

Original Article

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT POTENTIAL OF *TRIBULUS TERRESTRIS* FRUIT AND *MESUA FERREA* FLOWER EXTRACTS: A COMPARATIVE STUDY

DAKSHAYINI P. N., MAHABOUB BASHA P.*

Research Scholar Department of Zoology, Bangalore University, Bangalore-560 056, India. *Department of Zoology, Bangalore University, Bangalore 560056, India
Email: pmbashabub@rediffmail.com

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ABSTRACT

Objective: This study aimed to investigate the antioxidants and free radicals scavenging properties of ethanolic extracts from *Tribulus terrestris* fruit (TTF) and *Mesua ferrea* flower (MFF).

Methods: The antioxidant and free radical scavenging activities were measured by using standard protocols against phytoextracts used at 50, 100, 150, 200 and 250 µg/ml concentration. Total phenolic and flavonoid content were estimated using Folin-Ciocalteu and aluminium-chloride reagent assay methods.

Results: Among the extracts, MFF showed the highest total antioxidant ability followed by TTF. With regard to various scavenging activities, MFF had the highest radical scavenging activity than TTF extract closely resembled the standards. Also, the reducing capacity on ferrous ion was high in MFF than TTF. The quantified phenolic contents of TTF and MFF ethanolic extracts were 27.0±0.36 and 57.0±0.32 mg gallic acid equivalent per gram extract respectively. A positive correlation (p value<0.001) was observed between phenolic content and free radical scavenging efficiencies.

Conclusion: The results confirm that both MFF and TTF are important sources of natural antioxidants and serves as an effective free radical scavengers and/or inhibitors. Hence, both extracts are suitable plant-based pharmaceutical products can be used to handle 'free radical-mediated diseases.

Keywords: Polyphenols, Antioxidants, *Tribulus terrestris*, *Mesua ferrea*

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INTRODUCTION

Of late, plant-based pharmaceuticals have gained popularity and much interest has been focused not only on the discovery of new biologically active molecules by the industry but also on the adoption of crude extracts of plants such as infusions, for self-medication by the general public to handle free radical based adverse effects. A current research on free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of adverse effects [1]. Resultantly, natural antioxidants, which are ubiquitous in fruits, leaves, and flowers, have received great attention and have been studied extensively [2], since they are effective free radical scavengers and are presumed to be less toxic than synthetic antioxidants. Phytomedicines are well tolerated, with fewer side effects; in contrast, synthetic drugs can be highly effective, their usage is often hampered by severe side effects [3]. Additionally, synthetic pharmaceuticals have single chemical, contrarily many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. Therefore, considering the importance of natural product-based antioxidants in treating several human ailments, the present study was focused towards evaluation of antioxidant properties of *Tribulus terrestris* and *Mesua ferrea* plant extracts.

Tribulus terrestris (TT) known as Gokhru, a perennial creeping herb is an annual plant of the family 'Zygophyllaceae' and being used for generations to energize, vitalize, and improve sexual function and physical performance in men. Its usage has been indicated to treat several ailments due to its unique property of healing cuts, wounds and boils. Literature survey revealed that this plant and its fruits have been evaluated for antihyperglycaemic and antioxidant effects [4-5]. Though fruit extract contains a series of terpenoids and steroidal saponins as bioactive components along with di-p-

coumaroylquinic acid derivatives [6], there is a dearth of the literature on the antioxidant potential of its contents.

Mesua ferrea (MF) popularly known as Ceylon ironwood or Indian rose chestnut of the family 'Cluciaceae'. Many parts of this plant have been indicated to offer relief to certain ailments such as asthma, leprosy, cough, fever, and vomiting. Available literature has shown its richness in having xanthenes, phenyl coumarines, triterpenoids and its flower possesses flavonoids, mesuol and mesuone. Furthermore, the usage of MF flower extract has been indicated in handling diabetic and cancer complications [7-8]. However, there is a lacuna on the antioxidant potential of its flower contents.

