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Total Phenolic, Total Flavonoid and Free Radical Scavenging Potential of Moringa Oleifera Leaf and Tribulus Terrestris Fruit Extracts

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Abstract: ***This study investigated the antioxidant potential and free radical scavenging ability of ethanolic extracts from Tribulus terrestris fruit (TTF) and Moringa oleifera leaf (MOL) ethanolic extract. The antioxidant and free radical scavenging activities were measured by using standard protocols for the doses using 50, 100, 150, 200 and 250 µg/ml concentrations. Total phenolic and flavonoid content were estimated using the Folin-Ciocalteu method. Among the extracts, MOL showed the highest total antioxidant ability followed by TTF. With regard to various scavenging activities, MOL had the highest radical scavenging activity than TTF extract closely resembled the standards. Also, the reducing capacity on ferrous ions was high in MOL than TTF. The phenolic contents of TTF and MOL ethanolic extracts were quantified. A positive correlation (p value<0.001) was observed between phenolic content and free radical scavenging efficiencies. The results confirm that both MOL and TTF are important sources of natural antioxidants and serves as an effective free radical scavenger and/ or inhibitors. Hence, both extracts are suitable plant-based products that can be used as pharmaceutical agents to handle 'free radical-mediated diseases.***

Keywords: Antioxidant potential, free radical scavenging ability, Moringa oleiferaleaf extract, Tribulus terrestrisfruit extract, pharmaceuticals

1. Introduction

Traditional plant medicines have accorded mankind with a wide range of treatment options for illness. For thousands of years, India's herbal medicinal system has been used, and it has had a significant impact on human health It is estimated that 80% of the world's population relies on medicinal plants for self-treatment / self- medication (Kunwar and Adhikari, 2005). Plants of medicinal importance not only provide a major resource base for the traditional medicine and herbal industry, but also a source of income and health security for a large portion of the Indian population (Rahman et al., 2011). The attention paid by health authorities for the use of herbal medicines has dramatically increased thereby paving way to several research institutes to conduct research on a wide variety of plants to explore their therapeutic properties and medicinal applications.

2. Literature Review

The antioxidant properties of *Tribulus terrestris*(TT) and *Moringa olifera*(MO) plant extracts were evaluated in this study. TTalso known as Gokhru, is an annual plant of the 'Zygophyllaceae' family that has been used for generations to energise, vitalize, and improve sexual function and physical performance in men. It is a highly effective diuretic and tonic (Selvam, 2008). The whole plant extract of TT has been shown to have tremendous antioxidant activity as well as to provide streptozotocin-induced diabetic rats from oxidative stress (Amin et al, 2006). TT extract administration restored endogenous myocardial antioxidant status or free radical scavenging activity in cardiomyopathy (Ojha et al., 2008).

Likewise, *Moringa oleifera* (MO), commonly known as drumstick tree or horseradish tree, is a dietary plant native to the Indian subcontinent. Belonging to the member of the Moringacaea family it is a fast-growing, drought-resistant tree common throughout northern India's sub-Himalayan tracts and found worldwide in the tropics and sub-tropics (Fuglie, 1999; Basha et al., 2021). The bark, leaves, flowers, and fruits of the plant have all been used in the Ayurvedic medicinal system (Anwar and Rashid, 2007; Rakesh et al., 2021). The leaves of this tree are considered to be high in nutritional value and are used to make traditional Indian dishes. More importantly, the leaves have anti-infective, anti-inflammatory, anti-fever, anti-asthmatic, anti-ulcer, and anti-wound-healing properties, as well as anti-diarrhea, anti-blood-pressure, anti-paralysis, anti-diabetes, and anti-diabetes-like properties (Seshadri and Nambiar, 2003; Anwar et al., 2007). In experimental studies, the leaf extract was shown to have anticancer (Sreelatha et al., 2011), antioxidant (Bharali et al., 2003), and radioprotective (Rao et al., 2001) properties. The importance of antioxidants in human health has become increasingly clear as research into the mechanisms of their interaction with oxidants has advanced dramatically. Furthermore, human epidemiological studies have revealed that natural antioxidants may have health benefits. However, there is a paucity of literature on the antioxidant potential of the aforementioned phytoextracts. Considering the importance of these two native plants assessments were made to explore the phytoconstituents, antioxidant potential and free radical scavenging activity of ethanolic extracts of TT and MO by employing various in vitro assay systems, i.e. 2,2-diphenyl-1- picrylhydrazyl radical (DPPH) and nitric oxide radical scavenging, reducing power, scavenging activity of superoxide and hydrogen peroxide, in order to understand

the usefulness of aforesaid plant extracts as food and in medicine.

3. Materials and Methods

Chemicals

Gallic acid, butylated hydroxyl toluene (BHT), trichloroacetic acid (TCA), ferric chloride, nicotinamide adenine dinucleotide (NADH), nitro blue tetrazolium (NBT), phenazine methosulphate (PMS)sulphanilamide, naphthyl ethylene-diamine dihydrochloride, quercetin and ascorbic acid were obtained from Merck India Ltd, Mumbai.

Plant material

Tribulus terrestris fruits ethanolic (TTFE) and *Moringa olifera* leaves ethanolic (MOLE) extract were from different locations of Jnanabharati, Bangalore (longitude of 77 °30′ 05.604″E and latitude of 12 °56′ 57.608″N) and washed to remove periphyton, dust and sediment particles. Fruits and leaves of both the plants were cleaned, shade dried for around 25-30 day at room temperature and then crushed to fine powder. Both the plant samples were subjected to Soxhlet extraction system for 24 hours using 70 % ethanol and filtrates were made use for phytochemical analysis.

