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# Antioxidant activity of herbal formulation containing *Tinosporia cordifolia, Moringa oleifera* and *Allium sativum*

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***Abstract*---**The aim of this study was to screen herbal formulation containing *Tinosporia cordifolia, Moringa oleifera* and *Allium sativum* to display potent antioxidant activity *in vitro* in order to find possible sources for future novel antioxidants in food and pharmaceutical formulations. Data from present results revealed that herbal formulation containing *Tinosporia cordifolia, Moringa oleifera* and *Allium sativum* act as an antioxidant agent due to its free radical scavenging and cytoprotective activity.

***Keywords*---**Antioxidant Activity, Herbal formulation *Tinosporia cordifolia, Moringa oleifera, Allium sativum.*

## Introduction

Free radicals are thought to have a significant role in the development of tissue damage and pathological events (Floyd, 1990; Volterra et al., 1994). Phenolic chemicals are present in a wide range of plants, both edible and non-edible, and they have been shown to have a variety of biological effects, including antioxidant activity. Crude extracts of phenolic-rich fruits, herbs, vegetables, grains, and other plant materials are gaining popularity in the food industry because they prevent lipid oxidation and hence increase food quality and nutritional content (Lattanzio, 2013; Boudet, 2007). As the future trend moves toward functional food with particular health benefits, scientists, food manufacturers, and consumers are becoming more interested in the antioxidant contents of plant materials in the preservation of health and protection from coronary heart disease and cancer (Schinella et al., 2002; Cheynier, 2012; Kähkönen et al.,1999). Flavonoids and other phenolic compounds have been linked to a reduction in the risk of cancer and heart disease. The antioxidant status of plasma in humans has been demonstrated to improve after consuming alcohol-free red wine or a phenolic

component combination derived from red wine (Tungmunnithum et al., 2018; Rauha et al., 2000).

*Tinospora cordifolia* Miers (Menispermaceae) is a glabrous climbing succulant shrub, commonly found in hedges. It is native to India, thrives easily on tropical region (Prince & Menon, 1999). *Moringa oleifera* is a member of Moringaceae, and it is grown extensively in many Southeast Asian countries particularly in Thailand, India, Philippines and Pakistan (Charoensin, 2014; Fuglie, 2001). For thousands of years, garlic (*Allium sativum*) has been utilised in international cuisines as well as herbal medicine, and it has been believed to help prevent anything from high cholesterol to cancer (Rahman et al., 2012). The aim of this study was to screen herbal formulation containing *Tinosporia cordifolia, Moringa oleifera* and *Allium sativum extract*s of Finnish origin with respect to antioxidant activity in order to find new potential sources of natural anti-oxidants.

**Material and Methods**

Extraction methods used for getting the active components from the plants. The active component such as alkaloids, flavonoids, terpenoids, glycosides, fatty acids, etc. are found to be responsible for the activity of plants. Various solvents like aqueous, ethanol, methanol, petroleum ether, benzene, chloroform, etc. were used to obtain different types of extracts. Depending on the polarity of these solvents, various extraction procedures reported for particular plants (Doss et al., 2011).

**Collection and Authentication of Test Plants**

The raw plant's materials of *Moringa oleifera* (leaves), *Tinospora cordifolia* (aerial parts) and *Allium sativum* (cloves) were collected from the Jaipur, Rajasthan. The authentication and identification have done by Dr. Sunita Sharma, Head, Raw Material and Herbarium, Rajasthan University, Jaipur.

**Methodology of Extraction of Plant Materials**

The raw materials of *M. oleifera* (leaves), *T. cordifolia* (aerial parts) and *A. sativum* (cloves) collected and authenticated. The raw materials were air dried for few weeks under shade and pulverized using mechanical blenders until it becomes the coarsepowder. Finally, these coarse powders stored in airtight container for future use. Ultimately, by yield, the type of extract was selected for the study.

**Sequential Extraction Procedure for Experimental Plant Materials**

In this method, freshly collected plant material (*M. oleifera*, *T. cordifolia,* and *A. sativum*) chopped, shade dried and grounded separately for each plant sample. Through, the dried coarse plant material, different extracts prepared for each plant sampleindividually (Krakowska-Sieprawska et al., 2022).

