloroform fraction (MCF), Moringa ethyl acetate fraction (MEF) and Moringa butanol fraction (MBF) [9].

2.3. Phytochemical analysis

The methanolic extracts of *Moringa oleifera* leaves were subjected to preliminary phytochemical test for the identity of diverse phytochemical constituents as according to standard procedures by [9-11].

2.4. Qauntitative analysis

2.4.1. Evaluation of total phenolic content:

The amount of phenolic content in MCE and all fractinds was estimated by Folin-Ciocalteu reagent method [12]. This method uses Gallic acid as a reference standard for plotting the calibration curve.A quantity of 1.5 ml of Folin-Ciocalteu reagent (diluted 1:10) was introduced to 0.5 ml of the plant extract in test tubes and had been neutralized with 3 mL of sodium carbonate solution (7.5%, w/v). The tubes were saved in dark at room temperature for 30 min with intermittent shaking for colour improvement. The absorbance was read at 765 by the usage of double beam UV-Vis spectrophotometer.The content of total phenolic compounds

was calculated as mean ±SD (n=3) and expressed as mg/g Gallic acid equivalent (GAE) of extract weight(EW).

2.4.2. Evaluation of total flavonoid content:

The total flavonoids content of MCE and all fractions was valuated through aluminum chloride colorimetric method[13]. Quercetin was used as a standard to assemble the calibration curve. 2 ml of plant extract was added to 2 ml of 2% AlCl3 ethanol solution. After staying at room temperature for 30 minutes, the absorbance was measured at 420 nm by double beam UV-Vis spectrophotometer. The content of total flavonoids was calculated as mean ± SD (n = 3) and expressed as mg/g quercetin equivalents (QE) of the extract weight (EW).

2.4.3. Evaluation of total tannin content:

The total tannin content of MCE and all fractions was calculated by colorimetric method [14].Catechin was used as a standard to construct the calibration curve.3 ml of 4% ethanol vanillin solution and 1.5 ml of concentrated hydrochloric acid were introduced to 0.4 ml of the plant extract.The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm with double beam UV-Vis spectrophotometer. The content of total tannin compounds was calculated as mean ± SD (*n*=3) and expressed as mg/g Catechin equivalents (CE) of the extract weight (EW).

2.5. Total antioxidan capacity

2.5.1. DPPH scavenging activity

The free radical scavenging activity of MCE and all fractions was determined by using DPPH procedure [15] with slight modifications. 10 µl of diluted plant extract was added to 190 µl of a 0.250 mmol/l $DPPH^{\bullet}$ ethanol solution. The solutions were placed in the dark at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm by the usage a UV spectrophotometer. Inhibition of DPPH radical was calculated as follows:

$$\text{DPPH scavenging effect} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 and A_1 are the absorbance at 517 nm of the control and the sample, respectively.

2.5.2. Phosphomolybdenum capacity

Total antioxidant capacity of MCE and all fractions was determined by the phosphomolybdenum assay [16] a volume of 2 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was combined to 0.2 ml of various concentrations of the extract plant. The resulting solutions were incubated in a boiling water bath at 95°C for 90 min. The combination was left to cool at room temperature then the absorbance of the mixture was read at 695 nm, using ascorbic acid as a positive control, and the results have been expressed as mM equivalent ascorbic acid.

2.5.3. Superoxide anion scavenging activity

The superoxide anion scavenging activity of MCE and all fractions can be measured as described by [17] with slight modifications. A sample solution (0.5 mL) at concentration of 0.003 mg/ml and 4.5 mL of 50 mmol/L phosphate buffer (pH 8.2) were added into freshly prepared 10 µL of 45 mmol/L pyrogallol. The inhibition rate of pyrogallol auto-oxidation was measured at 320 nm every 0.5 min for 4 min. The percentage inhibition of auto-oxidation of pyrogallol is calculated through using the following equation:

$$I = \frac{\Delta A_0 - \Delta A}{\Delta A_0} \times 100$$

Where A0 is the rate of autoxidation of pyrogallol in the absence of antioxidant and A is the rate of autoxidation of pyrogallol in the presence of antioxidant.

3. RESULTS AND DISCUSSION

3.1. Percentage yield (%)

The yield of all fractions and Moringa crude extract was decided as:

$$\text{Yield (\%)} = (W_1 * 100)/ W_2$$

Where W_1 is the weight of the extract after evaporation of the solvent, and W_2 is the weight of the dry plant material

Differents yield of Moringa crude extract and fractions obtained are demonstrated in table 1.