Quantification of phytochemical constituents

Total phenolic content

Soluble phenolic content of TTFE and MOLE extracts were determined according to the modified method of Singleton et al., 1999 by using the Folin-Ciocalteu reagent. About 1 ml of plant extract was mixed with 0.5 ml of folin-ciocalteu reagent (1:10) followed by 1.5 ml of Na_2CO_3 (0.7M). Subsequently, the mixture was shaken for 2 hours at room temperature and absorbance was measured at 760 nm. The total phenolic content was calculated from standard calibration curve of gallic acid and was expressed as equivalents of gallic acid/g extract.

$$C = \frac{(c \times v)}{m}$$

Where,
C=total phenol content, mg of gallic acid equivalents/g extract.
c= concentration of gallic acid established from the calibration curve(mg/ml).
v =volume of extract in ml, andm = weight of extract in gram.

Total flavonoid content

The total flavonoid content was determined based on the formation of flavonoid-aluminium complex according to the modified method of Zhishen et al., 1999. To 1 ml of extract, 0.1 ml $NaNO_2$ (5%) was added and incubated for 5 min at room temperature, then 0.1 ml of $AlCl_3$ (10%) was added and continued incubation for further 5 min, later the reaction mixture was treated with 0.6 ml of NaOH (1 mmol). Finally, the reaction mixture was diluted to 5 ml with distilled water and the absorbance was measured at 510 nm. The flavonoid content was calculated from standard quercetin curve and results expressed as equivalents of quercetin per gram extract.

$$C = \frac{(c \times v)}{m}$$

Where,
C= total phenol content, mg of gallic acid equivalents/g extract.
c= concentration of gallic acid established from the calibration curve(mg/ml),
v =volume of extract in ml, and m = weight of extract in gram.

DPPH free radical scavenging assay

Free radical scavenging activity of TTFE and MOLE extracts against 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was investigated spectrophotometrically by modified method of Chan et al., 2007. Different aliquots of both standard and sample solutions (50-250µg/ml) were mixed with 1 ml of DPPH (0.2 mmol) solution. The mixtures were incubated in dark for 30 min at room temperature and the absorbance was measured at 517 nm. The absorbance of the control sample containing the same amount of solvent and DPPH solution was measured. Ascorbic acid was used as standard and the percent inhibition of activity was calculated using formula.

$$DPPH\ scavening\ activity(\%) = \frac{[\text{Abs (control)} - \text{Abs (test)}]}{\text{Abs (control)}} \times 100$$

Where, Abs (control): Absorbance of the control and Abs (test): Absorbance of the extract/standard.

Superoxide radical (O^{2-}) scavenging assay

Superoxide radical scavenging activity of TTFE and MOLE extracts were evaluated using nitro blue tetrazolium (NBT) reduction using the modified method of Nishikimi et al., 1972.In the assay, auto oxidation of phenazine metho-sulphate (PMS, in phosphate buffer pH 7.4) generates superoxide anions which reduce the yellow dye nitro blue tetrazolium to blue coloured formazan. The reaction mixture consisted of 1 ml NBT solution (156 µM) and sample solutions of different concentrations (50-250 µg/ml). The reaction was started by adding 100 µl of PMS (60 µM) to the reaction mixture and incubated for 5 min at 25°C, absorbance was measured at 560 nm against blank. Ascorbic acid was used as the standard and percent (%) inhibition activity was calculated using the formula.

$$Superoxide\ scavening\ activity(\%) = \frac{[\text{Abs (control)} - \text{Abs (test)}]}{\text{Abs (control)}} \times 100$$

Where, Abs (control): Absorbance of the control and Abs (test): Absorbance of the extract/standard.

Hydrogen peroxide radical ($H_2O_2^-$) scavenging assay;

Hydrogen peroxide radical scavenging ability of TTF and MOL extracts were assessed by adopting the method given by Ruch et al., 1989. A solution of hydrogen peroxide (2 mmol) was prepared in phosphate buffer (0.2 M, pH 7.4). Extracts in different concentrations (50-250 µg/ml) were added to a hydrogen peroxide solution (0.6 ml, 2 mmol). The absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing

the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both extracts and standard compounds were calculated using formula.

$$\text{Hydrogen peroxide } scavening\ activity(\%) = \frac{[\text{Abs (control)} - \text{Abs (test)}]}{\text{Abs (control)}} \times 100$$

Where, Abs (control): Absorbance of the control and Abs (test): Absorbance of the extract/standard.

Nitric oxide radical (NO^-) scavenging assay;

Nitric oxide radical scavenging ability of extracts was measured by adopting the modified method described by Rao, 1997. In the assay, sodium nitroprusside generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions. To 1 ml of sodium nitroprusside (SNP) solution (10 mmol), 1 ml of extract at different concentrations (50-250 µg/ml) was added and incubated at room temperature for 2 hours at 27 °C. An aliquot (1 ml) of the incubated solution was taken and diluted with 0.5 ml of griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthyl ethylene diamine dihydrochloride) and the absorbance of pink coloured chromophore formed was read immediately at 550 nm and compared with standard, butylated hydroxyl toluene (BHT). Percent inhibition activity was calculated using formula.

$$\text{Nitric oxide } scavening\ activity(\%) = \frac{[\text{Abs (control)} - \text{Abs (test)}]}{\text{Abs (control)}} \times 100$$

Where, Abs (control): Absorbance of the control and Abs (test): Absorbance of the extract/standard.

Ferric reducing/antioxidant power (FRAP) assay

Ferric reducing/antioxidant power assay of TTFE and MOLE extracts were determined according to the method described by Benzie and Strain, 1996. FRAP working solution was prepared by mixing 2.5 ml of 10 mmol ferric tripyridyltriazine (Fe^{3+}-TPTZ) solution, 2.5 ml of 20mmol ferric chloride ($FeCl_3$). $6H_2O$ and 25 ml of 0.3M sodium acetate buffer (pH 3.6). To 1 ml of extract having different concentrations (50-250 µg/ml) 3 ml of FRAP reagent was added and incubated at 37°C for 30 min in water bath. The increase in absorbance of the coloured product ferrous tripyridyltriazine complex (Fe^{2+}-TPTZ) was measured spectrophotometrically at 593 nm. Fresh ferrous sulphate ($FeSO_4$) working solution was used to plot the calibration curve. Based on the ability to reduce ferric ions by the extracts, the antioxidant capacity was calculated using linear calibration curves and results are expressed as µmol Fe^{2+} gram equivalent of sample.

$$\mathbf{y = 0.0033x + 0.1817}$$

Where, y = absorbance and x = concentration in µg/ml

Statistical analysis

Statistical analysis was performed using one-way Analysis of Variance (ANOVA) with least significant difference (LSD) post hoc (at $P<0.01$) and linear regression analysis was used to calculate IC_{50} values by using 'SPSS software package 20.0'. Correlation and graphical preparations were plotted using 'Origin Pro software 9.0'. Results are shown as the Mean±SEM of six measurements.

