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Research Article

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF TRADITIONAL INDIAN LEAFY VEGETABLES: *MUKIA MADERASPATANA* AND *SOLANUM TRILOBATUM*

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ABSTRACT

In ancient times, humans used medicinal plants for protecting from various diseases. Various reports prove that antioxidants in medicinal plants will be responsible to prevent or cure these diseases. Hence, this present study was taken to examine the antioxidant and antimicrobial activity of two leafy green vegetables, *Mukia maderaspatana* and *Solanum trilobatum*. Among both the samples, *S. trilobatum* exhibited higher phenolics (11 %), tannin (10.5 %), flavonoid (16 %) $DPPH^{\cdot}$ (0.84 g/g DPPH), $ABTS^{\cdot+}$ (269 mmol/kg), hydroxyl (23 %), superoxide (94 %), FRAP (36 µg/mmol Fe (II)), phosphomolybdenum (2230 µmol/g) and metal chelating activity (10 mg EDTA/g). Interestingly both the samples exhibit equal activity on antihaemolysis (54 %) where *M. maderaspatana* (89 %) showed higher inhibition on linoleic acid peroxidation. *M. maderaspatana* showed higher inhibition zone against *Staphylococcus aureus* (14 mm), *Klebsiella pneumoniae* (12 mm), *Pseudomonas aeruginosa* (12 mm) and *Escherichia coli* (14 mm) while *Salmonella typhi* was resistant to both of these extracts. These findings showed that the intake of these leafy vegetables in the diet increases the resistance towards oxidative damage and pathogenic food borne microbes and confirmed its traditional therapeutic value.

Keywords: *M. maderaspatana, S. trilobatum*, Antibacterial, Antioxidant, Polyphenols

INTRODUCTION

Plants, our gift of nature which is the sources of bioactive constituents have been used traditionally to cure various ailments in Ayurvedha, Unani and Siddha. During last few years, synthetic drugs occupy the position for curing various diseases. Due to their side effects, scientists are now focusing to explore the potentiality of traditional medicines. Due to prooxidative enzyme systems, lipid oxidation, irradiation, inflammation, smoking, air pollutants and glycoxidation, reactive oxygen species or free radicals (superoxide, hydroxyl, hydrogen peroxide) are highly produced and exceeds the normal enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants (ascorbic acid, α-tocopherol, glutathione, carotenoids and flavonoids) in the body. Hyper physiological burden of free radicals causes the imbalance between free radicals and antioxidants and results in the oxidation of biomolecules (protein, amino acids, lipids and DNA) (i.e., oxidative stress) and lead to cell injury and death[1]. This have been implicated in a number of degenerative diseases like atherosclerosis, diabetes mellitus, ischemia/reperfusion (I/R) injury, Alzheimer's disease, inflammatory diseases, carcinogenesis, neurodegenerative diseases, hypertension, ocular diseases, pulmonary diseases and haematological diseases[2]. Hence, there is a need to supply exogenous supply of antioxidants, where synthetic antioxidants like butylated hydroxylanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylated hydroquinone (TBHQ) were reported to be carcinogenic[3].

Due to the increasing failure of chemotherapeutics, resistance of many infectious microorganisms and many antibiotics are associated with hypersensitivity, immune suppression and allergic reactions[4]. The associated problems with synthetic ones push this research to focus on evaluating the antioxidant and antimicrobial activity of two leafy green vegetables namely, *Solanum trilobatum* and *Mukia maderaspatana*. *Solanum trilobatum* L. (Family-Solanaceae), a potential rejuvenator drug and nutraceutical vegetable, occurs in Southern India and has been used traditionally in Siddha system of medicines to treat various diseases[5]. This plant is well known in Ayurveda and Siddha system as 'Alarka' and 'Tuduvelai'. Previous pharmacological studies confirm that it possess antibacterial, antifungal, antioxidant, anti-tumor, anti-asthmatic, anti-ulcerogenic, anti-inflammatory, analgesic, counteracts snake poison and cures lung disease[6]. *Melothria maderaspatana* (Linn.) Cogn. (Syn. *Mukia maderaspatana*, *Cucumis maderaspatana* or *Mukia scabrella*) (Family- Cucurbitaceae), a plant drug of Siddha medicine found throughout India and in China, Taiwan, Malaysia, Australia, New Zealand and in Africa[7]. It has antioxidant, diuretic, stomachic, gentle aperients, antipyretic, antiflatulent, antibronchitis, hepatoprotective, anti-rheumatic, analgesic, antibacterial, stimulant, anti-inflammatory, anticancer, anti-hypertensive, anti-diabetic, expectorant, cures toothache and recommended in vertigo and biliousness[8]. A large population in Southern India use these *M. maderaspatana* leaves for making chutney and ingredient in dosa preparation[9]. The rural people consume the cooking liquor of both of these leaves with the spices to relieve cold and throat pain.

MATERIALS AND METHODS

Plant material

Two leafy green vegetables namely *Solanum trilobatum* and *Mukia maderaspatana* were procured from natural vicinity of Coimbatore city, India. The aerial parts of these plants were separated, shade dried, powdered and stored in air tight container at room temperature. The moisture content of these fresh and powdered samples were analysed using moisture analyser (Sartorius-MA 35, Germany).

Preparation of plant extracts

The dried and ground samples of *Mukia maderaspatana* (MME) and *Solanum trilobatum* (STM) were defatted with petroleum ether for 24 h, air dried and extracted with 100 % ethanol for *M. maderaspatana* and 80 % methanol for *S. trilobatum* in the ratio 1:10 at room temperature for 48 h. The residue was re-extracted under the same condition until the extraction solvents became colourless. The obtained extract was filtered, air dried and percent recovery was calculated. The dried extract was stored at 4° C for further analysis.

Chemicals

Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), potassium ferricyanide, 2,2'-diphenyl-1-picryl-hydrazyl ($DPPH^{\cdot}$), nitro blue tetrazolium (NBT), linoleic acid, ethylenediamine tetra acetic acid (EDTA), potassium persulfate, ferrous chloride, ascorbic acid, Tween 20, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Himedia, Merck and Sigma. All chemicals used were of analytical grade.

Microorganisms and culture conditions

The microorganisms like *Staphylococcus aureus* (MTCC 3160), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 424), *Salmonella typhi* (MTCC 3215) and *Escherichia coli*

(MTCC 40) were procured from MTCC, IMTECH, Chandigarh, India. They were further subcultured and maintained on nutrient agar medium at 4°C.