**Extraction of *Moringa oleifera***

The *M. oleifera* leaves raw material was collected, cleaned, washed and shade dried. The raw materials were homogenized to a fine powdered and stored in an airtight bottle. The extraction was conducted by weighing 100 g of *M. oleifera* dried leaf powder and then it's extracted using aqueous, methanol, chloroform, petroleum ether in subsequent extraction process by using soxhlet apparatus. In every step, the marc dried which results from the previous step of sequential extraction. The extractive was filtered through Whatman No.1 filter paper to eliminate any debris and underivable substances dissolved in the extraction solvent. The resultant extractive solvent removed at their respective temperature by using rotary evaporator under vacuum. Finally, the extracts weighed, and their percentage yield was evaluated using the formula and recorded. Then, the extract was stored in a refrigerator at 4 $^{o}$C until further use (Krakowska-Sieprawska et al., 2022).

Table 1: Details of sequential extraction procedure for *M. oleifera*

| Solvent Used | Sample (gram) | Boiling Point (°C) | Duration of Extraction (hours) |
|---|---|---|---|
| Aqueous | 90 | 90 | 5 |
| Methanol | 78 | 65 | 7 |
| Chloroform | 70 | 62 | 6 |
| Petroleum Ether | 62 | 69 | 3.5 |
| Residue left | - | - | - |

**Extraction of *Tinospora Cordifolia***

*T. cordifolia* raw material was collected, cleaned, washed and shade dried. The raw materials were homogenized to a fine powdered and stored in an airtight bottle. The extraction process begins by weighing 100 g of *T. cordifolia* dried aerial part powder and extracted using aqueous, methanol, acetone, petroleum ether in subsequent extraction process by using soxhlet apparatus. In every step, the marc dried which results from the previous step of subsequent extraction. The extractive was filtered through Whatman No.1 filter paper to eliminate any debris and unextractable substances that didn't dissolve in the extraction solvent. The resultant extractive solvent removed at their respective temperature by using rotary evaporator under vacuum. Finally, the extracts weighed, and their percentage yield was evaluated using the formula and recorded. Then, the extract is stored in a refrigerator at 4$^{o}$C until further use (Krakowska-Sieprawska et al., 2022).

Table 2: Details of sequential extraction procedure for *T. cordifolia*

| Solvent Used | Sample (gram) | Boiling Point (°C) | Duration of Extraction (hours) |
|---|---|---|---|
| Aqueous | 94 | 90 | 6.0 |
| Methanol | 85 | 65 | 8.0 |

| | | | |
|---|---|---|---|
| Chloroform | 80 | 55 | 7.0 |
| Petroleum Ether | 61 | 69 | 4.0 |
| Residue left | - | - | - |

**Extraction of *Allium sativum***

*A. sativum* raw material was collected, cleaned, washed and shade dried. The raw materials were homogenized to a fine powdered and stored in an airtight bottle. The extraction process is started by weighed 100 g of *A. sativum* cloves powder and extracted using aqueous, methanol, ethyl acetate, dichloromethane in subsequent extraction process by using soxhlet apparatus. In every step, the marc dried which results from the previous step of subsequent extraction. The extractive was filtered through Whatman No.1 filter paper to eliminate any debris and unextractable substances that didn't dissolve in the extraction solvent. The resultant extractive solvent removed at their respective temperature by using rotary evaporator under vacuum. Finally, the extracts weighed, and their percentage yield was evaluated using the formula and recorded. Then, the extract was stored in a refrigerator at 4°C until further use (Krakowska-Sieprawska et al., 2022).

