

Antioxidant and antimicrobial properties of *Tamarindus indica* L.

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Abstract

In this study, *In Vitro* antioxidant and antimicrobial activities of aqueous and Hydro alcoholic extracts of leaves *Tamarindus indica* were investigated. *In-vitro* antioxidant assay was performed by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, Hydroxyl radicals scavenging assay, Ferric reducing assay, (and) Nitric oxide radical scavenging assay. Percentage of free radical scavenging potential was determined using ascorbic acid as a standard in each method. In Ferric reducing antioxidant power assay, antioxidant property was determined by measuring ferric reducing values. It was observed that hydro alcoholic extracts were having more potential antioxidants. Hydro alcoholic extract at a dose level of 500 µg/ml shown maximum FRAP value 0.76±0.08. IC₅₀ values were determined for each extracts. Lowest IC₅₀ value was exhibited by HAETIL in DPPH, Hydroxyl radical scavenging, Ferric reducing and NO scavenging methods are: 195.30µg/ml, 182.02µg/ml, 196.23µg/ml respectively. These results indicated that the anti oxidant capacities depends on concentration and nature of extracts. Antimicrobial activities were evaluated by determining inhibition zone diameter and calculated MIC values. Aqueous extracts of *T. indica* leaves shown maximum inhibition zone diameter against gram positive bacteria *Staphylococcus aureus* 17.33±1.5 mm and was exhibited by Ciprofloxacin used as standard 36.0±2.0mm. In gram negative bacteria *Pseudomonas aeruginosa*, Hydro alcoholic extracts of *T. indica* leaves shown 24.66±.51mm and Gentamicin used as a standard 37.0±1.73. Aqueous extracts of leaves shown good antimicrobial activity against Gram positive bacteria and the hydro alcoholic extracts of leaves shown in case of Gram negative bacteria. Minimum Inhibitory Concentration shown by different extracts were in the range of 8-20mg/ml. Hydro alcoholic extracts of leaves shown lowest minimum Inhibitory concentration 8mg/ml against *Pseudomonas aeruginosa*.

Keywords: *Tamarindus indica*, antioxidant, Nitric oxide, Hydroxyl radical, *Staphylococcus aureus*

Introduction

Tamarindus indica is of medium to large in size, it is evergreen tree, 12-18 m. high in height and 7m. in girth. The latest morphologic and molecular analyses and continued study will clarify the exact positioning of *T.indica* in relation to its putatively related genera [1]. Traditionally plant has been used in treatment of wound, fever, malaria, abdominal pain, leprosy, conjunctivitis, stomachic, preservative, anthelmintic, bacterial infection, diabetes, oxidative stress, asthma [2]. It has been also reported to possess activities like Antidiabetic [3,5], anti-oxidative [4,6,8] hypolipidemic and weight reducing [7,8] and antimicrobial [9,10]. Flavonoids and other polyphenols have been found in tamarind leaves. These compounds have recorded as antimicrobial agents in many other plants. [2].

Generally oxidative stress results if an imbalance between the generations of oxygen derived radicals and the organism's

antioxidant potential [11]. Oxidative stress leads to various complications like cardiovascular disease, nephropathy, neuropathy, retinopathy, erectile dysfunction [12].

Irrational use of antibiotics now a responsible for development of drug resistance against microbes which are responsible for infectious disease. Scientists are searching various novel natural antimicrobial agents which obtained from plant source to handle this drug resistance [13]. The reason for this study is that antimicrobial activities of *T.indica* leaves extract have not been reported in the literature. Till date, people have been using it as a food and medicine since a long time. Considering the traditional claim, chemical constituents and traditional reported activities of *T.indica* we were carried out the present work to evaluate the *in vitro* antioxidant and antimicrobial potentials of hydro alcoholic and aqueous leaves extracts of *T.indica* on several microorganisms of medicinal importance and prove it scientifically.



Materials and Methods

Instruments used

UV Visible Spectrophotometer (Systronics 2203), Laminar Air Filter (Jyoti scientific, Gwalior) Soxhlet assembly, Incubator, Autoclave (Khera Instrument Pvt. Ltd).

Chemicals and Reagents

1, 1-Diphenyl 2-Picryl Hydrazyl radical (DPPH), Tripyridyl triazine (TPTZ), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) obtained from (Sigma Aldrich Mumbai). Ethanol from (Fisher Scientific, Mumbai). Thiobarbituric acid (TBA), Ascorbic acid and Sodium nitroprusside from (Hi-media laboratories Pvt. Ltd. Mumbai). Ferric chloride (High Purity Laboratory Chemicals Pvt. Ltd, Mumbai) and all other reagents used are of analytical grade.