4. Results

Natural antioxidants that are present in medicinal herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoid and phenolic compounds. In the present study, the antioxidant potential of ethanol extract of leaf and fruit extracts of TTandMOplants was evaluated.

DPPH free radical scavenging activity

The data shown in Table-(1.1) and Fig-(1.1) represents the DPPH radical scavenging activity analyzed to establish the antioxidant potential of different concentrations of TTFE and MOLE extracts used. As a positive control, the ascorbic acid was used to represent IC50 value of 116.86µg/ml, while MOLE showed IC50 value of 413.84µg/ml and TTFE exhibited IC50 value of 221.51µg/ml respectively (Table-1.5).

Superoxide radical scavenging activity

The data projected in Table-(1.2) demonstrated the superoxide anion radical activities of TTFE and MOLE extracts. During the analysis, it was observed that the phytoextracts viz., TTFE and MOLE exhibited a dose-dependent response (activity) and increased the aggregation of blue NBT and were able to inhibit the superoxide anions. The highest superoxide anion radical activity was obtained for MOLE extract than TTFE extract (Table-1.2). The percent inhibition of superoxide radicals generated by both MOLE and TTFE extracts was found increasing in a concentration-dependent manner (Fig-1.2), wherein MOLE showed an IC_{50} value of 188.77µg/ml and TTFE showed 264.26µg/ml respectively when compared to the IC_{50} value 8.66µg/ml of ascorbic acid, the standard (Table- 1.5).

Nitric oxide radical scavenging activity

Both TTFE and MOLE extracts exhibited a concentration-driven scavenging capacity of nitric oxide radicals and data represented in Table-(1.3) and Fig-(1.3). Both phytoextracts demonstrated to have a substantial role in nullifying the nitric oxide radical scavenging activity at an increasing concentration. The TTFE extract showed higher nitric oxide radical scavenging activity with IC_{50} value of 219.02µg/ml while MOLE showed 282.27µg/ml. However, the IC_{50} values of both extracts were found to be significantly higher than the IC_{50} value of BHT, the standard showed a value of 140.26µg/ml (Table- 1.5).

Hydrogen peroxide radical scavenging activities

Both phytoextracts viz., TTFE and MOLE exhibited a dose-dependent increase in hydrogen peroxide radical scavenging activity and the data represented in Table-(1.4) and Fig-(1.4). The MOLE extracts showed IC_{50} value of 422.9 and TTFE showed 259.59µg/ml whereas ascorbic acid showed 52.25µg/ml (Table-2.5). Both the phytoextracts, viz., TTFE and MOLE, were capable of nullifying hydrogen peroxide. However, TTFE extract was found more potent in scavenging peroxide radicals compared to MOLE.

Ferric reducing/antioxidant activity

The reducing ability of the extracts MOLE and TTFE was established based on their capability to reduce the ferric to ferrous TPTZ complex in the reaction mixture and the data shown in Table-(1.5) and Fig-(1.5). The FRAP value was achieved by plotting a graph standard curve of $FeSO_4$ (50-250µg/ml) and in the present study, the standard curve was found to be linear (y=0.0033x+0.1612, R= 0.998) as shown in Fig-(1.5). The result demonstrated that the TTFE extract possesses a higher antioxidant capacity compared to the MOLE extract. FRAP value of the TTFE extract being 151.2µmol Fe^{2+} equivalents per gram extract(s) while the FRAP value of the MOLE extract being 113.1µmol Fe^{2+} equivalents per gram extract, respectively. Total phenolic content (TPC) and total flavonoid content (TFC)

The amounts of TP and TF contents in both leaves and fruit are found to be much higher, the results of the present study showed that the mean amounts of TP content in TTFE and MOLE was found to be 28.72 and44.9 mg of eq gallic acid/gm extract. Similarly, TFC in both the extracts were found to 39.6 and 36.67 mg of eq quercetin/gm extractrespectively (Fig 1.6 and 1.7).

Correlation between total phenolic content and antioxidant activity

The data are shown in Table-(1.6) depict the Pearson's correlation coefficient (R) values calculated for the total phenolic content v/s antioxidant ability. A strong positive and significant (P<0.01) correlation was witnessed between the total phenolics and DPPH activity with the usage of MOLE extract (R = 0.998). The free radical scavenging activity of MOLE extract exhibited a strong positive correlation with phenolic content and it was found as H_2O_2>NO->O_2->FRAP activities. By the usage of TTFE extract, the strongest positive correlation was held between the total phenolics and NO- scavenging activity (R = 0.998) at p<0.01 followed in the following order H_2O_2 (p<0.05) scavenging activities. The free radical scavenging activity of TTFE extract showed a strong positive correlation with NO->H_2O_2>FRAP>O_2->DPPH activities against phenolic content. Thus the correlates established a highly positive association between the total phenolic content and different antioxidant/free radical scavenging capacities of both extracts.

Table 1.1: Determination of DPPH radical scavenging activity of *Moringa oleifera* leaf (MOLE), and *Tribulus terrestris* fruit (TTFE) extracts at different concentrations

	% inhibition		
Concentrations	Standard AA	MOLE	TTFE
50	33.23±0.46	5.08±0.18	14.70±0.34
100	43.61±2.44	20.10±0.15	21.39±0.24
150	54.09±0.23	23.63±0.40	37.12±0.40
200	78.82±0.43	25.31±0.24	47.16±0.41
250	88.68±0.46	30.10±0.21	54.42±0.12

Values are Mean ± SEM'µg/ml' (n=3, p<0.05 for all tested dosages), AA-Ascorbicacid, MOLE- *Moringa oleifera* leaf ethanolic extract, TTFE- *Tribulus terrestris* fruit ethanolic extract.