The importance of antioxidants on human health has become increasingly clear owing to spectacular advances in understanding mechanisms of their reaction with oxidants. Furthermore, some human epidemiological studies have shown that natural antioxidants have potential health effects [9-10]. However, there is a dearth of the literature with regard to antioxidant potential of aforesaid phytoextracts. Keeping in view 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 MF 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 foodstuff as well as in medicine.

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 (TTF) were collected in the morning hours (7.00-10 AM) 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 of these plants were cleaned, shade dried for around 25-30 d at room temperature and then crushed to fine powder. Crude extract of *Mesua ferrea* flower (MFF) dry powder (Batch No: KH/MF/001/14) was procured from Konark herbals and healthcare, Daman, India. Both the plant samples were subjected to Soxhlet extraction system for 24 h using 70 % ethanol and filtrates were made use for phytochemical analysis.

Quantification of phytochemical constituents

Total phenolic content

Soluble phenolic content of TTF and MFF extracts were determined according to the modified method of Singleton *et al.* [11] 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 Na₂CO₃ (0.7 M). Subsequently, the mixture was shaken for 2 h 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, and m = 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.* [12]. To 1 ml of extract, 0.1 ml NaNO₂ (5%) was added and incubated for 5 min at room temperature, then 0.1 ml of AlCl₃ (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 flavonoid content, mg of quercetin equivalents/gram extract,

c = concentration of quercetin 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 TTF and MFF extracts against 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was investigated spectrophotometrically by modified method of Chan *et al.* [13]. Different aliquots of both standard and sample solutions (50-250 µg/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,

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

Where, Abs (control): Absorbance of the control and

Abs (test): Absorbance of the extract/standard.

Superoxide radical (O₂⁻) scavenging assay

Superoxide radical scavenging activity of TTF and MFF extracts were evaluated using nitro blue tetrazolium (NBT) reduction using the modified method of Nishikimi *et al.* [14]. 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.

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

Where, Abs (control): Absorbance of the control and

Abs (test): Absorbance of the extract/standard.

Hydrogen peroxide radical (H₂O₂) scavenging assay

Hydrogen peroxide radical scavenging ability of TTF and MFF extracts were assessed by adopting the method given by Ruch *et al.* [15]. 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.

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

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 Sreejayan Rao [16]. 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 h 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% H₃PO₄ and 0.1% naphthyl ethylenediamine 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.

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

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 TTF and MFF extracts were determined according to the method described by Benzie and Strain [17]. FRAP working solution was prepared by mixing 2.5 ml of 10 mmol ferric tripyridyltriazine (Fe³⁺-TPTZ) solution, 2.5 ml of 20 mmol ferric chloride (FeCl₃).6H₂O 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 (Fe²⁺-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 $\mu\text{mol Fe}^{2+}$ gram equivalent of sample.

$$y = 0.0033x + 0.1817$$

Where, y = absorbance and x = concentration in $\mu\text{g/ml}$

Statistical analysis

Statistical analysis was performed using one-way Analysis of Variance (ANOVA) with least significant difference (LSD) *post hoc* (at $P < 0.01$) by SPSS software package 20.0. Linear regression analysis was used to calculate IC_{50} values by using 'Graph pad prism software 6.0'. Correlation and graphical preparations were plotted using 'Origin Pro software 8.0'. Results are shown as the mean \pm SEM of six measurements.

RESULTS

Total phenolic and flavonoid content

From the results it was evident that both, TTF and MFF extracts were found to be good source of polyphenols. In comparison, MFF extract showed more amounts of phenolic and flavonoid contents compared to TTF extract. The quantified phenolic contents of TTF and MFF ethanol extracts were found to be 27.0 ± 0.36 and 57.0 ± 0.32 mg gallic acid equivalent per gram extract; similarly, the quantified flavonoid contents were found to be 34.6 ± 0.55 and 72.3 ± 0.76 mg quercetin equivalent per gram extract respectively.