Plant material

The leaves of *T. indica* were collected from local region around Raipur, Chhattisgarh, India in the month of November- December. Leaves were washed under running tap water followed by rinsed with distilled water for five minutes. leaves as well as seeds of *T. indica* were identified and authenticated by Dr. P. C. Panda .Principal Scientist Taxonomy Division of "Regional Plant Resource Centre, Bhubaneswar, Odisha, India" (Voucher specimen no: BM - 1).

Extractions

The collected leaves were dried under shade and then pulverized by an electrical blender. The coarse powder was then passed through sieve No. 20. The powdered material (500 g) was extracted with boiling water for 10 hrs. The resulted extract was filtered using Whatman filter paper no. 4 and concentrated by lyophilizer (yield: 5.34 % (w/w) with respect to dried plant material). The powdered material (500 g) was also extracted with ethanol and water (8:2) for 2 days. The resulted hydroalcoholic extract was filtered through Whatman filter paper no. 4 and concentrated by rotary evaporator under reduced pressure and low temperature to yield 8.87 % (w/w) with respect to dried plant material [14].

Test organisms

Antimicrobial activities of *T. indica* leaves extracts were evaluated against four pathological isolated strains provided by MTCC (details address) Chandigarh. The bacterial strains used to assess the antimicrobial properties were, (using) two Gram-positive strains: *S. aureus* (MTCC3160), *B. subtilis* (MTCC3053) and two Gram-negative strains: *E. coli* (MTCC3221), *P. aeruginosa* (MTCC3163).

Methods

Antioxidant activity assays

DPPH radical scavenging assay

DPPH assay was done according to the method of (Vani T *et al.*). The anti-oxidant activities of HAETIL and AETIL of leaves were measured with scavenging capacity of 1, 1-diphenyl 2-picryl hydrazyl radical DPPH radical. 10 mg of dried HAETIL and AETIL extract dissolved in 10 ml of methanol to give concentration of 1mg/ml. Separately all the samples were diluted in 10 ml volumetric flask with methanol to give varying from 50 µg - 500 µg/ml. DPPH solution was prepared by dissolving 4.3mg of DPPH in 3.3 ml methanol, it was protected from light by covering the samples with aluminium foil and keeping in dark areas. 150µl of DPPH solution was added to 3 ml methanol and absorbance was taken by UV spectrometer immediately at 516 nm for control. The assay mixture contain 0.3 ml of 1.0 mM DPPH solution, 2.4 ml of 99% ethanol, and 0.3 ml of extract solution of required concentration. The solutions were rapidly mixed and scavenging capacity was measured by Spectrophotometrically by monitoring the decrease in absorbance at 516 nm [15]. Lower absorbance value of reaction mixture indicates higher degree of free radical scavenging activity.

$$\text{DPPH scavenging effect (\%)} = (1 - A_s / A_c) \times 100$$

Where A_c is the absorbance of control and A_s is the absorbance in the presence of sample.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging assay was done according to the method of Uma Devi P *et al.*/2002. The formation of hydroxyl radicals (OH \cdot) from Fenton reagents was quantified using 2 deoxyribose oxidative degradation. The principle of the assay is the quantification of the 2-deoxyribose degradation product, malonaldehyde by its condensation with Thiobarbituric acid (TBA). Reaction Mixture consisted of 100µl Deoxyribose (33.6mM), 50µl FeCl $_3$ (300µM), 50µl EDTA (1.2mM), 100µl H $_2$ O $_2$ (33.6mM) in 550µl PO $_4$ buffer saline (pH=7.4) Plant extract at concentration 50-500 µg/ml was added to the reaction mixture to make a final volume of 1ml. Ferric chloride and EDTA were premixed just before addition to the reaction mixture. Reaction mixture was incubated for 1 hr at room temperature. 0.5 ml of 2.5% Thiobarbituric acid (TBA) and Trichloroacetic acid (TCA) were added to the reaction mixture. Mixture was then incubated for 20 min in a boiling water bath and then cooled and centrifuged at 3200 rpm. After a cooling period, Thiobarbituric acid reactive substances (TBARS) formation was measured at 532 nm against an appropriate blank. The hydroxyl radical-scavenging activity was determined by comparing absorbance of the control with that of test compounds. The test tube with Phosphate buffer saline was considered as a blank and Ascorbic acid was used as a standard. The results were expressed as % inhibition of free radicals [16].