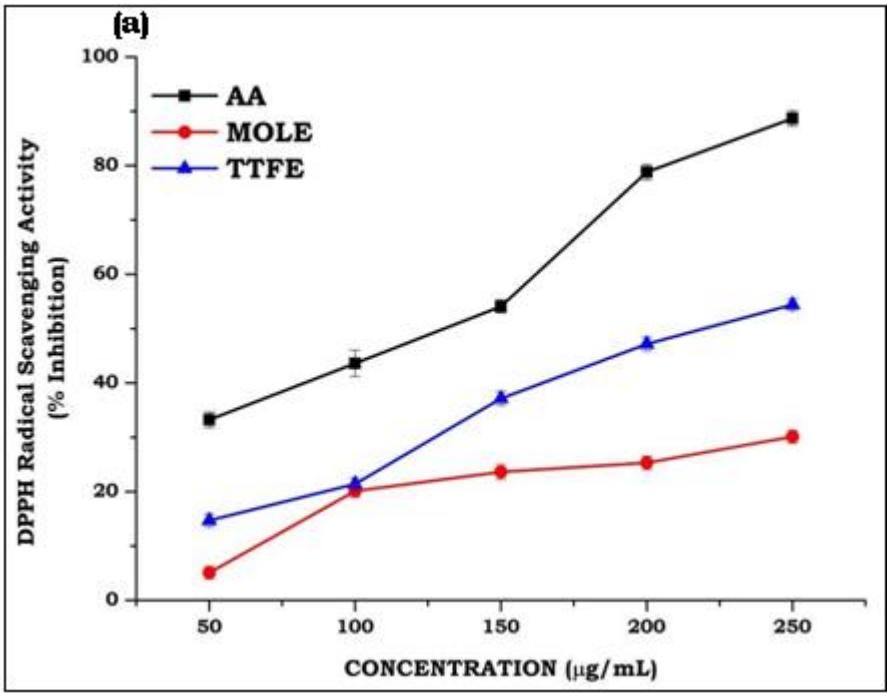


Figure 1.1: Determination of DPPH radical scavenging activity of *Moringa oleifera* leaf (MOLE), and *Tribulus terrestris* fruit (TTFE) extracts at different concentrations.

Table 1.2: Determination of Superoxide radical scavenging activity of *Moringa oleifera* leaf (MOLE), and *Tribulus terrestris* fruit (TTFE) extracts at different concentrations.

Concentration	% Inhibition		
	Standard AA	MOLE	TTFE
50	60.99±0.32	12.89±1.12	20.89±1.39
100	68.99±0.20	34.01±0.88	27.72±1.69

150	76.89±0.35	43.74±0.61	36.17±1.02
200	89.70±0.28	52.96±0.83	42.45±1.08
250	95.37±0.31	61.42±0.51	46.76±1.39

Values are Mean ± SEM 'µg/ml' (n=3, p<0.05 for all tested dosages), AA -Ascorbic acid, MOLE- *Moringa oleifera* leaf ethanolic extract, TTFE- *Tribulus terrestris* fruit ethanolic extract.

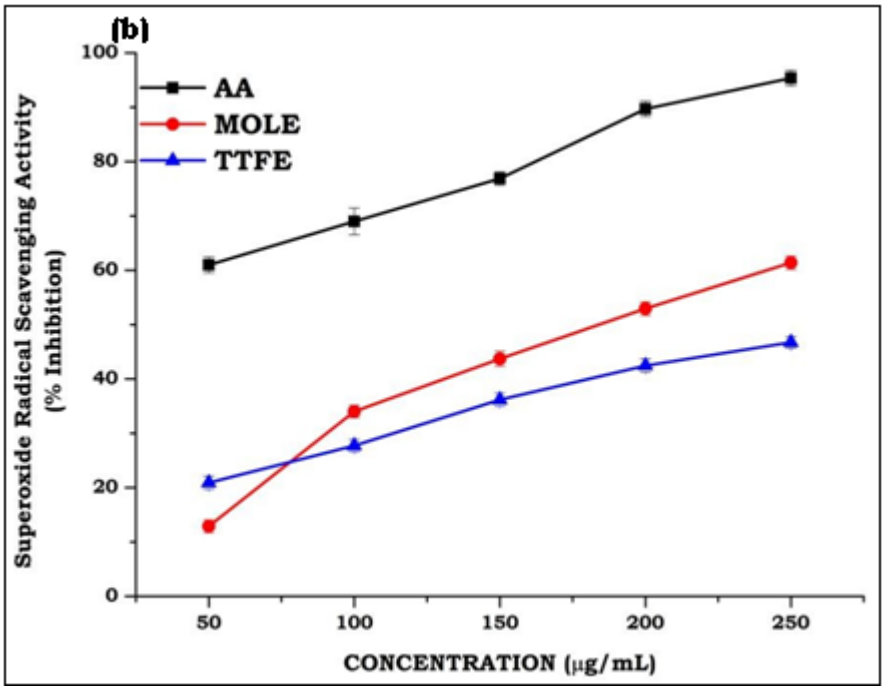


Figure 1.2: Determination of Superoxide radical scavenging activity of *Moringa oleifera* leaf (MOLE), and *Tribulus terrestris* fruit (TTFE) extracts at different concentrations.