DPPH free radical scavenging activity

The antioxidant potential of TTF and MFF extracts were analysed by DPPH radical scavenging assay and both the plants extracts exhibited scavenging ability in a concentration dependent manner. In comparison, MFF extract exhibited a higher scavenging ability (54.96%) with IC_{50} value of $131.0 \mu\text{g/ml}$ than TTF extract wherein

the IC_{50} value was $142.2 \mu\text{g/ml}$ (51.4% inhibition) [fig. 1] and the standard (ascorbic acid) value being $126.2 \mu\text{g/ml}$.

Superoxide and hydrogen peroxide radical scavenging activities

The percent inhibition of superoxide radical generation by both TTF and MFF ethanolic extracts was found increasing in a concentration dependent manner showing IC_{50} value of $152.6 \mu\text{g/ml}$ (53.4% inhibition) and $118.2 \mu\text{g/ml}$ (59.36% inhibition) respectively when compared to the standard, ascorbic acid (IC_{50} value $112.7 \mu\text{g/ml}$) [fig. 2 and 3]. With regard to peroxide radical activity MFF extract exhibited high inhibition (58.63% with IC_{50} value of $130.3 \mu\text{g/ml}$) compared to TTF (53.26% inhibition with IC_{50} value of $190.4 \mu\text{g/ml}$) when taken into account the IC_{50} value of standard, ascorbic acid ($111.7 \mu\text{g/ml}$).

Nitric oxide radical scavenging activity

Though both the extracts, TTF and MFF were found to possess scavenging ability of nitric oxide radicals in concentration dependent manner, only MFF extract showed significant increase in nitric oxide radical scavenging activity with an increasing concentration of extract and exerted 71.16% inhibition with IC_{50} value of $158.7 \mu\text{g/ml}$ when compared to the TTF extract wherein 57.3% inhibition with IC_{50} value $172.6 \mu\text{g/ml}$ [fig. 4] was observed. However, the IC_{50} values of both these extracts were found to be lesser than the IC_{50} value of BHT, the standard $109.2 \mu\text{g/ml}$.

Ferric reducing/antioxidant activity

The reducing capacity of TTF and MFF extracts were measured based on their ability to reduce the ferric tripyridyltriazine (Fe^{3+} TPTZ) to ferrous tripyridyltriazine (Fe^{2+} TPTZ) complex in the reaction mixture. Here, the absorbance of both extracts significantly increased with an increasing concentration and were found to possess concentration dependent scavenging activity [fig. 5]. The ferric reducing ability was calculated using standard curve (FeSO_4) and antioxidant activity of MFF and TTF extracts were found to be 157.36 and $133.12 \mu\text{mol Fe}^{2+}$ equivalent per gram extract respectively.

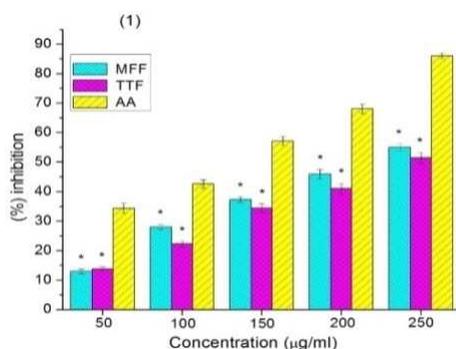


Fig. 1: Concentration dependent percentage inhibition of DPPH radicals by *Mesua ferrea* flower (MFF), *Tribulus terrestris* fruit (TTF) extracts and the Standard, ascorbic acid (AA). All data expressed as mean \pm SEM (n=6). * $P < 0.01$ compared to respective standard

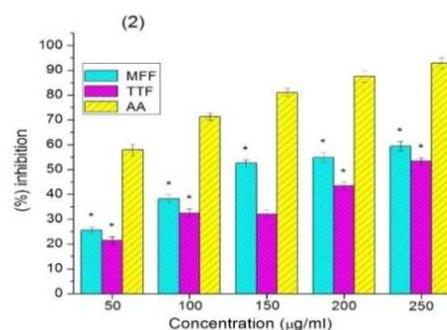


Fig. 2: Concentration dependent percentage inhibition of superoxide radicals by *Mesua ferrea* flower (MFF), *Tribulus terrestris* fruit (TTF) extracts and the Standard, ascorbic acid (AA). All data expressed as mean \pm SEM (n=6). * $P < 0.01$ compared to respective standard