% scavenging of OH = $[(A_0 - A_1)/A_0] \times 100$,

Where A_0 absorbance of the control and A_1 is the absorbance in presence of sample.

Fe³⁺ reducing antioxidant power (FRAP assay)

The total antioxidant potential of extract was determined by using Fe³⁺ reducing anti-oxidant power (FRAP) by the assay of *Benzie et al 1999* and *Strain et al., (1999)* FRAP assay uses antioxidants as reductant in a redox - linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric. At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue color) can be monitored by measuring the change in absorption at 593 nm. FRAP reagent (300 mM acetate buffer, pH = 3.6, 10 mM TPTZ (tripyrindyl-s-triazine) in 40 mM HCl and 20 mM FeCl₃.6H₂O in the ratio of 10:1:1) and incubated at 37 C for 10 min. at a ratio of 10:1:1. Aliquots (200µl) of HAETIL and AETIL were added to 3 ml of the FRAP solution allowed to react for 90 min at 37°C, before reading the absorbance at 593 nm [17].

The data were expressed as µM ferric ions reduced to ferrous form per ml (FRAP value).

FRAP value of sample (µM) = (Change in absorbance of sample from 0 to 4 minute / Change in absorbance of Standard from 0 to 4 minute) X FRAP value of standard (1000 µM).

FRAP value of Ascorbic acid is 2 µM.

NO scavenging assay

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent (*Marcocci et al. 1994*) Sodium nitroprusside (SNP 10mM) in phosphate buffer saline (PBS 10mM p^H7.4) was prepared immediately before the experiment. Sodium nitroprusside (final concentration 5 mM, 1ml) in PBS was mixed with 3 ml of different (50-500) µg/ml of the extract samples, diluted in PBS and incubated at 25 C for 150 min. Samples with 0.5 ml concentration varying from (50- 500 µg/ml) were removed and diluted with 0.5 ml of greiss reagent (1% sulfanilamide, 2% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride). The inhibition of NO generation was estimated by comparing the absorbance values of control with HAETIL and AETIL. The absorbance of the chromophores formed during the diazotization of Nitrite with Sulphanilamide and subsequent coupling with naphthylethylenediamine dichloride was measured at 546 nm. Ascorbic acid was used as standard antioxidant also as positive control treated in the same way with Griess reagent [18-21]

Nitric Oxide scavenged (%) = $\frac{A_{Control} - A_{Test}}{A_{Test}} \times 100$

Where, A control = Absorbance of control reaction and
A test = Absorbance in the presence of the samples of extracts.

Determination of antibacterial activity

Disc diffusion assay

Disc assay diffusion method described by Collins CH *et al.*, 1995 was used for determination of antimicrobial activity of plant extracts as follows. Required apparatus and materials were sterilized in autoclave and placed them in a laminar airflow cabinet under pathogen free condition. The nutrient agar media used for the antimicrobial test was prepared (Hi-Media Laboratories Ltd., Mumbai, India). By streaking with loop, microorganisms were inoculated in nutrient broth and incubated at 35°C for 12 hr. Nutrient agar media was prepared and poured in disc and kept for drying. Swab cotton was dipped in broth having microbial growth and gently squeezed against the inside of the tube to remove excess fluid. Inoculated the dried surface of agar plate by streaking the swab over the entire sterile agar surface. Repeated this procedure two more times and rotated the plate 60 angel each time to ensure an even distribution of inoculums. The disc top has been replaced and allows 3 to 5 minutes but no longer than 15 minutes for any excess surface moisture to be absorbed before applying the test and antibiotics disks. Paper disc of Whatman filter paper no.1 of diameter 5mm. Disc were dipped in concentration of extracts (100mg/ml) and air dried in laminar air flow. Paper Discs were placed appropriately on the surface of the agar plate. Inverted the plate and placed them in an incubator at 35 C within 15 minutes after disc were applied. After 12 hrs of incubation, examined each plate and measured the diameters of complete inhibition zone [22, 23].