Table 1.3: Determination of Nitric oxide radical scavenging activity of *Moringa oleifera* leaf (MOLE), and *Tribulus terrestris* fruit (TTFE) extracts at different concentrations

	% Inhibition		
Concentration	Standard BHT	MOLE	TTFE
50	24.54±1.06	8.12±0.37	11.60±1.60
100	32.37±0.95	12.05±0.56	16.70±0.44
150	51.40±0.38	19.93±0.74	32.98±1.06
200	72.00±0.64	30.38±0.56	46.68±0.74
250	85.39±0.32	40.71±0.77	57.72±0.64

Values are Mean ± SEM 'µg/ml' (n=3, p<0.05 for all tested dosages), BHT -Butylated hydroxyl toluene, MOLE- *Moringa oleifera* leaf ethanolic extract, TTFE- *Tribulus terrestris* fruit ethanolic extract.

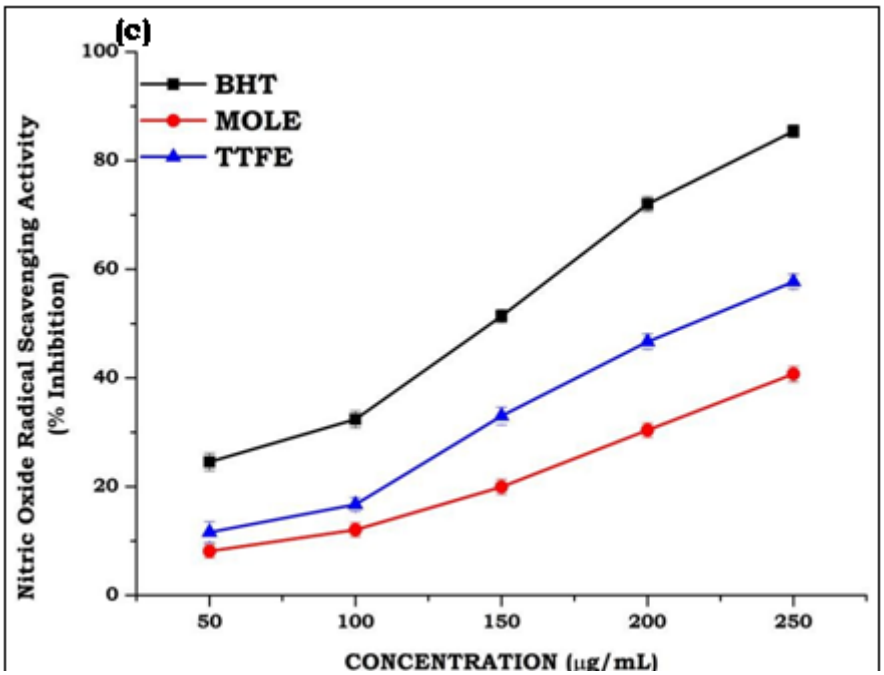


Figure 1.3: Determination of Nitric oxide radical scavenging activity of *Moringaoleifera* leaf (MOLE), and *Tribulus terrestris* fruit (TTFE) extracts at different concentrations.

Table 1.4: Determination of Hydrogen peroxide radical scavenging activity of *Moringa oleifera* leaf (MOLE), and *Tribulus terrestris* fruit (TTFE) extracts at different concentrations.

Concentration	% inhibition		
	Standard AA	MOLE	TTFE
50	48.59±0.53	5.17±0.29	15.60±0.50
100	59.63±0.76	13.41±0.44	20.68±0.25
150	69.68±0.61	19.64±1.20	31.91±0.49
200	75.42±4.78	24.90±0.44	38.19±0.77
250	86.79±3.24	28.45±0.57	49.70±0.66

Values are Mean ± SEM 'µg/ml' (n=3, $p<0.05$ for all tested dosages), AA-Ascorbicacid, MOLE- *Moringa oleifera* leaf ethanolic extract, TTFE- *Tribulus terrestris* fruit ethanolic extract.

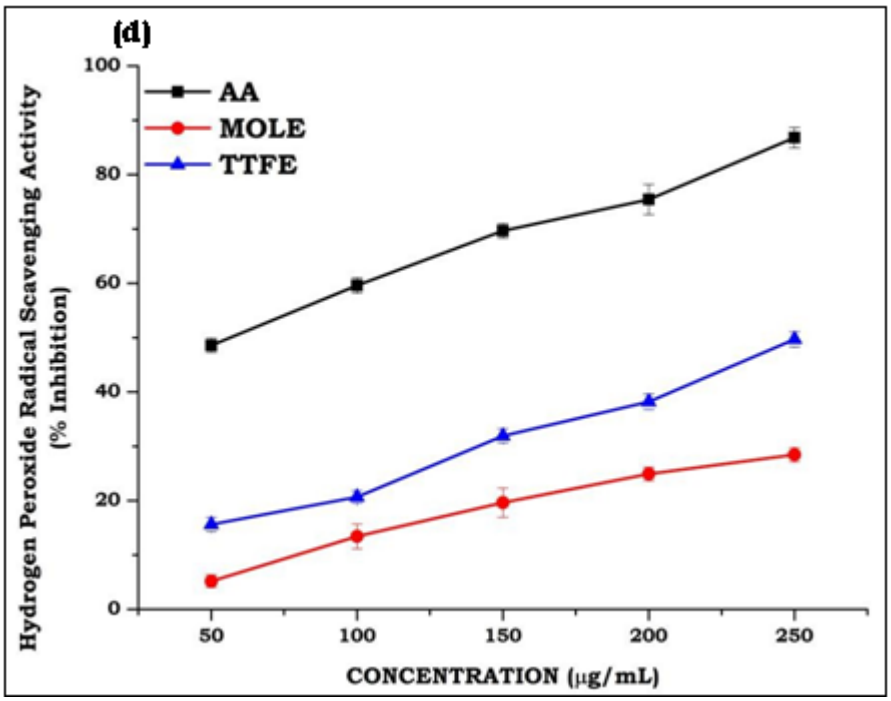


Figure 1.4: DeterminationofHydrogenperoxideradicalscavengingactivityof*Moringa oleifera* leaf (MOLE), and *Tribulus terrestris* fruit (TTFE) extracts at different concentrations

Table 1.5: Comparison of IC50 values derived upon phytoextracts usage (MOLE and TTFE) relative to free radical quenching.