Determination of total phenolics and tannin contents

Total phenolics and tannins were measured as tannic acid equivalents from tannic acid standard curve (3-15 µg range) [10]. One mL of the sample extract was transferred to a test tube and 0.5 mL of Folin-Ciocalteu reagent and 2.5 mL of sodium carbonate solution (20 %) were added. After an incubation period of 40 minutes in dark and the absorbance was recorded at 725 nm against the reagent blank. Using the same extracts, the tannins were estimated after treatment with polyvinylpolypyrrolidone (PVPP).

Estimation of total flavonoids

Sample was added with 0.3 mL of 5 % $NaNO_2$, after 5 min mix with 0.3 mL of 1 % $AlCl_3$ and 2 mL of 1 M NaOH was added after 6 min and make up the volume to 10 mL with water, mixed thoroughly and the absorbance was measured at 510 nm. Total flavonoids were measured from rutin standard graph and expressed as mg rutin equivalents[11].

Free radical scavenging activity on 2, 2-diphenyl-1-picrylhydrazyl (DPPH $^{\cdot}$)

The antioxidant activity of extracts and standards (ascorbic acid, BHA, BHT and rutin) was measured in terms of hydrogen donating ability using a stable, commercially available organic nitrogen radical $DPPH^{\cdot}$[12,13]. Different concentrations (0.1 mL) of sample extracts prepared in methanol was mixed with 3.9 mL of $DPPH^{\cdot}$ (0.025 g/L) and incubated in dark for 30 min. The absorbance was measured at 515 nm which showed the remaining DPPH concentration. A calibration curve was plotted by taking sample concentration on X-axis and absorbance value on Y-axis. IC_{50} value of crude extract was calculated from the above graph and DPPH standard graph as amount of extract required to scavenge 1 g of DPPH. The antioxidant activity of the extract was expressed as mg/g of DPPH.

Antioxidant activity by the $ABTS^{\cdot+}$ assay

The $ABTS^{\cdot+}$ radical cation decolourization assay was performed to evaluate the radical scavenging ability of crude extracts[14, 15]. ABTS radical cation ($ABTS^{\cdot+}$) was generated by adding 2.45 mM potassium persulphate to 7 mM ABTS and incubated in dark at room temperature for 12–16 h. This stock solution of $ABTS^{\cdot+}$ was diluted with ethanol to give an absorbance of 0.70 (± 0.02) at 734 nm, which act as a working solution. 10 µL of crude extract (prepared in ethanol) was mixed with 1.0 mL of diluted $ABTS^{\cdot+}$ solution and incubated at 30 °C for 30 min. The absorbance value was estimated at 734 nm. Trolox standards were also prepared (in ethanol: 0-1.5 mM) to get the concentration response curve. The unit of Trolox equivalent antioxidant activity (TEA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as µmol/g of extracts. The TEA of ascorbic acid, BHA, rutin and tannic acid were also measured by $ABTS^{\cdot+}$ method for comparison.

Superoxide anion radical scavenging assay

All the solutions used for this assay should be prepared in 0.05 M phosphate buffer (pH =7.8). Sample extract (150 µg/mL) prepared in phosphate buffer was mixed with 1 mL of NBT (10^{-4} M), 1 mL of methionine (10^{-2} M) and 3 mL of riboflavin (10^{-6} M) solution. The mixtures were kept in an aluminium foil lined box with two 20 W fluorescent lamps. The reactants were kept in such a way that the light should reach the contents with approximately 4000 lux intensity[11, 16]. Control was also (assay mixture without sample) treated as above. All the samples and standards (rutin, quercetin, BHA and BHT) were run in triplicates and in both illuminated and non-illuminated conditions. The differences in sample absorbance (A) and control (A_1) between the illuminated and non-illuminated condition was recorded in order to avoid interferences. The degree of superoxide radical scavenging activity was calculated as,

$$\% \text{ SRSA} = [(A - A_1) / A] \times 100$$

Hydroxyl radical scavenging activity

Sample extract (150 µg/mL) was mixed with 1 mL of iron – EDTA solution (0.13 % ferrous ammonium sulphate in 0.26 % EDTA), 0.5 mL of 0.018 % EDTA and 1 mL of DMSO solution (0.85 % in 0.1 M phosphate buffered saline, pH=7.4). The reaction was initiated by the addition of 0.5 mL of 0.22 % ascorbic acid and incubated at 80–90 ºC in water bath for 15 min. The reaction was terminated by the addition of 1 mL of ice cold TCA (17.5 %). Nash reagent (3 mL) was added to the above mixture and allowed to stand at room temperature for 15 min and the absorbance values were recorded at 412 nm[17]. Sample control was also run with the substitution of phosphate buffer instead of ascorbic acid. Reaction mixture without samples was used as control. The per cent of hydroxyl radical scavenging activity (HRSA) was calculated using the following formula,

$$\text{HRSA \%} = [(A \text{ of sample} - A \text{ of Control}) / A \text{ of Control}] \times 100$$

Linoleic acid emulsion assay

The scavenging ability of the extracts and standards (BHA, BHT, rutin, quercetin and trolox) on lipid peroxidation was assessed by ammonium thiocyanate method[18, 19]. About 500 µg of extract in 0.5 mL of ethanol was mixed with 2.5 mL of linoleic acid emulsion (0.284 g of linoleic acid, 0.284 g of Tween 40, 50 mL of Phosphate buffer 0.02 M, pH=7.0) and 2.0 mL of 0.2 M phosphate buffer (pH=7.0) and incubated at 37 °C. Aliquots of 0.1 mL from the above mixture was taken at regular intervals (every 12 h) and mixed with 4.7 mL of 75 % ethanol, 0.1 mL of 30 % ammonium thiocyanate and 0.1 mL of ferrous chloride (0.02 M in 3.5 % HCl). The contents were mixed well and allowed to stand for 3 min and the absorbance values were recorded at 500 nm. A control was run with linoleic acid but without samples. The degree of oxidation was measured for every 12 h until 48 th h after the absorbance of the control reached its maximum. The net value of sample (A) and control (A_1) was calculated from the difference between absorbance at 48 h and absorbance at 0 h. Percentage of lipid peroxidation inhibition (LPI) was calculated as,

$$\text{LPI \%} = [1-(A/A_1)] \times 100$$

Ferric reducing antioxidant power assay (FRAP)

FRAP assay can be used to evaluate the electron donating ability of antioxidants [20, 21]. An aliquot of 30 µL sample was mixed with 90 µL of water and 900 µL of FRAP reagent (2.5 mL of 20 mM of TPTZ in 40 mM of HCl, 2.5 mL of 20 mM of ferric chloride, 25 mL of 0.3 M of acetate buffer (pH=3.6)) and incubated at 37 °C for 30 min. After incubation the absorbance values were recorded at 593 nm. Known ferrous sulphate concentrations ranging from 400-2000 µmol were used to generate the calibration curve. From the graph, the ferrous ions reduced by the sample were calculated using regression equation. The antioxidant activity is expressed as amount of extract required to reduce 1 mM of ferrous ions. The antioxidant activity of sample was compared with the following standards: tannic acid, rutin, BHA and ascorbic acid.