Table 3: Details of sequential extraction procedure for *A. sativum*

| **Solvent Used** | **Sample (gram)** | **Boiling Point (°C)** | **Duration of Extraction (hours)** |
|---|---|---|---|
| Aqueous | 95 | 90 | 6.0 |
| Methanol | 88 | 65 | 8.0 |
| Chloroform | 76 | 77 | 7.0 |
| Petroleum Ether | 69 | 70 | 4.0 |
| Residue left | - | - | - |

**Preliminary Phytochemical Analysis**

The determination of phytochemicals done by the standard methods (Kokate, A. 1999). These chemical tests can be done by experimenting various phytochemical agents present in the extracts. Phytochemical screening performed for the analysis of tannins, phenols, alkaloids, flavonoids, glycosides, saponins, terpenoids, carbohydrates and proteins through subsequent standard approaches as given underneath

**Formulation of Polyherbal Extracts**

*M. oleifera* (leaves), *T. cordifolia* (aerial parts) and *A. sativum* (cloves) methanolic extracts used in following proportions to prepare polyherbal formulations:

Table 4: Details for the preparation of polyherbal formulations

| Polyherbal formulation codes | Proportion of methanolic extracts (*M. oleifera* (MM), *T. cordifolia* (TM), and *A. sativum* (AM)) |
|---|---|
| PF-1 | 1:1:1(1MM:1TM:1AM) |
| PF-2 | 2:1:1(2MM:1TM:1AM) |
| PF-3 | 2:2:1(2MM:2TM:1AM) |

**Antioxidant Activities**
**TEAC (Trolox equivalent antioxidant capacity) assay**

**Material Required:** Methanolic extracts of *M. oleifera*, *T. Cordifolia*, *A. sativum*, their polyherbal formulation PF-1, PF-2 and PF-3, and 7 mM ABTS solution (gently mixing 5 ml of 7 mm ABTS solution and 88 µl of 140 mM potassium persulfate solution), analytical grade chemicals (Benzie & Strain, 1999).

**Preparation of Sample Solution**
Approximately 1 g of dry powder test samples weighed in the test tube. In each test tube, 10.0 ml of 80 % methanol was poured and kept in an incubator for around 24h. The sample in the test tube was filtered via Whatman filter paper No. 41 (Merck, India). The filtrate utilized for further investigations (Benzie & Strain, 1999).

**Procedure**
2,2' Azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) radical cation was prepared by reacting 5 ml ABTS stock solution with 88 µl Potassium persulfate. The mixture was allowed to stand in dark at room temperature for 12-16 hours before use. Stability of the radical is 2 days under dark conditions. Appropriate dilutions of test items were prepared.
**Step. 1:** ABTS radical solution was mixed with methanol to get an absorbance of 0.70-0.80 at 734 nm.
**Step. 2:** 30 µl of diluted ABTS radical solution and 3 ml of methanol (ABTS reagent) were mixed prior to the addition into the plate.
**Step. 3:** The reaction mixture was made by addition of 10 µl test substance and 290 µl of ABTS reagent to reach a final volume of 300 µl and absorbance was noted (Benzie & Strain, 1999).

**Antioxidant Activity-DPPH Scavenging Capacity Assay**
**Material Required:** Methanolic extracts of *M. oleifera*, *T. Cordifolia*, *A. sativum*, their polyherbal formulation PF-1, PF-2 and PF-3, Standard 100 µg/ml ascorbic acid solution (10 mg of ascorbic acid dissolved in 100 ml of sterile distilled water) and 1mM DPPH (394g DPPH in 1000 ml of 80 % methanol kept in the dark), analytical grade methanol.

**Preparation of Sample Solution**
Approximately 1 g of dry powder test samples weighed in the test tube. In each test tube, 10.0 ml of 80 % methanol was poured and kept in an incubator for around 24h. The sample in the test tube was filtered via Whatman filter paper

No. 41 (Merck, India) (Benzie & Strain, 1999). The filtrate utilized for further investigations.

**Procedure**

DPPH radical scavenging assay estimated the free radical scavenging activity of different concentrations of PF-1, PF-2, PF-3, MM, TM, and AM and standard ascorbic acid. The concentrations of 10, 25, 50, 100 and 200 µg/ml of each test samples preparedby dissolving in 80 % methanol. The various testing extracts and standard ascorbic acid solution of 1 ml were taken in separate test tubes and added with 1ml of 1.0 mmol/L DPPH radical solution. The solution was quickly mixed, vortexed for 20 seconds and made to stand in the dark at 37 °C for 30 min. The methanol with DPPH used as negative control, and only methanol acts as blank. All the samples tested in triplicates, and the decrease in absorbance of each test samples was observed at 517 nm using UV-Vis spectrophotometer.
The percentage of radical scavenging activity of tested extracts and positive control ascorbic acid calculated by using the following formula:

$$\text{Free radical scavenging activity (\%)} = \frac{[A_c - A_s]}{A_c} \times 100$$

Where,
$A_C$= Absorbance of the control (2 ml DPPH solution +1 ml of 80 % methanol solution)
$A_S$= Absorbance of the DPPH solution in the presence of extract (2 ml DPPH+1 ml of test compound) (Benzie & Strain, 1999).