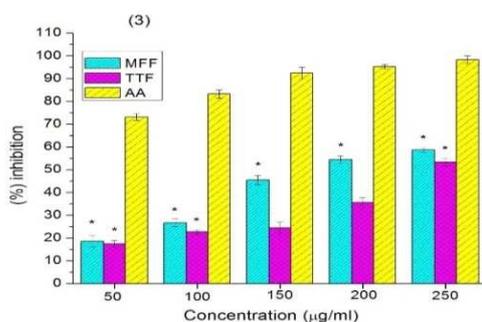


Fig. 3: Concentration dependent percentage inhibition of hydrogen peroxide radicals by *Mesua ferrea* flower (MFF), *Tribulus terrestris* fruit (TTF) extracts and the Standard, ascorbic acid (AA). All data expressed as mean \pm SEM (n=6). * $P < 0.01$ compared to respective standard

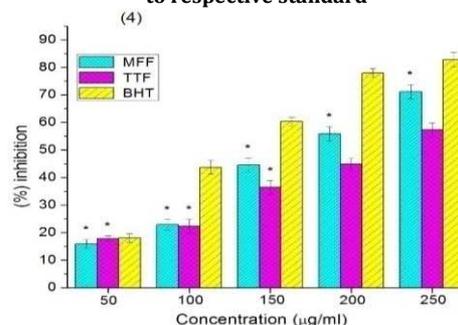


Fig. 4: Concentration dependent percentage inhibition of nitric oxide radicals by *Mesua ferrea* flower (MFF), *Tribulus terrestris* fruit (TTF) extracts and the Standard, BHT. All data expressed as mean \pm SEM (n=6). * $P < 0.01$ compared to respective standard

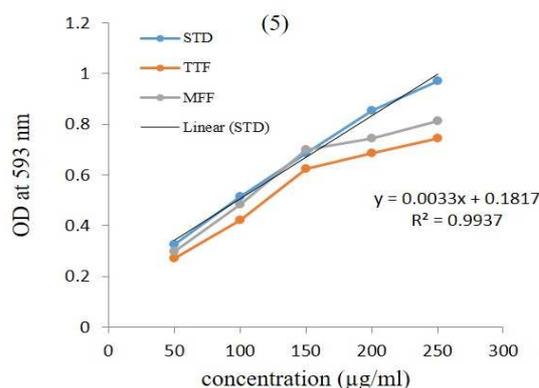


Fig. 5: FRAP assay results showing concentration dependent ferric reducing activity of *Mesua ferrea* flower (MFF), *Tribulus terrestris* fruit (TTF) ethanol extracts and the standard, ferrous sulphate (FeSO_4)

Correlation between total phenolic content and antioxidant activity

Pearson's correlation coefficients (r) values measured by the total phenolic content and antioxidant activities of both phytoextracts are

shown in table 1 and 2. Here the observed ' r ' values of MFF were found to be more significant when compared to TTF ethanolic extract. The results showed a strong positive correlation between the total phenolic content and antioxidant activities (DPPH, O_2^- , H_2O_2 , NO^- and FRAP assays) of both the extracts.

Table 1: Correlation between total phenolic content and antioxidant activities of *Mesua ferrea* flower ethanol extract

| | Total phenol | DPPH | O_2^- | H_2O_2 | NO^- | FRAP |
|------------------------|--------------|---------|----------------|------------------------|---------------|------|
| DPPH | 0.993** | - | | | | |
| O_2^- | 0.975** | 0.974** | - | | | |
| H_2O_2 | 0.977** | 0.974** | 0.980** | - | | |
| NO^- | 0.991** | 0.974** | 0.949* | 0.983** | - | |
| FRAP | 0.975** | 0.977** | 0.998** | 0.989** | 0.964** | - |

Values represent the correlation factors, **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level