Phosphomolybdenum assay

An aliquot of 0.1 mL sample solution was mixed with 1mL of phosphomolybdenum reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) and incubated in water bath at 95 °C for 90 min. After incubation, the samples were read at 695 nm[22]. The results were expressed as ascorbic acid equivalents in µmol/g extract. Butylated hydroxyanisole (BHA), rutin, tannic acid and trolox standards were also estimated for comparison.

Metal chelating activity

The chelating of ferrous ions and standards like butylated hydroxyanisole (BHA), rutin, tannic acid and trolox was estimated[23]. An aliquot of 0.1 mL sample, 0.6 mL of distilled water and 0.1 mL of 0.2 mM $FeCl_2$ were mixed well and incubated for 30 s. Then, 0.2 mL of 1 mM ferrozine was added to the above mixture and incubated for 10 min at room temperature and the absorbance was recorded at 562 nm. EDTA (0-100 µg) was used as standard for the preparation

of calibration graph. Metal chelating ability of antioxidant was expressed as mg EDTA/g sample.

Antihaemolytic assay

Antihaemolytic assay is used to determine the antioxidant potential of plant extracts against H_2O_2 induced oxidative degradation of membrane lipids which leads to the lysis of RBC cells[24]. Cow blood (obtained from the local veterinary hospital) was centrifuged with phosphate buffered saline until the supernatant obtained was colourless. The sedimented erythrocytes were collected and diluted to 4 % suspension with phosphate buffered saline. Sample extract (500 µg/mL) were mixed with 2 mL of RBC solution and the mixture was made up to 3.5 mL with phosphate buffered saline and incubated at 37 ºC for 5 min. After 5 min, 0.5 mL of H_2O_2 solution of appropriate concentration (prepared in buffered saline) was added to the tubes and incubated at 37 ºC. Concentration of H_2O_2 in the reaction mixture was adjusted in such a way that it should reach 80–90 % haemolysis after 240 min at 37 ºC. The reaction mixture was centrifuged and extent of haemolysis was determined by measuring at 540 nm which corresponds to haemoglobin liberation. Antihaemolytic activity of BHA, rutin and tannic acid was also estimated for comparative analysis. Antihaemolytic activity (AA) of extract was calculated as,

$$AA\ \% = [(A \text{ of sample} - A \text{ of Control}) / A \text{ of Control}] \times 100$$

Antibacterial activity

The antibacterial activity of extracts was analyzed via agar disc diffusion method and determination of MIC (Minimum Inhibitory Concentration) [25-28]. One mL of overnight bacterial culture was inoculated into 100 mL of nutrient broth and incubated for 3 h at 37 °C to get the exponential phase culture. They were diluted to the absorbance (600 nm) ranging from 0.150-0.250. Fresh bacterial cultures (approximately 10^6 CFU/mL) were inoculated into sterile Muller Hinton agar plates using a sterile spreader. Sterile discs (6 mm) were loaded with 750 µg/mL of extracts dissolved in 5 % dimethyl sulfoxide (DMSO) and were left to dry for 1 to 2 h under sterile conditions. The dried discs were placed on the inoculated plates with the positive controls of standard antibiotics such as Gentamycin, Ciprofloxacin, Amikacin, Tetracycline and Streptomycin (10 µg/disc) and negative control as sterile disc treated with DMSO. The plates were then incubated at 37 ºC for 24-36 h. Diameter of inhibition zones around each discs were measured and recorded at the end of the incubation time.

The MICs of the extracts were determined by broth dilution method. Two fold serial dilutions were made to obtain a range of concentrations by transferring 1 mL of freshly prepared stock solution of the extracts into sterile tube containing 2 mL Muller Hinton broth and mixed thoroughly. One mL aliquot from this tube was transferred to the next tube containing 2 mL Muller Hinton broth and it was continued up to five to ten dilutions depending upon the concentration of the extracts. Test organism (approximately 10^6 CFU/mL) was inoculated to each tube and incubated at 37 ºC for 24 h. The MIC was determined by visual inspection as the absence of growth in above diluted concentrations and it was considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria after 24 h.

Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple-range test ($p < 0.05$) using SPSS 13. Values expressed are means of three replicate determinations ± standard deviation.

RESULTS AND DISCUSSION

Nowadays, research is going in around the world to exploit the traditional medicinal plants for therapeutic value scientifically. The qualities like low toxicity, inexpensive and potent pharmacological activities made medicinal plants very useful to mankind. Due to the complexity of oxidation-anti-oxidation process, multi-method approach is necessary to assess antioxidant activity of samples. In this present study, the antioxidant and antimicrobial activity of *Solanum trilobatum* and *Mukia maderaspatana* were compared and discussed with their related species. The antioxidant activity of fresh and dry mass of these leafy vegetables were evaluated and expressed in the present study which is essential to include in our daily diet.

Recovery percent

Recovery percent of *S. trilobatum* and *M. maderaspatana* were shown in Table 1. In our present study, the yield percent (23.56 %) of *S. trilobatum* methanolic extract were within the range of previous reports, *S. torvum* and *S. nigrum* leaves and fruits (3.7-71.7 %)[29], higher than the *S. trilobatum* chloroform extract (1.66 %) and *S. surattense* leaf extract (0.8-4.7 %)[30,31]. The yield percent of *M. maderaspatana* (5.72 %) were higher than the previous reports where the same plant of different parts yield 0.7-4.7 % and *Trichosanthes cucumerina* leaves (4.8 %) [32, 33]. The variation might be due to the use of different solvents, different plant parts, time, temperature, mode of extraction as well as on the chemical nature of the sample.