**Statistical Analysis**

The test results specified as Mean ± SEM (Standard error of the mean) of triplicates. The evaluation can do by one- way analysis of variance (ANOVA) followed by post hoc analysis using Dunnett's multiple range test (Graph Pad Software). The experimental values at $p < 0.05$ considered statistically significant as related to control. The concentration of sample required to scavenge 50 % of DPPH free radical ($IC_{50}$) evaluated from dose-response curve plotted between percentage inhibition and respective concentrations. The logarithmic equation assessed for calculating the half maximal inhibitory concentration ($IC_{50}$) of test sample required to scavenge DPPH radical by 50 % (Benzie & Strain, 1999).

**Results and Discussion**
**Extraction of *M. oleifera*, *T. Cardifolia* and *A. sativum***

Extraction of *M. oleifera* leaves, *T. Cardifolia* aerial parts, and *A. sativum* cloves were done using the Soxhlet apparatus with different solvents. Table 5, 6 and 7 display the percent yield of each extract respectively.
The formula for the calculation of the percentage of yield: Percentage of yield = (Weight of crude (g) x 100)/ (Weight of raw material (g))
The result expressed w/w.

Table 5: The percentage yield (%) and extracts colour of *M. oleifera* leaves after solvent evaporation

| Solvent Used | Yield/100g | Yield (%) | Extract Colour |
|---|---|---|---|
| Aqueous | 9.80 | 15.8 | Brown powder |
| Methanol | 13.55 | 19.3 | Brown powder |
| Chloroform | 6.43 | 8.2 | Yellow-brown |
| Petroleum Ether | 4.77 | 5.3 | Dark green |

The yield extracted successively from *M. oleifera* leaves studied using different solvents. The recorded return is noted to be highest for methanol and trailed by aqueous, chloroform and petroleum ether in succession. Thus, by yield, the extract of methanol of the leaves of *M. oleifera* was selected for further study.

Table 6: The percentage yield (%) and color of the extracts of *T. Cardifolia* aerial parts after solvent evaporation

| Solvent Used | Yield/100g | Yield (%) | Extract Colour |
|---|---|---|---|
| Aqueous | 8.5 | 13.9 | Brown powder |
| Methanol | 13.0 | 16.2 | Brown powder |
| Acetone | 7.0 | 8.2 | Brown powder |
| Petroleum Ether | 3.5 | 3.8 | Dark green powder |

The yield attained for each following extracts of the parts of *T. Cardifolia* in the study using aqueous, methanol, acetone and petroleum ether. This result was recorded to be highest for methanol followed by aqueous, acetone and petroleum ether used in succession. Thus, the methanolic extract of the aerial part of *T. Cardifolia* selected for further study.

Table 7: The percentage yield (%) and extracts colour of *A. sativum* bulbs after solvent evaporation

| Solvent Used | Yield/100g | Yield in % | Colour of extract |
|---|---|---|---|
| Aqueous | 9.2 | 13.3 | Light yellowish-brown powder |
| Methanol | 11.6 | 15.2 | Light yellowish-brown powder |
| Ethylacetate | 8.2 | 9.3 | Yellow-brown |
| Dichloro Methane | 6.0 | 6.3 | Dark green |

The yield attained for each following extracts of the bulbs of *A. sativum* in the study using aqueous, methanol, ethyl acetate and dichloromethane. This result was recorded to be highest for methanol followed by aqueous, ethyl acetate and dichloromethane used in succession. Thus, by yield, cloves of *A. sativum* methanolic extract was selected for further study.