Table 2: Correlation between total phenolic content and antioxidant activities of *Tribulus terrestris* fruit ethanol extract

| | Total phenol | DPPH | O_2^- | H_2O_2 | NO^- | FRAP |
|------------------------|--------------|---------|----------------|------------------------|---------------|------|
| DPPH | 0.997** | - | | | | |
| O_2^- | 0.959** | 0.951* | - | | | |
| H_2O_2 | 0.940* | 0.927* | 0.970** | - | | |
| NO^- | 0.991** | 0.993** | 0.945* | 0.945* | - | |
| FRAP | 0.967** | 0.984** | 0.902* | 0.848 | 0.965** | - |

Values represent the correlation factors, **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level

DISCUSSION

Plant polyphenols are significant compounds that act as free radical scavengers and can exert multiple biological effects, including antioxidant and free radical scavenging abilities [18]. Similarly, flavonoids are of great importance and helps in fighting against diseases. The ability of flavonoids to act as potent antioxidants depends on their molecular structures, the position of the hydroxyl group and other features in its chemical structure. It is evident from present study results that both TTF and MFF extracts are good source of polyphenols and in comparison MFF extract showed higher phenolic and flavonoid contents than TTF. In brief, MFF extracts showed high levels of total phenolic content (57.0 mg of equivalent gallic acid per gram) followed by total flavonoids (72.3 mg of equivalent quercetin per gram extract), while the quantified phenolic and flavonoid contents of TTF extract were comparatively less (27.0 and 34.6 mg respectively). Earlier findings of Khaleel et al. [19] reported the presence of total phenolic and flavonoid contents in crude ethanolic extracts of MF seeds in the range of 10.1 and 12.9 mg respectively. Likewise, Chaitanya et al. [20] reported 37.34 mg of phenolic content in ethyl acetate extract of MF bark. 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, the ethanolic extracts of both plants exerted a significant scavenging activity on the DPPH radical which was found to be increasing with the increasing concentration. At 250 $\mu\text{g/ml}$ concentration, the exerted values of MFF and TTF extracts were found to be 54.96% (with IC_{50} 131.0 $\mu\text{g/ml}$) and 51.46% (with IC_{50} 142.27 $\mu\text{g/ml}$) respectively when compared to ascorbic acid, the standard (86.10%). The preponderance of studies on ethyl acetate and methanolic extracts of MF stem bark and leaf exhibited a dose-dependent increase in scavenging activity [20-21]. Similar results highlighting scavenging ability of TT fruit and leaf ethanolic extracts were reported [22-23]. Furthermore, the boiled TTF extracts was shown to have radical scavenging ability [24] nevertheless the maximum scavenging ability was recorded in hexane extract of TTF among acetone, isopropanol and aqueous extracts [25]. Thereby, the results of present study confirm that the ethanolic extracts of both plants are effective with

respect to DPPH radical scavenging activity and in comparison, MFF has better antioxidant potential than TTF.

Superoxide anion radical (O_2^-) is generated by four-electron reduction of molecular oxygen into water. This radical also formed in aerobic cells due to electron leakage from the electron transport chain. Superoxide radicals (O_2^-) are also formed by activated phagocytes such as monocytes, macrophages, eosinophil's and neutrophils and the production of O_2^- is an important factor in the killing of bacteria by phagocytes. In living organisms, O_2^- is removed by the enzyme called superoxide dismutase (SOD). In this study, assessments were made to confirm *in vitro* superoxide anion scavenging activity of both extracts. At 250 $\mu\text{g/ml}$ concentration, the exerted values of MFF and TTF extracts were found to be 59.36% (with IC_{50} 118.2 $\mu\text{g/ml}$) and 53.4% (with IC_{50} 152.6 $\mu\text{g/ml}$) respectively when compared to ascorbic acid, the standard (112.7%). Similar results were observed in earlier investigations wherein TT fruit and MF leaf extracts exhibited superoxide radical scavenging activity when tested with different solvents such as, aqueous, hexane and methanol [24-26]. The present study results rely only on ethanol solvent wherein a concentration-dependent increase in quenching superoxide radicals witnessed to high extent. Comparatively, MFF ethanol extracts possess strong radical scavenging ability than TTF.