Radical scavenging assay	µg/ml		
	Standards	Mole	TTFE
DPPH	116.86±0.416[a]	413.84±0.86[c]	221.51±1.15[b]
Superoxide	8.66±0.17[a]	188.77±0.57[c]	264.26±0.8[b]
Nitric oxide	140.26±0.29[c]	282.27±0.4931[a]	219.02±1.453[b]
Hydrogenperoxide	52.25±0.32[a]	422.9±0.393[c]	259.59±0.32[b]

Values are Mean ± SEM 'µg/ml' (n=3, $p<0.05$ for all tested dosages), where different superscripts (a, b, and c) in a row indicate significant ($p<0.05$) differences among extracts and standard by using DMRT post hoc. MOLE- *Moringa oleifera* leaf ethanolic extract, TTFE- *Tribulus terrestris* fruit ethanolic extract.

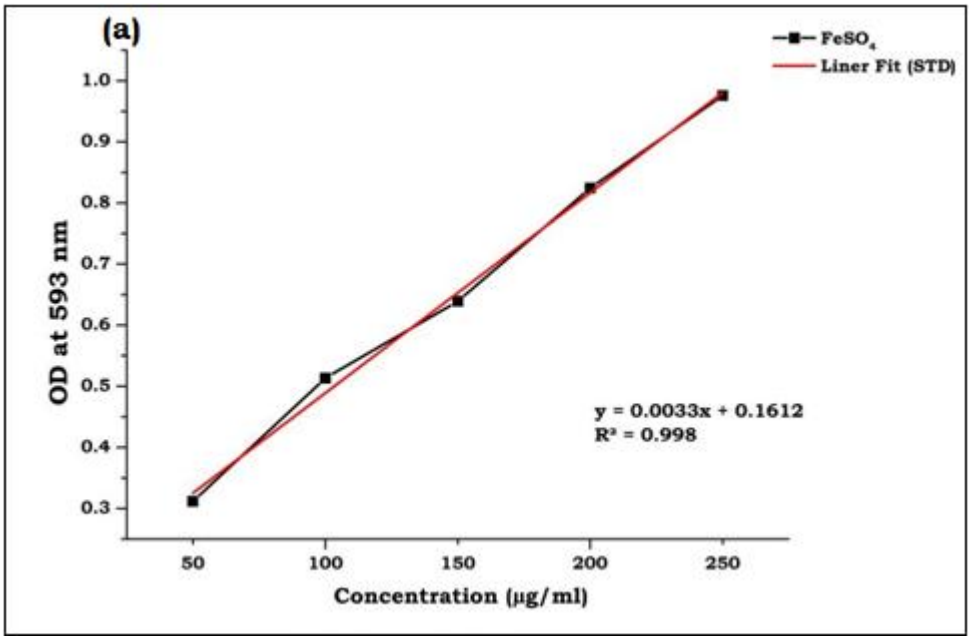


Figure 1.5: Determination of Ferric reducing /antioxidant power (FRAP) of *Moringa oleifera* leaf (MOLE) and *Tribulus terrestris* fruit (TTFE) extracts
(a) Standard graph for FRAP assay (b) Ferric reducing /antioxidant power (FRAP) assay: Results showing FRAP value of *Moringa oleifera* leaf (MOLE) as 151.2µM Fe^{2+} equivalents/g, and *Tribulus terrestris* fruit (TTFE) as 113.1µM Fe^{2+}equivalents/g.