Table 1. Extraction yield % of *Moringa oleifera* leaves

Extract	Yield(w/w)
Crude extract	32,77 %
Chloroform fraction	1.81%
Ethyl acetate fraction	0.13%
n-butanol fraction	6.40%

3.2. Phytochemical analysis

Qualitative estimation of *Moringa oleifera* plant confirmed that the leaves have been wealthy in most important classes of secondary metabolites such as tannins, phenols, flavonoids, Steroids, Coumarins and glucosides, (Table 2). While have been poor in Alkaloids and steroids. The existence of these compounds has been proved and isolated in Moringa oleifera like *N*,α-L-rhamnopyranosyl vincosamide, 4-(α-L-rhamnopyranosyloxy) phenylacetonitrile (niazirin), pyrrolemarumine 4″-*O*-α-L-rhamnopyranoside, 4′-hydroxy phenylethanamide-α-L-rhamnopyranoside (marumoside A) and its 3-*O*-β-D-glucopyranosyl-derivative (marumoside B) and methyl4-(α-L-rhamnopyranosyloxy)-benzylcarbamate [18,19].However, their amounts in the leaves are still unknown.

Steroids and Alkaloids have additionally been discovered in Nigeria, suggesting that the pharmacological interest of plant from this climatic zone may also vary [20].The findings of other authors confirmed that these compounds have antimicrobial activity [21].

Table 2. Results of Phytochemical analysis of methanolic extract

Phytochemical test	Methanol extract
Tannins	+
Steroids	+
Flavonoids	+
Alkaloids	-
glucosides	+
Coumarins	+
Phenols	+
Saponins	+
terpenoids	-

+: Detected; -: Not detected.

Terpenoids were detected in fresh leaves of *Moringa oleifera* which have been reported to be active against antibacterial activity [22]

The richness of medicinal plants for active compounds suggests their therapeutic and preventive properties, natural flavonoids because of their multiple properties are solutions to cardiovascular disease, diabetes, cancers, venereal and microbial illnesses [23]. Tannins have confirmed their biological effects as antioxidants,antimicrobial and antiviral While Saponins proven their capacity of precipitating and coagulating red blood cells, anti-inflammatorys , antifungals and protective activities of veins and capillaries [24-26].

3.3. TPC

In this research TPC of MCE and all fractions were expressed as Gallic acid in mg/g extract weight, recorded among 32.985 ± 0.07 and 309.418± 0.71mg GAE/g EW, The highest content of phenols was observed in MEF and the lowest was registered in MCF. (MCF < MBF < MCE < MEF) (Table 3).

3.4. TFC

In this study TFC of MCE and all fractions were expressed as quercetin equivalent in mg/g extract weight, varied among 44.695 ± 0.23 and 5.192 ± 0.17 mg QE/g EW,The best value of

flavonoids were found in MEF,while the lowest had been found in MCF.(MCF < MCE < MBF < MEF) (Table 3).

3.5. TTC

In this research TTC of MCE and all fractions were expressed as catechin equivalent in mg/g extract weight, varied among 15.148 ± 0.66 and 3.864±0.07(mg QE/g EW), the best amount of tannin was determined in MCF, while the lowest had been present in the MCE.(MCE < MBF < MEF< MCF) (Table 3).

Table 3. Total phenolics, flavonoid and tannins content of *Moringa oleifera* leaves

Compounds	**Total phenolics (mg/g)**	**Total flavonoids (mg/g)**	**Total tannins (mg/g)**
Crude extract	117.863 ±0.22	17.738 ±0.04	3.864±0.07
Chloroform fraction	32.985 ±0.07	5.192 ± 0.17	15.148 ± 0.66
Ethyl acetate fraction	309.418±0.71	44.695 ± 0.23	10.430 ± 0.06
n-Butanol fraction	102.215±0.53	18.263 ± 0.03	4.986 ± 0.05

We have observed that amounts of polyphenols are bigger in polar fractions as compared to non-polar fractions, similar results were found by [13] These results indicate that phenolics contents were influenced by the solvents used in extracting the quality and quantity of these compounds and their solubility which depends in particular at the number of hydroxyl groups, the molecular weight and the length of the primary carbon chain of polyphenols [27].When comparing these results with those of *Moringa peregrina* from Al-Batinah Region of Sultanate of Oman [13]. We found that chloroforme fraction of *Moringa peregrina* contain more TPC and TFC than the same fractions of *Moringa oleifera*. Whil ethyl acetate fraction of *Moringa oleifera* is richer than those of *Moringa peregrina.* The content of total phenol is 81.26mg/g of ethyl acetate and 8.39 mg/g of chloroform fraction, the content of flavonoid is 75.53 mg/g of ethyl acetate and 6.55mg/g of chloroform fraction.Which benefits the richness of *Moringa oleifera* genus.