Hydrogen peroxide (H_2O_2) plays a role as a signalling molecule in the regulation of a wide variety of biological processes. It can cross the cell membranes rapidly, and can react with iron and copper ions to form hydroxyl radical which is the origin of many toxic effects. In this study, the ethanolic extracts of both plants exerted a significant scavenging activity on peroxide radical which was found to be increasing with the increasing concentration. At 250 $\mu\text{g/ml}$ concentration, the exerted values of MFF and TTF extracts were found to be 58.63% (with IC_{50} 130.3 $\mu\text{g/ml}$) and 53.26% (with IC_{50} 190.4 $\mu\text{g/ml}$) respectively when compared to ascorbic acid, the standard (111.7 $\mu\text{g/ml}$). Earlier findings of Alakh *et al.* [27] on methanolic extract of MFF showed a satisfactory H_2O_2 radical scavenging activity when it was compared to *Argyrea speciosa*. Similarly, boiled TTF extracts showed an increase in percentage inhibition at higher concentrations when compared to the standard [24]. From the results it is clear that both extracts have scavenging ability and MFF ethanol extract showed strong percentage inhibition and its efficacy was much higher than the TTF when compared to their IC_{50} values.

Nitric oxide, a strong pleiotropic inhibitor of physiological processes, diffusible free radical, reacts with superoxide anion radical and forms to a potent cytotoxic oxidant molecule, the peroxynitrite ($ONOO^-$). In this study, MFF ethanol extract was found scavenging on nitrous oxide (IC_{50} value-158.7 $\mu\text{g/ml}$), and showed 71.16% inhibition at higher concentration which was close when compared to BHT, the standard (82.73% inhibition with IC_{50} value-109.2 $\mu\text{g/ml}$). Similarly, TTF ethanol extract exerted a concentration-dependent scavenging activity, and showed 57.3% inhibition with IC_{50} value of 172.6 $\mu\text{g/ml}$. Similar results were observed in earlier investigations wherein MF stem bark extracts (ethyl acetate and methanol) [20-21] exhibited a dose-dependent nitric oxide radical scavenging ability. MFF ethanol extract showed potent inhibition against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines [28]. Even TTF boiled extracts showed significant scavenging activity against nitric oxide radicals in a dose-dependent manner whereas, least scavenging activity was observed in ethanolic extract when compared to hexane [24-25].

In ferric reducing antioxidant power assay, Fe^{3+} -TPTZ complex is reduced to Fe^{2+} -TPTZ in presence of phytoextracts. In the reaction, antioxidants act as reductants and donate electrons to break and stabilize free radical chain. Since the reducing capacity of a compound serve as a significant indicator of its potential (antioxidant activity) the reducing ability of phytoextracts are measured. The reductive ability of both (MFF and TTF) extracts were calculated using standard curve ($FeSO_4$) and exhibited values were found to be 157.36 and 133.12 μmol of equivalent Fe^{2+} per gram extract respectively. Preponderance of studies made on MF stem bark, seeds and pericarp with methanol and petroleum ether

extracts have shown considerable reducing ability [21], while MFF ethanolic extract has better potential compared to other parts and solvents. Similarly, the reducing ability of TT aerial parts of herbal preparations was shown to have less ability than fruit [29].

The correlation coefficients (r) assessed indicates the existence of linear relationship 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 [30].

CONCLUSION

The ethanolic extract of MFF possess high total phenolic and flavonoid contents and exhibited significant antioxidant potential in scavenging free radicals than TTF. The results confirm that both phytoextracts are important sources of natural antioxidants and helps to curb free radicals such as ROS/RNS and highly recommended as substitutes to handle oxidative damage to various biological macromolecules including DNA, lipids, and proteins and results also provide promising perspectives on future novel therapeutic agent's development.

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AUTHORS CONTRIBUTIONS

Basha Mahaboob P designed the experimental study and Dakshayini PN carried out analysis and coordinated in preparing the manuscript and revision. Both the authors have read and approved the final manuscript.

CONFLICTS OF INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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