The preliminary qualitative analysis of sequential extraction of *M. oleifera*, *T. Cardifolia,* and *A. sativum* was carried out. Presence of triterpenoids, carbohydrates, tannins, phenols, alkaloids, anthraquinone glycosides, flavonoids, & saponins confirmed in the methanolic & aqueous extracts but absent in chloroform & petroleum ether extracts of the leaves extract of *M. oleifera*. The methanolic, petroleum ether & chloroform extracts have indicated cardiac

glycoside while the cardiac glycoside was utterly absent in the aqueous extracts of *M. oleifera*. Presence of carbohydrates, tannins, phenols, flavonoids, alkaloids, and proteins confirmed in the methanolic as well as aqueous extracts of *T. Cardifolia.* The methanolic, acetone and aqueous extracts showed flavonoids are present while the flavonoids were utterly absent in the petroleum ether extracts of *T. Cardifolia.* Steroids were present in all the extracts except aqueous extract. Saponins are present only in methanolic, and petroleum ether extracts but utterly lacking in aqueous and acetone extracts. Presence of proteins, alkaloids, carbohydrates, phenols, flavonoids, tannins, & saponin confirmed in the methanolic & aqueous extracts but absent in dichloromethane & ethyl acetate extracts of the cloves of *A. sativum*. The methanolic, aqueous & ethyl acetate extracts showed alkaloids while the alkaloids were utterly absent in the dichloromethane extract of *A. sativum*.

**Antioxidant Activities and Chromatogram Identification**
**DPPH Radical Scavenging Activity**

The DPPH assay determined the antioxidant activity of *M. oleifera*, *A. sativum*, PF-1, PF-2, and PF-3. The antioxidant activity measured by the DPPH assay in test samples presented in the Figure 1. The maximum DPPH free radical scavenging activity determined ($p<0.01$) in the *A. sativum* and PF-3 samples. In case of DPPH+ radical activity, the $IC_{50}$ values of PF-1, PF-2, and PF-3 found to be 181.27, 83.54 and 45.06 µg/mL of the polyherbal formulations. It's observed that PF-3 has the significant highest free radical scavenging capacity when compared to the other formulations. The $IC_{50}$ values of methanolic extract of *M. oleifera* (MM), methanolic extract of *T. Cardifolia* (TM) and methanolic extract of *A. sativum* (AM) were 74.72, 735.42 and 13.12 µg/ml. *A. sativum* possesses significant high free radical scavenging capacity as compared to *M. oleifera* and *T. Cardifolia*. The lower of $IC_{50}$ value is, the higher of free radical scavenging activity of an experimental sample.
The antioxidant activities of polyherbal formulation PF-1 and *T. Cardifolia* were considered to be very low compared to a standard Ascorbic acid having $IC_{50}$ 10.16 µg/ml. However, the differences in the polyherbal formulation could be due to the synergistic effect of different plants in formulations. The antioxidant effect of the experimental extracts may vary by the quantity of various phytochemicals like polyphenols flavonoids eTM. presence that has shown antioxidant properties. The mode of the reaction in between the DPPH and free radicles depends upon the physical and chemical reactions or confirmation of the antioxidant. Hence, the antioxidant action of test compounds can be ordered in the following: *A. sativum* > PF-3 > *M. oleifera* > PF-2 > PF- 1> *T. Cardifolia*. The present study exhibits the synergistic effect of these polyherbal formulations on the antioxidant activity. The PF-3 showed highest antioxidant activity in comparison to PF-2 and PF-1, and it showed significant scavenging capacity to DPPH radical assay.