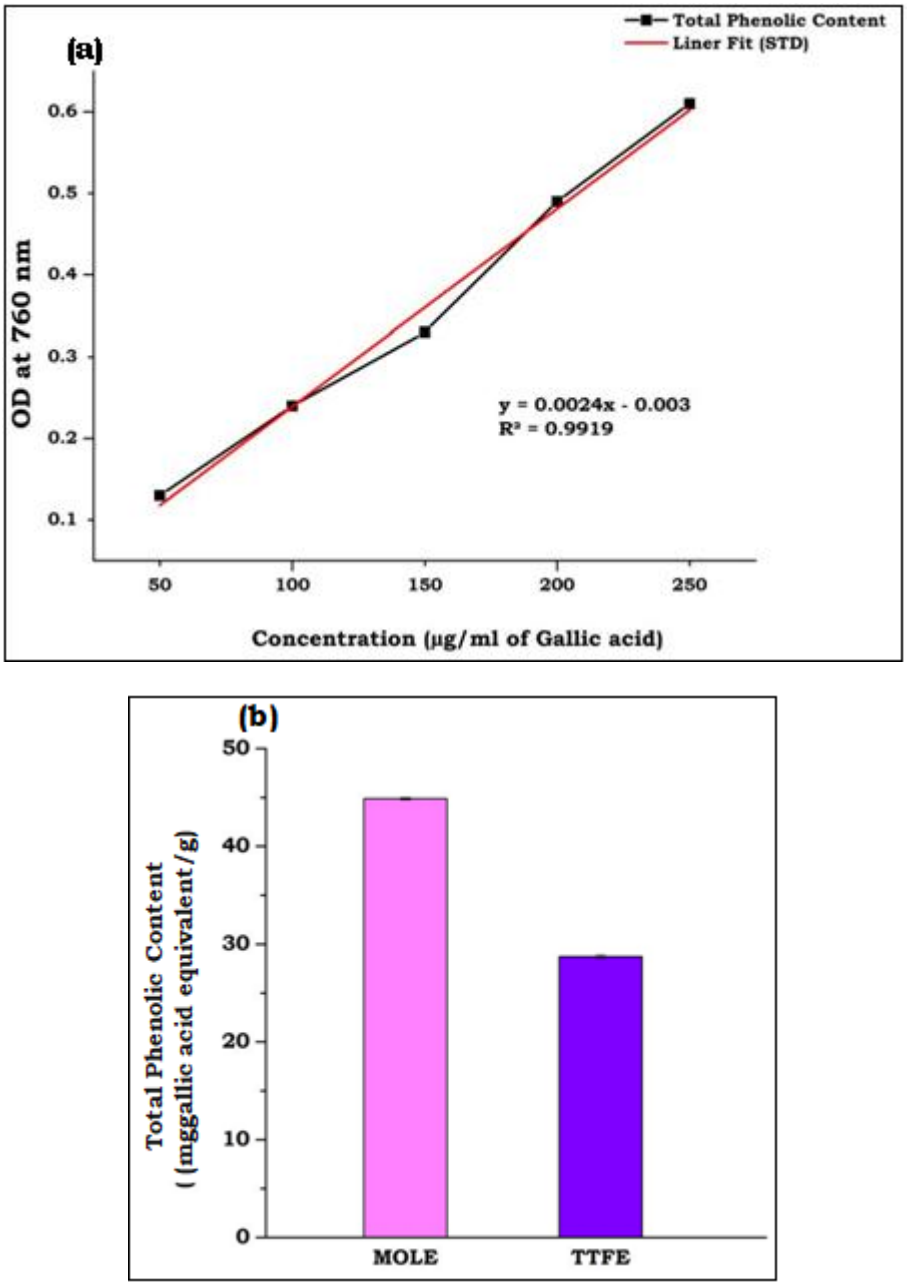
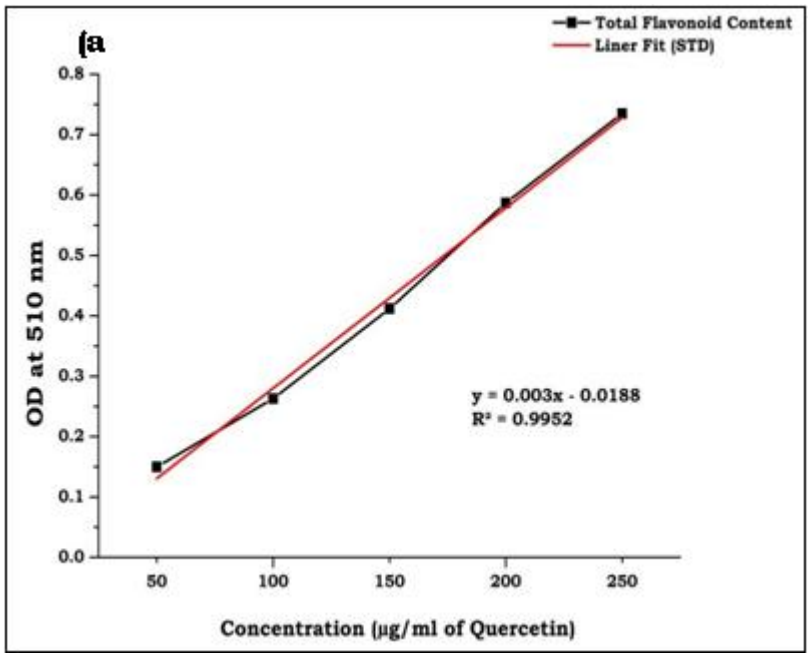


Figure 1.6: Determination of Total Phenolic Content of *Moringa oleifera* leaf (MOLE) and *Tribulus terrestris* fruit (TTFE) extracts.
(a) Standard graph of gallic acid (standard) (b) Total Phenolic Content of *Moringa oleifera* leaf (MOLE) as 44.87±0.118mg gallic acid equivalent/g extract and *Tribulus terrestris* fruit (TTFE) as 28.72±0.184 mg gallic acid equivalent/g extract.



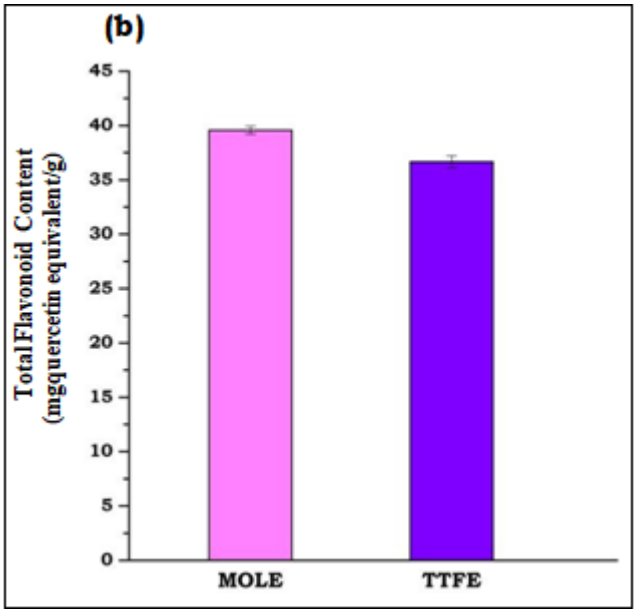


Figure 1.7: Determination of Total Flavonoid Content of *Moringa oleifera* leaf (MOLE) and *Tribulus terrestris* fruit (TTFE) extracts. (a) Standard graph of quercetin (standard) (b) Total Phenolic Content of *Moringa oleifera* leaf (MOLE) as 36.66±0.55mg quercetin equivalent/g and *Tribulus terrestris* fruit (TTFE) as 39.55±0.366mg quercetin equivalent/g.

Table 1.6: Correlates (r) relative to total phenolic content and free radical quenching activities of MOLE and TTFE

Total phenolv/s	Phytoextracts	
	MOLE	TTFE
DPPH	0.998**	0.961
O^{2-}	0.971	0.982
NO^{-}	0.990*	0.998**
H2O2	0.998*	0.997*
FRAP	0.908	0.990*

Values represent the correlation factors (r); **Correlation values are significant at 0.01 level; *Correlation values are significant at 0.05 level.

DPPH: Diphenyl-picrylhydrazyl radical scavenging activity; O2-: Superoxide radical scavenging activity; H2O2: Hydrogen peroxide scavenging activity; NO-: Nitric oxide scavenging activity and FRAP: Ferric reducing/antioxidant activity.