Determination of total phenolics and tannins

Phenolics acting as primary antioxidants or free radical terminators that are much stronger than those of vitamin C and E. Tannins or polymeric polyphenolics are also potent antioxidants than simple monomeric phenolics and thus may be important dietary antioxidants[34]. So, it is reasonable to determine the phenolics and tannins in selected plant extracts. Total phenolics and tannins of MME and STM were presented in Table 1.

The total phenol content of *M. maderaspatana* (0.19 g/100g DM) (3.15 g/100g extract) was comparable with *Mukia scabrella* (0.16 g/100g DM), *C. grandis* leaf fraction (2.7-6.3 g/100g extract) and lower than the different parts of this same plant (4.6-19.7 g/100g extract) [32, 35, 36]. The phenolics content of *S. trilobatum* (114 mg/g extract) (2.88 g/100g DM) was higher compared to all parts of *S. diphyllum* (26-68 mg/g extract), leaf of *S. torvum* and *S. nigrum* (0.4-5.01 g/100g extract) and lower than the chloroform extract of *S. trilobatum* (69.8 g/ 100 g) [29,30,37].

The tannin content of *M. maderaspatana* (2.51 g/100g extract) (150 mg/100g DM) showed similar reports within the range of all parts of this plant (0.1-8.3 g/100g extract) and higher than the leaves of *Coccinia grandis* (17.7 mg/100g DM) [32, 36]. The tannin content of *S. trilobatum* (10.5 g/100g extract) were similar than *S. surattense* leaf extract (9-10.7 g/100g extract) [31].

The variation in phenolics and tannins are due to the difference in species, solvents, plant parts used and different agroclimatic regions. The ortho-dihydroxy groups in phenols were the most important structural feature for the high antioxidant activity. Other structural features (e.g., OH position and number, glycosylation, methoxylation) played a modified role in enhancing or reducing the activity. The significant reactivity of the ortho-dihydroxy structural system is possibly to due to the smaller dissociation energy of the O–OH bond in comparison with other structural systems (non-ortho-structural systems, e.g., 1, 3; 2, 4; 1, 5), and owing to the greater stability of the transient radical involved, in which the oxygen-centered unpaired p-orbital is conjugated with a lone pair on the adjacent oxygen atom[38]. Natural tannins act as powerful antioxidant agents due to the presence of higher number of hydroxyl groups, especially many ortho-dihydroxy or galloyl groups[39].

Determination of flavonoid content

The flavonoid content of *S. trilobatum* (41.7 mg/g DM) (9.34 mg/g FM) were expressed in Table 2 which is higher than that of *S. nigrum* (0.004-0.78 mg/g DM) and *S. grandiflorum* stem (0.25 mg/g FM)[40,41]. Flavonoids were not detected in *M. maderaspatana* methanol extract but its presence was observed in *M. maderaspatana* leaves[9], it might be due to the poor extractability of flavonoids in ethanol. Flavonoids contains several number of phenolic hydroxyl compounds (flavonols, flavones, flavanols and isoflavones) mainly glycosides are important contributor of antioxidant activity[38].

Table 1: Recovery percent, total phenolics and tannins of *S. trilobatum* and *M. maderaspatana*

Sample	Extract yield (%)	Total phenolics (mg TAE/g)[A]			Total tannins (mg TAE/g)[B]		
		Extract	DM	FM	Extract	DM	FM
MME	5.72	31.5 ± 0.15	1.9 ± 0.01	0.23 ± 0.01	25.1 ± 0.16	1.5 ± 0.01	0.18 ± 0.01
STM	23.56	113.6 ± 0.14	28.8 ± 0.02	6.4 ± 0.01	105.1 ± 0.28	26.6 ± 0.07	6.0 ± 0.01

[A] Total phenolics (mg equivalent tannic acid) [B] Tannins (mg equivalent tannic acid)

All values are expressed as Mean ± SD for three determinations; MME - *Mukia maderaspatana* ethanol extract; STM - *Solanum trilobatum* methanol extract

Table 2: Total flavonoid content of *S. trilobatum* and *M. maderaspatana*

Samples	Total flavonoids (mg rutin/g)		
	Extract	DM	FM
MME	-	-	-
STM	164.53 ± 2.8	41.72 ± 0.71	9.34 ± 0.16

All values are expressed as Mean ± SD for three determinations; MME - *Mukia maderaspatana* ethanol extract; STM - *Solanum trilobatum* methanol extract

Free radical scavenging activity on 2, 2-diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$)

DPPH$^{\cdot}$ nitrogen centered radical provides an easy and highly reproducible way to assess the antioxidant ability of plant extracts to donate electron or labile hydrogen atoms to radicals. When compared to standards the plant extract require higher amount to scavenge 50 % of DPPH (Table 3). *M. maderaspatana* (IC_{50} - 1.2 mg/mL) exhibit more activity than *Momordica charantia* leaf and stem (IC_{50} - 9.7 & 18 mg/mL) and comparable to all parts of *M. maderaspatana* (IC_{50}: 0.12-1.8 mg/mL) and less activity than *Coccinia grandis* different fractions (0.145-0.6 mg/mL) [32, 36, 42]. *S. trilobatum* extract showed higher DPPH scavenging activity (0.15 mg - 86 %) (IC_{50} - 76.23 µg/mL) than whole parts of *S. trilobatum* chloroform extract (0.15 mg - 35 %), *S. surattense* leaf (IC_{50}: 180-390 µg/mL) and *S. nigrum* leaf (1 mg: 72-96 %) [30, 31,40]. The number of DPPH$^{\cdot}$ radical is reduced by available hydroxyl groups in the sample extract. Nitrogen centered radicals such as DPPH$^{\cdot}$ react with phenols (ArOH) via two different mechanisms: (i) a direct abstraction of phenol H-atom (HAT reactions) and (ii) an electron transfer process from ArOH or its phenoxide anion (ArO−) to DPPH$^{\cdot}$ (ET reactions) [43].

Table 3: DPPH activity of *Mukia maderaspatana* and *Solanum trilobatum*

Samples	DPPH (g/g DPPH)		
	Extract	DM	FM
MEE	13.62 ± 0.026	220.21 ± 0.415	1877.26 ± 3.535
STM	0.84 ± 0.009	3.30 ± 0.036	14.72 ± 0.159
ASC	0.10 ± 0.01	-	-
BHA	0.11 ± 0.02	-	-
BHT	0.25 ± 0.01	-	-
RUT	0.14 ±0.01	-	-

All values are expressed as Mean ± SD for three determinations; MEE - *Mukia maderaspatana* ethanol extract; SME - *Solanum trilobatum* methanol extract; ASC - ascorbic acid, BHA - butylated hydroxyanisole; BHT - butylated hydroxytoluene; RUT – rutin; DM – dry mass; FM - fresh mass.