Minimum inhibitory concentration

Broth dilution method was used for the determination of MIC according to Collins *et al.*, (1995). The micro-dilution broth susceptibility assay was used for the evaluation of minimal inhibitory concentration (MIC) as recommended by National committee for Clinical Laboratory Standard (NCCLS). The minimum inhibitory concentrations (MIC) of the extracts were estimated for each of the test organisms in triplicates. Nutrient broth was prepared according to the instructions of manufacturer's (Hi-Media Laboratories Ltd., Mumbai, India). 10ml of each of the broth was introduced into 5 screw-cap test tubes and sterilized at 121°C for 15 minutes and then allowed to cool. Two-fold serial dilution of the extract with nutrient broth was carried out to give different concentrations of the extracts (20.0, 18.0, 15.0, 10.0, 8.0, 5.0, 1.0 mg/ml). 0.1ml of the microorganism in each were inoculated into the dilutions and incubated at 37°C for 24 hours. The lowest concentrations of the extracts which show no turbidity represent the MIC [22, 23].

Statistical analysis



All the values are expressed as MEAN ± SD and calculated using Graph pad prism 5.0 statistical software and Windows XP 2007 Excel.

Results

The DPPH is considered to be model of stable lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipophilic auto-oxidation. Antioxidant react with DPPH reducing the number of DPPH radicals equal to that of number of their available hydroxyl groups. Therefore, the absorption at 516 nm was proportional to the amount of residual DPPH radicals [24]. It was visually noticeable as discoloration from purple to yellow. It was based on determination of percentage of scavenging of DPPH radicals. As shown in (Figure I), DPPH decolorization was increased by the HAETIL, AETI and ascorbic acid in a concentration dependent manner. Both extracts are good scavengers of DPPH radicals. Percentage scavenging effect of HAETIL, AETIS and ascorbic acid at a dose levels of 500 µg/ml are 73.7±0.57, 66.0±0.57, 97.0±0.57 respectively. IC₅₀ values are 301.83µg/ml, 346.63 µg/ml, and 56.70µg/ml. Hydro alcoholic extract shown more percentage of scavenging DPPH radical and less IC₅₀ value in comparison with aqueous extract.

Percentage of OH radical scavenging potential of *T. indica* leaves extracts and ascorbic were presented in (Figure II) able to scavenge % of OH radicals scavenging potential of HAETIL and AETIL at a concentration of 500 µg/ml were 70.00±0.57, 68.33±1.2, 91.0±0.57 and IC₅₀ values were 292.04 µg/ml, 346.63 µg/ml, and 50.70 µg/ml respectively. Both extracts were good scavengers of OH radicals. Hydroalcoholic extracts are potent scavenger of DPPH radical and less IC₅₀ value as compared with aqueous extract.

Table 1. IC₅₀ values of different extracts of *Tamarindus indica*

| Assay Methods | HAETIL(µg/ml) | AETIL(µg/ml) | Ascorbic acid |
|---------------|---------------|--------------|---------------|
| DPPH | 301.83 | 346.63 | 56.7 |
| OH | 292.04 | 346.63 | 56.7 |
| NO | 279.9 | 339.35 | 77.31 |

FRAP values of HAETIL, AETIL and ascorbic acid was shown in (Figure III). FRAP value of HAETIL and AETIL at a concentration of 500 µg/ml were 0.76±0.08, 0.33±0.03 and FRAP value of ascorbic acid standard was considered as 2. Results were revealed that at the same concentration FRAP values of HAETIL is more than AETIL. From this it can be concluded that the antioxidant activity shown by *T. indica* leaves extracts depends on concentrations and nature of extracts.

Percentage of NO radical scavenging potential of *T. indica* leaves extracts and ascorbic acid are presented in (Figure IV). Percentage

of NO radical scavenging potential of 500 µg/ml HAETIL, AETIL and ascorbic acid were 73.00±0.57, 66.0±0.57, 97.0±0.57 and IC₅₀ values were 279.90 µg/ml, 339.35 µg/ml, and 77.31 µg/ml respectively. Both the extracts were good scavengers of NO radicals. Hydro alcoholic extract shown more percentage of scavenging NO radical and less IC₅₀ values on comparison with aqueous extract. All the extracts had NO radical scavenging activity in concentration (dose) dependent manner.

Table 2 Antibacterial activity of *T. indica* leaves extract against gram positive bacteria.

| S. No. | Name of microorganisms | zone of inhibition (mm) | | |
|--------|------------------------|-------------------------|------------|------------|
| | | CP | HAETIL | AETIL |
| 1. | <i>S. aureus</i> | 36.0±2.0 | 11.33±1.52 | 17.33±1.52 |
| 2. | <i>B. subtilis</i> | 34.33±2.51 | 13.66±0.57 | 14.0±3.0 |

Values are expressed as Mean ± SD, CP: Ciprofloxacin, HAETIL: Hydroalcoholic extract of *T. indica* Leaf, AETIL Aqueous extract of *T. indica* Leaves.