5. Discussion

There are several biological effects exerted by plant polyphenols, including antioxidants and free radical scavenging abilities (Archana et al., 2015). As well, flavonoids have a great deal of importance in preventing diseases, the molecular structure of flavonoids, the position of the hydroxyl group in its chemical structure, and other features determines they are potent antioxidants. According to the findings of the current investigation, both TTFE and MOLE extracts are excellent sources of polyphenols, and MOLE extract outperformed TTFE in terms of phenolic content whereas and reverse trend was evident in flavonoid content.

The study revealed that TTFE and MOLE extracts exhibited significant phenolic contents of 44.9 and 28.7mg of gallic acid equivalent per gram, respectively. The total flavonoid content observed were 39.6 and 36.6mg of equivalent quercetin per gram extract in TTFE and MOLE extracts respectively. Several reports suggested that saponins, triterpenoids and alkaloids have a direct influence on the antioxidant efficacy of any herbal or phytoextracts (Othman et al., 2019, Gupta et al., 2014).Earlier findings of Boonyadist et al. 2012 reported the presence of total phenolic and flavonoid contents in crude ethanolic extracts of MOleaf found to be 53.5 g/chlorogenic acid equivalent and 25.1g/isoquercetin equivalent respectively. Likewise, Siddhuraju and Becker, 2003 reported 29.4 g/extract of phenolic content ethanol extract of MO leaves. Thereby, the findings of this study corroborate with the observations of previous studies. Further, the functional role of these secondary metabolites in the maintenance of anti-oxidative strategies is well exemplified. The antioxidant and radical

scavenging properties of plants are based on their medicinal value.

In this study, ascorbic acid was used as the standard and it showed an increase in the percent of inhibition of DPPH as the concentration of the compound increased. The TTFE extract showed IC_{50} at a concentration of 221.51µg/ml revealing its high DPPH scavenging efficiency. Besides, MOLE extract exhibited a significant dose-dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 413.84µg/ml while IC_{50} of ascorbic acid is 116.86µg/ml. The preponderance of studies on DPPH scavenging of *Moringa* and selected vegetables (spinach, cauliflower, cabbage and peas) has shown that the leaves of the plant and the flower of the plant are having more free radical scavenging activity than all the vegetables (Pakade et al., 2013). Similar findings have been published regarding the remarkable free radical scavenging potential of ethanol extracts of *Tribulus* (Hifnawy et al., 2015).Thereby, the results of present studyconfirm that the ethanolic extracts of both plants are effective with respect to DPPH radical scavenging activity and in comparison, MOLE has better antioxidant potential than TTFE.

During superoxide radical scavenging assay, superoxide anion formed from the coupling reaction of PMS-NADH eventually reduces NBT. When the herbal extract scavenges the superoxide anion, there would be a decrease in absorbance at 560 nm indicating the consumption of superoxide anion in the reaction mixture and determining the scavenging ability. In the present study, Ascorbic acid, MOLE and TTFE extracts were found to have a dose-dependent increase in percent inhibition of superoxide radical scavenging. MOLE extract showed an IC_{50} value of 188.77µg/ml indicating strong superoxide radical scavenging activity than TTFE which had an IC_{50} value of 264.26µg/ml.

Nitric oxide (NO) has a vital role in the regulation of physiological functions like inflammation. However, excessive NO levels can directly impair the tissues leading to oxidative injury. Further, NO reacts with superoxide radicals to form another reactive compound, peroxynitrite (ONOO-). During physiological functioning, peroxynitrite forms an adduct with carbon dioxide in body fluids which could eventually lead to oxidative injury of proteins causing several ailments (Szabó et al., 2007). In this study, MOLE was found to be less scavenging on nitric oxide (-282.27µg/ml), when compared to TTFE (IC_{50}–219.02µg/ml) and the reference compound, BHT (IC_{50} value-140.26µg/ml) (Table-1.5).

Hydrogen peroxide scavenging ability of phytoextract is important even though hydrogen peroxide is less harmful because of its weaker oxidizing and reducing capacities. But hydrogen peroxide can act as highly cytotoxic as it transforms into a hydroxyl radical in the presence of metal ions and superoxide anion and it also produces singlet oxygen while reacting with superoxide anion (Saumya and Basha, 2011). Both MOLE and TTFE extracts were found to have higher hydrogen peroxide scavenging activity as the concentration of the extract increased. MOLE extract was found to have a higher IC_{50} value of 422.9µg/ml indicating low scavenging ability than TTFE (IC_{50} value- 259.59µg/ml) and ascorbic acid (IC_{50} value 52.25µg/ml).

The FRAP test measures the electron-donating capacity of reducing agents (i.e., antioxidants), which results in the reduction of ferric (Fe^{3+}) complex to ferrous (Fe^{2+}) ions, resulting in a blue-green solution that is proportional to the quantity of Fe^{2+} in the reaction 44mixture (Ak and Gülçin, 2008). The Fe^{3+}-TPTZ complex is reduced to Fe^{2+}-TPTZ in the presence of phytoextracts, where electrons act as a catalyst. Using a standard curve ($FeSO_4$), the reductive ability of both extracts (MOLE and TTFE) was estimated and determined to be 113.1 and 151.2 µmol of equivalent Fe^{2+} per gram extract, respectively. The results indicated a better reducing ability of TTFE than MOLE extracts.

The correlation coefficients (r) assessed indicates the existence of linearrelationship between the amount of total phenolic content and antioxidant properties of both extracts. In brief, a strong correlation exists between total phenolic content and antioxidant activities of both extracts and results are in agreement with previous studies Dakshayini and Basha, 2018.

6. Conclusion

In conclusion, in vitro assessments carried out using phytoextracts support the fact that the ethanol extracts of *Moringa oleifera* leaves (MOLE) and *Tribulus terrestris* (TTFE) were having significant phenolic and flavonoid contents. Besides, both extracts exhibited have a higher free radical scavenging efficacy when compared to the reference compounds. Since the extracts have substantial in vitro antioxidant ability, they can be preferably used as protective and/or prophylactic agents in vivo models.

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