Antioxidant activity by the ABTS$^{\cdot+}$ assay

Proton radical scavenging is an important attribute of antioxidants. ABTS$^{\cdot+}$, a protonated radical which is generated from peroxidase substrate (ABTS) in the presence of potassium persulphate has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals[44]. The antioxidant capacity of test extracts is measured to decrease the colour reacting directly with ABTS radical and expressed in Table 4 relative to trolox equivalents. ABTS$^{\cdot+}$ is capable of reacting with both lipophilic tocopherols, carotenes, flavonoids, hydrophilic polyphenolic glycosides and phenolic acids[9].

M. maderaspatana scavenging activity which is less than the capacity of previous reports of *M. maderaspatana* leaves and whole parts[8, 32]. The difference in same species is due to the variation in plant parts, solvents and origin of different agroclimatic regions. *S. trilobatum* (15.3 µmol/g) (IC_{50} - 5 mg/mL) have highest ABTS radical scavenging activity than *Solanum grandiflorum* (5.9 µmol/g) and *S. pseudocapsicum* leaf extract (IC_{50} - 49.66 mg/mL) [41, 45]. The mechanism behind scavenging of ABTS$^{\cdot+}$ radical due to one molecule of ABTS radical cation abstracts an electron (or hydrogen atom) from the polyphenol and forms a semiquinone radical, regenerating the parent substrate, ABTS. Subsequently, the semiquinone radical reacts with another molecule of ABTS radical cation resulting in the formation of the first polyphenol-derived adducts (Ip and Ic). In pathway 1, the polyphenol derived adducts Ip and Ic undergo a degradation reaction and form adducts IIp and IIc, respectively, releasing a benzothiazolium ion. The benzothiazolium ion is known to be unstable, and easily undergoes hydrolysis and oxidation to form 3-ethyl-6-sulfonate-benzothiazolone [46].

Table 4: ABTS$^{\cdot+}$ and FRAP activity of *Mukia maderaspatana* and *Solanum trilobatum* extracts

Samples	ABTS$^{\cdot+}$			FRAP		
	TEA (mmol/kg sample)[A]			[µg/mmol Fe (II)] [B]		
	Extract	DM	FM	Extract	DM	FM
AA	971.4 ± 65.6	-	-	1.4 ± 0.01	-	-
BHA	1555.6 ± 51.0	-	-	2.3 ± 0.02	-	-
RUT	1301.0 ± 73.0	-	-	1.9 ± 0.04	-	-
TA	21087.7 ± 103.2	-	-	2.4 ± 0.05	-	-
MME	132.5 ± 12.8	8.2 ± 0.8	1.0 ± 0.1	49.5 ± 0 .01	800.5 ± 1.4	6824.2 ± 11.8
STM	268.9 ± 0.4	268.9 ± 0.4	15.3 ± 0.02	36.1 ± 0.1	142.2 ± 0.4	635.3 ± 1.7

All values are expressed as Mean ± SD for three determinations; AA - ascorbic acid; BHA - butylated hydroxyanisole; RUT - rutin; TA - tannic acid; MME - *Mukia maderaspatana* ethanol extract; STM - *Solanum trilobatum* methanol extract; DM - dry mass; FM - fresh mass

[A] Trolox equivalent antioxidant activity (mmol equivalent Trolox performed by using ABTS radical cation)

[B] Ferric reducing antioxidant power assay (concentration of substance having ferric-TPTZ reducing ability equivalent to that of 1 mmol Fe (II))

Superoxide anion radical scavenging assay

Superoxide anion radical (O_2^-), a highly toxic radical generated first after oxygen is taken into living cells by several enzymatic and non-enzymatic pathways, plays important role in the formation of other deleterious reactive oxygen species (hydrogen peroxide and hydroxyl) attacks a number of biological molecules and leads to unfavourable alterations of biomolecules including DNA [47]. The results of superoxide anion radical scavenging activity were presented in Fig 1.

The capacity of superoxide scavenging activity reveals that the extract possesses superoxide dismutase like activity. *S. trilobatum* extract efficiently scavenges superoxide scavenging activity than standards and *M. maderaspatana*. *S. trilobatum* exhibited higher superoxide radical scavenging activity (0.15 mg – 94 %) than *S. trilobatum* chloroform extract (0.1 mg - no activity) and *S. pseudocapsicum* leaf extract (IC_{50} - 1000 mg) [30, 45]. *M. maderaspatana* extract exhibited lower superoxide scavenging activity than *M. maderaspatana* aqueous leaf extracts[8]. The radical scavenging activity is also consistent with the high level of phenolic and tannins observed in *S. trilobatum* since phenolic compounds such as flavonoids and tannins are known to possess high superoxide anion scavenging abilities [48]. The presence of vitamin-C, flavonoids and superoxide dismutase in *S. trilobatum* may also responsible for the higher superoxide scavenging activity [49].

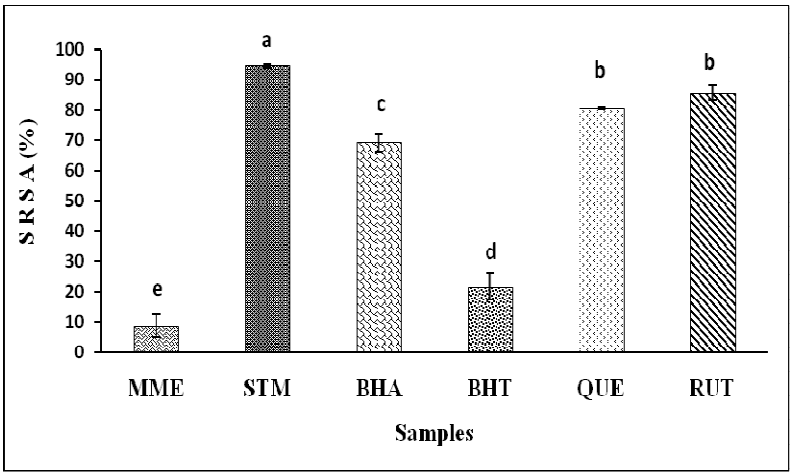

Fig. 1: Superoxide anion radical scavenging activity of *Mukia maderaspatana* and *Solanum trilobatum* extracts