According to [8,28,29] who reported that leaves of *Moringa oleifera* are a interesting source

of polyphenols compounds such as Gallic acid, Quercetin, Chlorogenic acid, o-Coumaric acid, Kaempferol, Myricetin, p-Coumaric acid, Condensed tannins, vanillin and Rutin.

Several studies have proven the factors influencing the total content of phenolic compounds including with geographical and climatic factors, plant maturity, the duration of storage, drying method and extraction technique used[24,30-33], this explains the wide range of mentioned values.

Table 4. Results of total antioxidan capacity of *Moringa oleifera* leaves

Compounds	**DPPH IC_{50} mg/ml**	**Molybdate ion Reduction assay mM**	**Anion superoxide $O_2^{\bullet-}$ %**
Crude extract	0.180±0.004	74.08±6.209	52.84±0.48
Chloroform fraction	0.545±0.039	36.238±0.912	70.67±0.64
Ethyl acetate fraction	0.076±0.005	42.412±2.720	86.24±0.10
n-Butanol fraction	0.082±0.0009	91.832±0.960	57.49±0.64
Quercetin	0.065±0.0003	-	87.49±0.6
BHA	0.108±0.006	0.868±0.123	-
Ascorbic acid	0.07±0.002	-	92.04±1.61

3.6. Total antioxidan capacity of *Moringa oleifera* leaves

The stability of the free radical DPPH to a stable molecule is related to accept an electron or hydrogen radical from the antioxidants [34]. The scavenging effect of MCE, all fractions and standard on the DPPH radical was decided by the decrease in absorbance at 517 nm. The results of DPPH inhibition expressed as IC_{50} and reported in (table 4). Highest value of IC_{50} (0.076±0.005 mg/ml) was detected in MEF Followed by MBF (0.082±0.0009 mg/ml) While the lowest value of IC_{50} (0.545±0.039 mg/ml) was detected in MCF. Although standard antioxidant had better scavenging activity, all extracts confirmed a very good antiradical activity.

All fractions and MCE additionally had been used to determined antioxidants capacities by phosphomolybdenum assay which is based on the reduction of Mo (VI) to Mo (V) by the

antioxidant compound and the formation of a green phosphate/Mo (V) complex at acid pH[34].The results of total antioxidants capacities from standard and all extracts showed that MBF had the strongest antioxidant activity with a value (91.832±0.960 mM) while MCF had the weakest antioxidant activity with a value (36.238±0.912 mM). After comparing, the results showed that the total antioxidants capacity of Moringa Oleifera leaves extracts were better than BHA, (Table 4).

Superoxide anion scavenging activity assay based on autoxidation of pyrogallol which used as an anion O_2^- source. The superoxide anions are scavenged by antioxidants and consequently decrease the rate of pyrogallol autoxidation or even inhibit it [35].The results of superoxide anion scavenging activity by MCE, standard and all fractions are reported in (table 4). The Highest scavenging activity was observed for the MEF (86.24±0.10%) followed by MCF (70.67±0.64%).The lowest scavenging activity was recorded in MCE (52.84±0.48%). Although standard antioxidant had better scavenging activity, all extracts confirmed a great scavenging activity.

The medicinal plants are wealthy of majority natural compounds active such as vitamin C, tocopherols, flavonoids and other phenolic compounds responsible of antioxidant activity many studies have confirmed that phenolic compounds have the first role in antioxidant activity and this determined by good correlation [8,34].

3.7. Correlation

There are a medium correlation between the phenolic, flavonoid and tannin contents and antioxidant capacity by the scavenging DPPH and scavenging $O_2^{\bullet-}$, whil there is a negative correlation between the phenolic and flavonoid contents with the capacity to reduce molybdate. The contribution of phenols and flavonoids in the DPPH scavenging activity was 45% and 53% respectively. We have found also 78% of the activity to reduce molybdate was due to tannins. The contribution of phenols, flavonoids and tannins in $O_2^{\bullet-}$ scavenging activity was 44%, 40% and 47% respectively. The higher antioxidant activity of the ethyl acetate fraction is correlated its higher content of phenolic compounds this results are attributed the influence of phenolic compounds as principal components in the antioxidant ability. Many studies showed positive correlation between the antioxidant Properties and total

content of phenolic compounds [23,34].

4. CONCLUSION

In conclusion, our study conclusively demonstrates that *Moringa Oleifera* leaves is a good source of major medically active compounds like tannins, phenols, flavonoids, Steroids, Coumarins and glucosides. The results reveal higher amounts of phenolic compounds and good antioxidant activities that justify its use in traditional system of medicine in Algeria and other countries.

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