Table 3 Antibacterial activity of *T. indica* leaves extract against gram negative bacteria.

| S. No. | Name of microorganisms | zone of inhibition (mm) Mean± SD | | |
|--------|------------------------|----------------------------------|------------|----------|
| | | GN | HAETIL | AETIL |
| 1. | <i>E. coli</i> | 30.66±1.15 | 19.33±1.15 | 16. ±1.0 |
| 2. | <i>P. aeruginosa</i> | 37.0±1.73 | 24.66±2.51 | 10.0±2.0 |

Values are expressed as Mean ± SD, GN: Gentamicin, HAETIL: Hydro alcoholic extract of *T. indica* leaf, AETIL Aqueous extract of *T. indica* leaf.

Table 4 Minimum inhibitory concentration (MIC) of different extract of *T. indica* against Gram positive and Gram negative bacteria.

| S. No. | Name of microorganisms | MIC (mg/ml) | |
|--------|------------------------|-------------|-------|
| | | HAETIL | AETIL |
| 1. | <i>S. aureus</i> | 20 | 15 |
| 2. | <i>B. subtilis</i> | 18 | 18 |
| 4. | <i>E. coli</i> | 10 | 15 |
| 5. | <i>P. aeruginosa</i> | 8 | 20 |

Values are expressed as mean



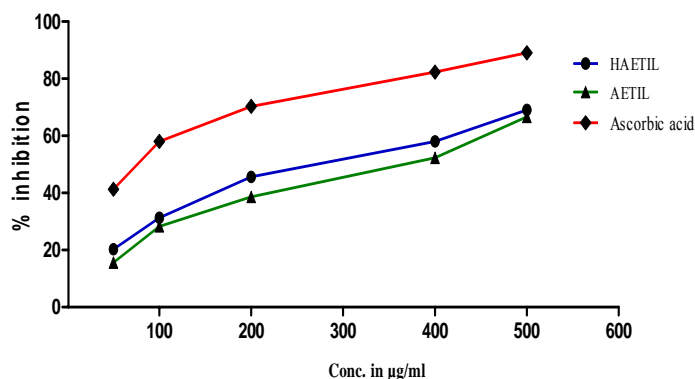


Figure 1 DPPH radical scavenging assay of hydro alcoholic and aqueous extracts of *T. indica* leaves and ascorbic acid (50-500µg/ml). Values represent the mean ± SD (n = 3).

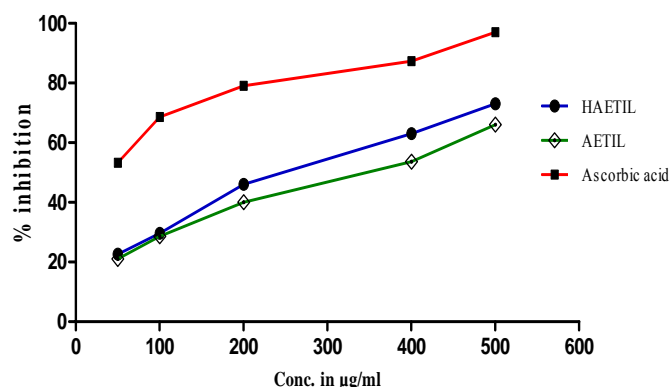


Figure 4 NO radical scavenging assay of hydro alcoholic and aqueous extracts of *T. indica* leaves and ascorbic acid (50-500µg/ml). Values represent the mean ± SD (n = 3).

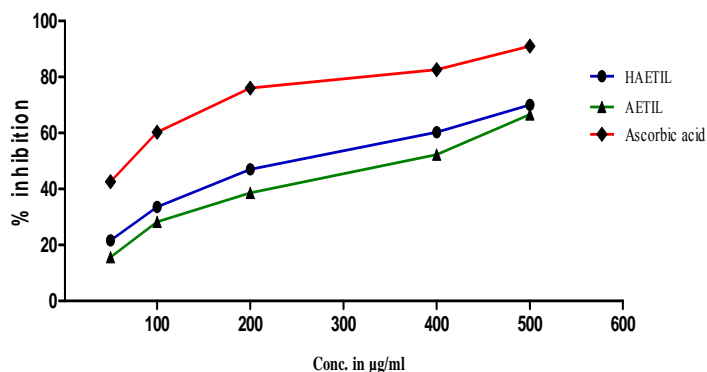


Figure 2 OH radical scavenging assay of hydro alcoholic and aqueous extracts of *T. indica* leaves and ascorbic acid (50-500µg/ml). Values represent the mean ± SD (n = 3)