MME - *Mukia maderaspatana* ethanol extract; STM - *Solanum trilobatum* methanol extract; BHA - butylated hydroxyanisole; BHT - butylated hydroxyl toluene; QUE - quercetin; RUT - rutin. Values are mean of triplicate determinations ± standard deviation. Bars having different alphabets are significantly different ($p < 0.05$)

Hydroxyl radical scavenging activity

Hydroxyl radicals are the major active oxygen species formed from the reaction of various hydroperoxides with transition metal ions and attack cell constituents including lipids, nucleic acid and proteins giving rise to many diseases including arthritis, atherosclerosis, cirrhosis, diabetes, cancer, Alzheimer's disease, emphysema and ageing[50]. Fig 2 showed *M. maderaspatana* and *S. trilobatum* have higher scavenging activity than standard, BHT. Hydroxyl radical scavenging activity of *M. maderaspatana* (150 µg - 15 %) is higher than *Eupatorium odoratum* leaf (200 µg - 11 %) [51], similar to *M. maderaspatana* leaf (150 µg - 18-20 %) but lower activity than *M. maderaspatana* aqueous leaf extract [8, 32]. The result of hydroxyl radical scavenging activity of *S. trilobatum* (0.15 mg – 23 %) was comparable to *S. surattense* leaf extract (0.1 mg – 18 %) and *S. trilobatum* chloroform extract (0.075 mg – 18 %) [30, 31]. The presence of phenolic compounds (ArOH) in samples might donate active hydrogen groups to hydroxyl radical and stabilizes it. The presence of flavonoids and vitamin C in *S. trilobatum* may be responsible for higher hydroxyl scavenging activity [49].

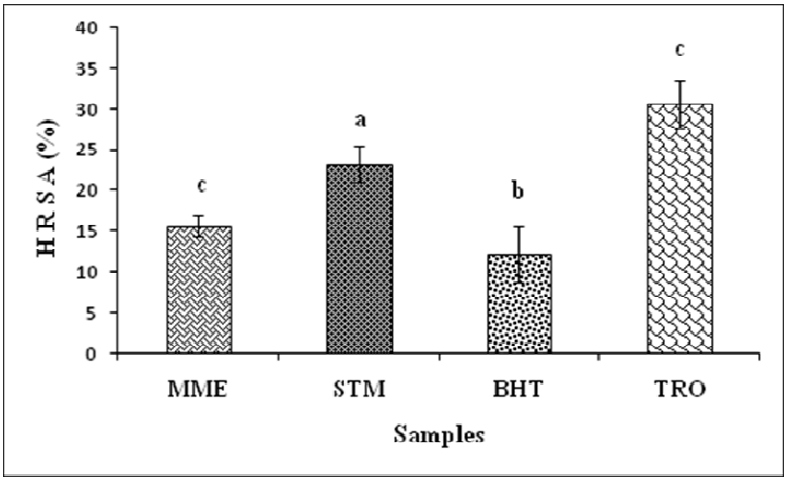

Fig. 2: Hydroxyl radical scavenging activity of *Mukia maderaspatana* and *Solanum trilobatum* extracts

MME - *Mukia maderaspatana* ethanol extract; STM- *Solanum trilobatum* methanol extract; BHT - butylated hydroxytoluene; TRO - trolox. Values are mean of triplicate determinations ± standard deviation. Bars having different alphabets are significantly different ($p < 0.05$)

Linoleic acid emulsion assay

Lipid peroxidation is a key process in many pathological events and it is one of the reactions induced by oxidative stress. The rearrangement of the double bonds in unsaturated lipids and the destruction of membrane lipids to produce breakdown products such as malondialdehyde, is known to be mutagenic and carcinogenic[52]. In the present study (Fig 3), it is observed that rutin and *M. maderaspatana* have similar activity in inhibiting lipid peroxidation. Both the extracts have strong antioxidant activity in controlling linoleic acid oxidation and oxidative deterioration of food. *S. trilobatum* (0.5 mg – 84 %) and *M. maderaspatana* (0.5 mg – 89 %) had significantly inhibited the lipid peroxidation than *Ervatamia coronaria* leaf (0.5 mg – 75 %) [53]. *M. maderaspatana* inhibited 50 % peroxidation activity at 0.32 mg/mL and it is comparable with previous report where *Coccinia grandis* showed 0.271-0.460 mg/mL[36]. The presence of conjugated ring structures and hydroxyl groups allows phenolics to actively scavenge free radicals and the presence of carboxylic acid groups can inhibit lipid peroxidation[54]. The presence of vitamin E, flavonoids and glutathione peroxidase in samples may be responsible for scavenging these lipid peroxides[49]. Surprisingly, in this assay, *M. maderaspatana* exhibited higher inhibition activity on linoleic acid peroxidation than *S. trilobatum*. The reason might be due to the contribution of predominant polyphenol compound, 7-O-β-D-glucopyranosyl-6-C-β-D glucopyranosyl apigenin (saponarin) in *M. maderaspatana*[9]. These saponarin phenols have been reported to possess hepatoprotective properties[55].

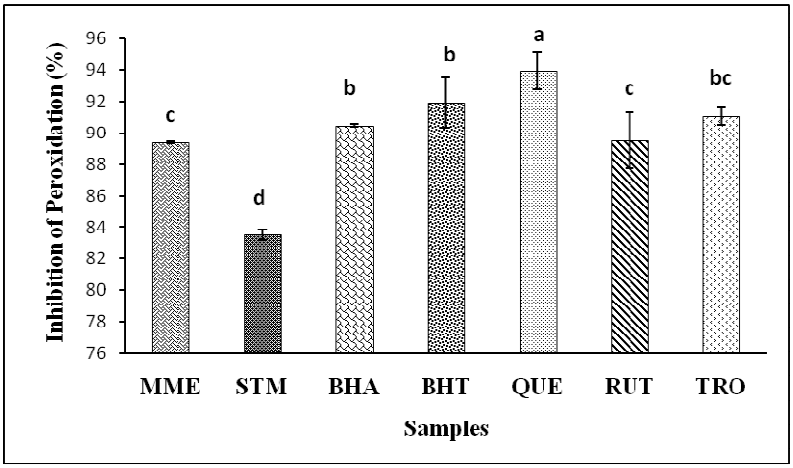

Fig. 3: Lipid peroxidation inhibition of *Mukia maderaspatana* and *Solanum trilobatum* extracts