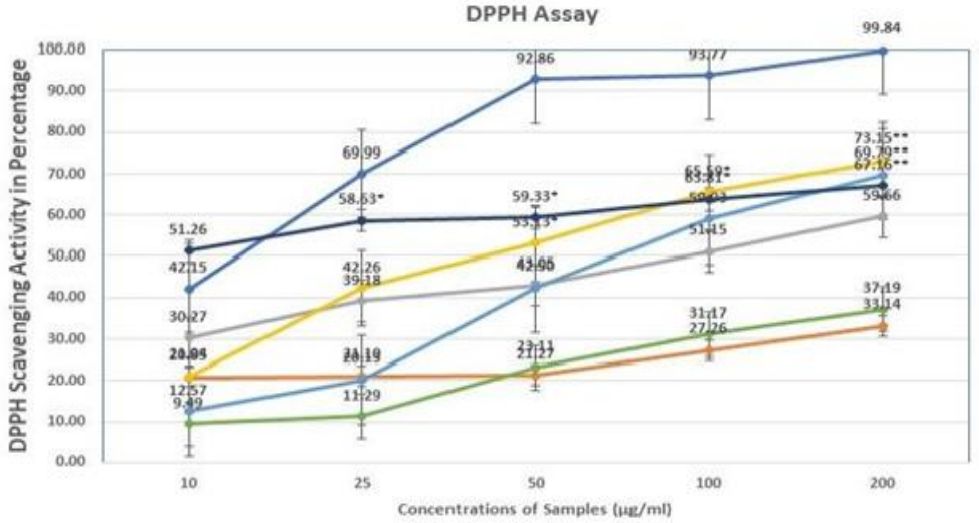


Figure 1: DPPH scavenging activity of Standard, PF-1, PF-2, PF-3, MM, TM, and AM. PF- Polyherbal formulation, MM- Methanolic extract of M. oleifera, TM- Methanolic extract of T Cordifolia, AM- Methanolic extract of A. sativum. Values are represented as Mean± SEM (in triplicates). Statistical analysis was done by one-way ANOVA surveyed by post hoc analysis using Dunnett's multiple range test. Here, ***p< 0.001, **p< 0.01, * p< 0.0

**Trolox Equivalent oxidant capacity**

The TEAC test, with a little modification, was used to measure free radical scavenging capability utilising the ABTS•+ radical cation. By scavenging the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS•+) radical, the relative antioxidant capacity of the test tissues was compared to that of Trolox (as the control). The ABTS•+ radical was created by mixing a 7 mM ABTS•+ solution with 2.45 mM $K_2S_2O_8$ solution. Prior to use, the ABTS•+ solution was diluted with water, yielding an absorbance of 0.70 to 0.80 at 734 nm. After incubating the 1.5 mL mixed solution for 6 minutes, the absorbance at 734 nm was measured using a Jasco V-550 UV-visible spectrophotometer after adding 1485 l of the diluted ABTS•+ solution to 15 l of tested sample or Trolox. The concentrations of the standard and samples were determined with respect to the calibration plot as the absorbance decreased (Duke, 1992). By comparing ABTS•+ decolorisation to Trolox decolorisation, the final TEAC value of the tested samples was computed. As a reference standard, the Trolox solution as utilised, and the findings were expressed in mmol equivalents.

Table 8: Free radical scavenging effects of PF, *M. oleifera*, *T. Cardifolia* and *A. sativum*, in ABTS assays

| **Sample** | **ABTS assay** | |
|---|---|---|
| | Concentration (µg/ml) | % Inhibition±SEM |
| PF1 | 30 | 15.60±0.82 |
| | 60 | 29.43±0.59 |

| | | |
|---|---|---|
| | 90 | 44.21±0.79 |
| | 120 | 54.96±0.51 |
| | 150 | 67.02±0.98 |
| | $IC_{50}$ values | 108.19±0.84 |
| PF2 | 30 | 12.11 ± 0.75 |
| | 60 | 22.43±0.43 |
| | 90 | 20.14±0.55 |
| | 120 | 45.66±0.21 |
| | 150 | 55.45±0.45 |
| | $IC_{50}$ values | 90.65±0.32 |
| PF3 | 30 | 10.15±0.76 |
| | 60 | 15.55±0.56 |
| | 90 | 10.11±0.24 |
| | 120 | 34.11±12 |
| | 150 | 45.55±60 |
| | $IC_{50}$ values | 80.11±45 |
| *M. oleifera* | 5 | 13.95±0.92 |
| | 10 | 27.78±1.09 |
| | 15 | 44.09±0.97 |
| | 20 | 57.33±0.86 |
| | 25 | 65.48±0.62 |
| | $IC_{50}$ values | 18.12±0.89 |
| *T. Cardifolia* | 10 | 13.95±1.31 |
| | 20 | 24.47±0.99 |
| | 30 | 35.70±1.22 |
| | 40 | 48.11±1.19 |
| | 50 | 62.29±1.47 |
| | $IC_{50}$ values | 41.66±1.18 |
| *A. sativum*, | 50 | 12.77±0.71 |
| | 100 | 23.05±0.88 |
| | 150 | 33.69±0.52 |
| | 200 | 46.22±0.61 |
| | 250 | 58.39±1.12 |
| | $IC_{50}$ values | 216.08±0.99 |