MME - *Mukia maderaspatana* ethanol extract; STM - *Solanum trilobatum* methanol extract; BHA - butylated hydroxyanisole; BHT - butylated hydroxytoluene; QUE - quercetin; RUT - rutin; TRO - trolox. Values are mean of triplicate determinations ± standard deviation. Bars having different alphabets are significantly different ($p < 0.05$)

Ferric reducing antioxidant power assay (FRAP)

FRAP measures the ferric reducing ability of the samples at a low pH, forming an intense blue colour as the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous (Fe^{2+}) form and absorbance is measured at 593 nm [56]. The reductants present in the sample extract causes the reduction of ferric to ferrous. The FRAP result was expressed in Table 4. *S. trilobatum* extract has more reducing activity (*A* at 25 µg is 0.373) than *S. trilobatum* chloroform extract (*A* at 50 µg - 0.1) [30], *S. diphyllum* (*A* at 100 µg - 0.12-0.24) and leaf of *S. torvum* and *S. nigrum* [29, 37, 40]. The reducing power of *M. maderaspatana* (*A* - 0.577) is higher than *Coccinia grandis* (*A*: 0.062-0.432), *Momordica charantia* leaf and stem and *M. maderaspatana* aqueous leaf extract[8, 42]. The presence of antioxidants like polyphenols in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The reducing power of extracts appears to be more related to the degree of hydroxylation and the extent of conjugation in polyphenols [57].

Phosphomolybdenum assay

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and a formation of a green phosphate Mo (V) complex at acidic pH which has the absorption maxima at 695 nm. The ascorbic acid equivalents of *S. trilobatum* and *M. maderaspatana* were shown in Table 5. *Mukia maderaspatana* have lower ascorbic acid equivalent than *C. grandis* vitamin E equivalents[36]. *S. trilobatum* extract (39.28 g/100g extract) has more reducing power than *S. surattense* leaf extract (25.7-32.9 g/100 g extract)[31]. Standards like BHA, rutin and tannic acid have high reducing activity whereas trolox showed less activity than the crude extract. The antioxidant activity by phosphomolybdenum method may mainly depend on the presence of polyphenols in the samples which may act as reducing agents, by donating the electrons and reacting with free radicals to convert them to more stable products, thus terminating the free radical chain reaction[58]. The results also correlate with the *Solanum* sample which has high polyphenol content and observed to have high reducing capacity.

Table 5: Metal chelating activity and phosphomolybdenum assay of *Mukia maderaspatana* and *Solanum trilobatum* extracts

Samples	Metal chelating (mg EDTA/g sample)[A]			Phosphomolybdenum AA (µmol/g sample)[B]		
	Extract	DM	FM	Extract	DM	FM
BHA	18.4 ± 10.5	-	-	24408.1 ± 48.9	-	-
RUT	3.8 ± 0.0	-	-	9749.1 ± 24.4	-	-
TA	227.1 ± 11.9	-	-	22122.5 ± 97.7	-	-
TRO	106.9 ± 5.9	-	-	654.7 ± 24.4	-	-
MME	3.3 ± 0.1	0.2 ± 0.01	0.02 ± 0.001	2056.5 ± 7.8	127.1 ± 0.5	14.9 ± 0.06
STM	10.2 ± 0.1	2.6 ± 0.1	0.6 ± 0.003	2230.3 ± 7.8	565.5 ± 2.0	126.6 ± 0.4

[A] Metal chelating activity (mg equivalent EDTA); [B] Antioxidant activity (µmol ascorbic acid equivalents performed by using phosphomolybdenum assay).

All values are expressed as Mean ± SD for three determinations; BHA - butylated hydroxyanisole; RUT - rutin; TA - tannic acid; TRO - trolox; MME - *Mukia maderaspatana* ethanol extract; STM - *Solanum trilobatum* methanol extract; DM - dry mass; FM - fresh mass

Metal chelating activity

Transition metal species such as ferrous iron (Fe^{2+}) can facilitate the production of ROS within animal and human systems, the ability of substances to chelate iron can be a valuable antioxidant capability[59]. Ferrozine in the reaction mixure can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of the rate of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator. Both the plant extracts showed chelating activity by their effectiveness in inhibiting the formation of ferrous and ferrozine complex. The metal chelating activity of samples was tabulated in Table 5.

S. trilobatum extract (39 %) (10.2 mg EDTA/g extract) has more metal chelating activity than *S. surattense* leaf extract (1.3 mg EDTA/g extract)[31], different parts of *S. diphyllum* (3-31 %) and less chelating power than *S. torvum* and *S. nigrum* [29,37]. *M. maderaspatana* (3.3 mg EDTA/g extract) have more metal chelating property than previous reports of all parts of *M. maderaspatana* (0.7-2.9 mg EDTA/g extract) but lower than *Coccinia grandis* leaf fraction [32, 36]. Chelating agents in sample extracts which form σ-bonds with a metal and they reduce the redox potential thereby stabilizing the oxidized form of the metal ion[60]. Standards like BHA, tannic acid and trolox act as a strong metal chelator than the plant extracts whereas *S. trilobatum* have highest metal chelating activity than rutin and *M. maderaspatana* exhibited similar activity to rutin. Flavonoids may be responsible for excellent ability to chelate ferrous ions as typical phenolic compounds due to their phenolic hydroxyl groups and properly oriented functional groups [61].

Antihaemolytic activity

Antihaemolytic assay, a biological test was based on free radical-induced erythrocytes lysis of cow blood. The hydroxyl radical is induced by the intensification of lipid and protein oxidation processes, resulting in several changes in membrane structures ultimately leading to the release of haemoglobin from cells[62]. Both the extracts showed similar activity in protecting cell membrane from hydrogen peroxide damage (Fig 4). Tannic acid has high inhibition activity on erythrocyte haemolysis whereas BHA and rutin have similar activity to both of these sample extracts. Antihaemolytic activity of *M. maderaspatana* (0.5 mg - 54.5 %) is within the range of previous reports of all parts of *M. maderaspatana* (0.5 mg - 43-76 %) [32]. *S. trilobatum* (0.5 mg – 55 %) prevented haemolysis from H_2O_2 damage similar to previous reports of *S. surattense* leaf acetone extract (0.5 mg – 52 %) and *S. torvum* leaf acetone and methanol extract (0.5 mg – 55 %) but lower than *S. surattense* leaf methanol extract (0.5 mg – 65 %)[29]. The presence of vitamin C, vitamin E and flavonoids in sample extracts will be responsible for scavenging hydrogen peroxide[49]. In addition, recent studies have suggested that the ability of certain phenolic compounds to partition in cell membranes, and the resulting restriction of their fluidity, could sterically hinder diffusion of free radicals, thereby, decreasing the kinetics of free radical reactions[63].