Values are expressed as mean±SEM, n=3

The amount of suppression of the ABTS radical's absorbance as a function of concentration against time was plotted to calculate the TEAC (1, 4 and 6 min). The TEAC was calculated by dividing the gradient of the plot of the percentage inhibition of the absorbance of the examined sample at each time point by the gradient of the plot for trolox (1 mM). At 1, 4, and 6 minutes, the trolox equivalent antioxidant capacity of the research samples at a dosage of 10 l was determined to be 0.93 to 2.60, 0.87 to 2.53 and 0.76 to 2.36 mM respectively (Shenoy et al., 2010).

Table 9: Trolox equivalent antioxidant capacity (TEAC) of PF, *M. Oleifera, T. Cardifolia* and *A. sativum*

| **Sample tested** | **% Inhibition±SEM** | | |
|---|---|---|---|
| | **At 1 min** | **At 4 min** | **At 6 min** |
| Trolox | 1.00 | 1.00 | 1.00 |
| PF1 | 1.37±0.05 | 1.29±0.02 | 1.24±0.09 |
| PF2 | 1.12±0.01 | 1.15±0.23 | 1.20±0.14 |
| PF3 | 1.09±0.01 | 1.10±0.45 | 1.11±0.23 |
| *M. oleifera* | 2.60±0.05 | 2.53±0.06 | 2.36±0.10 |
| *T. Cardifolia* | 1.97±0.02 | 1.91±0.06 | 1.89±0.03 |
| ***A. sativum*** | 0.93±0.05 | 0.87±0.07 | 0.76±0.01 |

Values are expressed as mean±SEM, n=3

The result shown that maximum TEAC activity find in *M. oleifera* at 1 min followed by 4 min and at 6 min. The minimum TEAC activity find in *A. sativum* at 6 min.

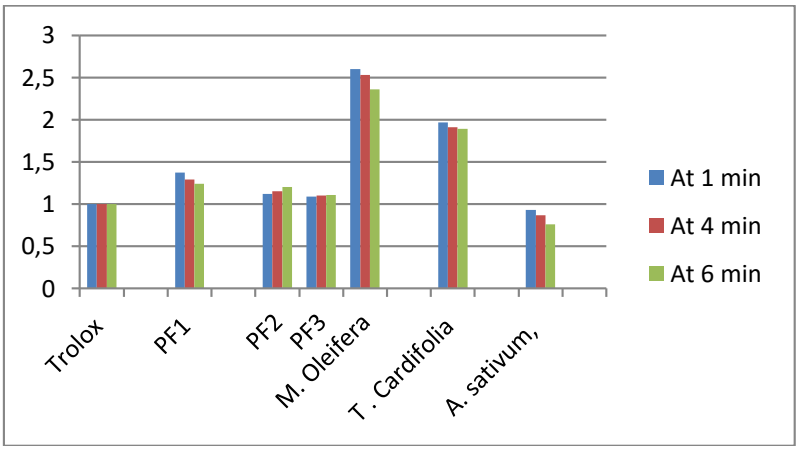


Figure 2: Trolox equivalent antioxidant capacity (TEAC) of PF, M. oleifera, T. Cardifolia and A. sativum

**Conclusion**

The current study of polyherbal formulations (*Moringa oleifera, T. Cordiafolia, Allium sativum*) PF-1, PF-2 and PF-3 establish antioxidant attributes. The investigation also helps to know the antioxidant activity of different combinations *M. oleifera, T. Cordiafolia, A. sativum* to form polyherbal formulation PF-1, PF-2, and PF-3. It is established that PF-3 polyherbal formulation is a probable source of antioxidants and has stronger antioxidant activity than in the PF-2 and PF-1. It also shows excellent scavenging capacity to DPPH radical assay. There is sure possibility for the application of this polyherbal formulation as a food improver, or a medicinal source.

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