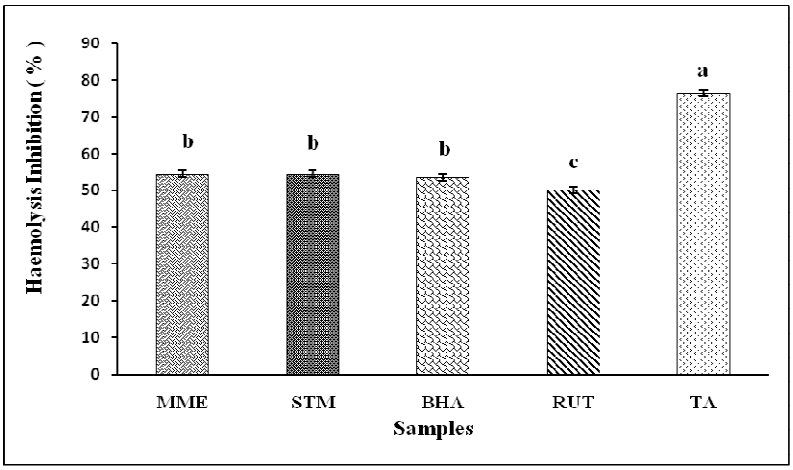

Fig. 4: Antihaemolytic activity of *Mukia maderaspatana* and *Solanum trilobatum* extracts.

MME - *Mukia maderaspatana* ethanol extract; STM - *Solanum trilobatum* methanol extract; BHA - butylated hydroxyanisole; RUT - rutin; TA - tannic acid. Values are mean of triplicate determinations ± standard deviation. Bars having different alphabets are significantly different ($p < 0.05$)

Antibacterial activity

Medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial and fungal growth[64]. Nowadays microbes are increasingly developing resistance against the drugs in use. To combat against these drug resistant microbes, a large library of novel compounds is required[65]. Five important infectious microorganisms like *K. pneumoniae, E. coli, S. aureus, S. typhimurium* and *P. aeruginosa* will able to cause various health related problems. Therefore, it is important to test the antibacterial activity of these extracts and these results were presented in Table 6. Some bacteria are resistant and some are sensitive towards commercial antibiotics like gentamycin, ciprofloxacin, amikacin, tetracycline and streptomycin. The activity of *M. maderaspatana* (750 µg) in the order of *S. aureus* > *E. coli* > *P. aeruginosa* > *K. pneumoniae*. All tested bacteria were susceptible towards both the extracts where *S. typhimurium* alone were resistant to both the extracts. This study agrees with the earlier findings showed more inhibitory activity on certain pathogens than the leaves and aerial parts of *Trichosanthes cucumerina*[33].

Solanum trilobatum methanol extract (750 µg) exhibited inhibition activity against gram positive and gram negative bacteria in the order of *E. coli* > *S. aureus* > *P. aeruginosa* > *K. pneumoniae*. The MIC of *M. maderaspatana* and *S. trilobatum* extract were 2.5 mg/mL for *S. aureus*, *P. aeruginosa*, *E. coli* and 3.5 mg for *K. pneumoniae*. While both extracts exhibited no activity on *S. typhimurium*.

When compared to standard antibiotics, both of these extracts showed less activity it might be due to the interference of other compounds in the crude extract. The results showed the effectiveness of extracts against pathogenic bacteria. With these results, it can suggest to promote these plant extract against water and food borne pathogens. Phenolic compounds are thought to be toxic to microorganisms, inhibiting the enzymes which are essential for the growth of microorganism[66].

The antioxidant activities of both the samples were also expressed in terms of dry and fresh mass. These values may be useful for consumers to understand the quantity and presence of antioxidants in it. With these results, it is concluded that the higher phenol, tannin and flavonoid in *S. trilobatum* contributed the higher antioxidant activity than *M. maderaspatana*. Surprisingly, *M. maderaspatana*

exhibited similar activity on antihaemolytic activity, higher inhibition of linoleic acid peroxidation and antimicrobial activity. The higher antihaemolytic, lipid peroxidation inhibition and antimicrobial activity in *M. maderaspatana* might be due to the contribution of cucurbitacin glycosides, higher amount of 7-O-β-D-glucopyranosyl-6-C-β-D-glucopyranosylapigenin (saponarin) [9].

Table 6: Antibacterial activity of successive extract of *Mukia maderaspatana*, *Solanum trilobatum* and comparison with standards

	Inhibition zone diameter (mm)							MIC (mg/mL)	
	MM	**ST**	**Gen**	**Cip**	**Tet**	**Ami**	**Str**	**MM**	**ST**
S. aureus	14	12	18	30	-	-	-	2.5	2.5
K. pneumoniae	12	8	-	-	15	25	-	3.5	3.5
P. aereuginosa	12	11	20	25	-	-	-	2.5	2.5
S. typhi	R	R	-	-	22	20	-	R	R
E. coli	14	13	14	-	-	-	12	2.5	2.5

MM - *Mukia maderaspatana*, ST - *Solanum trilobatum,* Gen - gentamycin, Cip - ciprofloxacin, Tet - tetracycline, Ami - amikacin, Str – streptomycin R - resistance of microorganisms to given concentration

CONCLUSION

Medicinal plants are an excellent reservoir to obtain various pharmaceutical constituents. Many modern medicines will cure diseases but it has side effects. Many peoples in developing countries practice only medicinal herbs due to their safety and capability of curing diseases. *S. trilobatum* showed efficient antioxidant activity than *M. maderaspatana* where antimicrobial activity was similar in both of these extracts. The intake of these plants may serve as safe alternative to synthetic ones and prevent free radical mediated diseases and protect from food and water borne pathogens. This study might provide foundation to identify various pharmaceutical products in future. The identification of active ingredients and mechanism of action responsible for both antioxidant and antimicrobial activity will be needed. Toxicity and certain *in vivo* studies will be required to explore these plants to food and pharmaceutical industry in upcoming days.

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