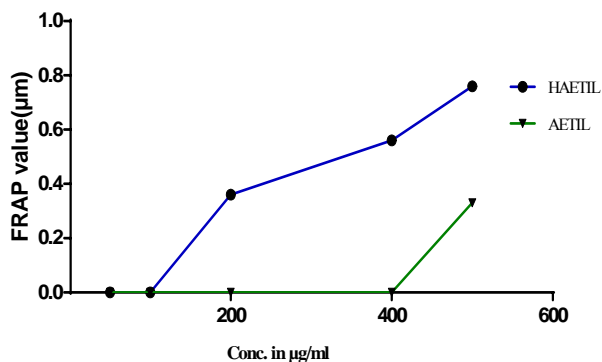


Figure 3 Fe³⁺ reducing antioxidant power assay of *T. indica* leaves extracts and ascorbic acid (50-500µg/ml) FRAP value of standard ascorbic acid (1000 µM) is 2. Values represent the mean ± SD (n = 3).

Antimicrobial assay

The antimicrobial effects of *T. indica* were tested against two species of gram positive and two species of gram negative bacteria results are summarized in (Table 2 & 3) using disc diffusion technique. Filter disc were dipped in to the concentration of extracts 100mg/ml. The maximum inhibitory activity was shown by AETIL against gram positive bacteria *S. aureus* 17.33±1.25 mm. HAETIL shown maximum inhibitory activity against gram negative bacteria *P. aeruginosa* and its inhibition zone diameter was 24.66±2.51 mm in. Ciprofloxacin is used as positive control for gram positive bacteria and its inhibition zone diameter was 36.0±2.0 mm against *S. aureus*. Gentamicin was used as a positive control for gram negative bacteria *P. aeruginosa* and its zone diameter was 37.0±1.73 mm. Results indicates that diameter of inhibition zone depend on nature of extract and bacterial strain tested.

The minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) of the extracts (HAETIL and AETIL) were determined in order to assess their antimicrobial activity. As shown in (Table 4), MIC values were 20 and 15 mg/ml against *S. aureus*; 18 and 18mg/ml against *B. subtilis*; 10 and 15 mg/ml against *E. coli*; 8 and 20 mg/ml against *P. aeruginosa* respectively for HAETIL and AETIL, in comparison to positive control Gentamicin and Ciprofloxacin.

Discussion

Medicinal plants, as the herbal remedies prepared from the whole plant are generally safe if used in the. The property of the plant



extract to scavenge the free radicals has been evaluated using separate assay for each type of reactive oxygen species. The mechanism of antioxidant action *in-vitro* involves direct inhibition of the generation of reactive oxygen species or scavenging the free radicals. Thus it is clear that a single method cannot give a comprehensive prediction of antioxidant so more than one method is recommended [25]. Free radicals which are involved in the lipid peroxidation are considered to play a major role in numerous chronic diseases like cancer and cardio vascular disease [26].

The anti oxidative capacities of the hydro alcoholic extracts of *T. indica* leaves and aqueous extracts of *T. indica* leaves and ascorbic acid were evaluated by the following four complementary assays DPPH, (OH), Fe⁺³ and NO radical scavenging assay. Ascorbic acid was used as a standard antioxidant. Percentage inhibition of the free radicals (DPPH, OH, Fe⁺³ and NO) were summarized in Figure I, II, III, and IV.

The DPPH free radical scavenging activity of plant extracts was due to neutralization of DPPH radical by transfer of hydrogen or an electron due to strong DPPH scavenging property of ascorbic acid it is used as standard antioxidant [27]. Many phenolic compounds have been reported to possess potent antioxidant activity, which vary according to the number and position of hydroxyl groups. Comparisons among the different classes of phenolic compounds showed that tannins were the most potential towards DPPH radical scavenging effect [28]. In the present study it was observed that both the extracts HAETIL and AETIL showed increase in percentage of free radical inhibition in concentrations dependent manner as comparable to standard ascorbic acid. It was also observed the free radical scavenging capacity of HAETIL is more than AETIL comparable to ascorbic acid. IC₅₀ values were also used for evaluation of antioxidant activities; in this study it was observed that IC₅₀ HAETIL is comparable to the standard ascorbic acid.

Hydroxyl groups of phenols are responsible for antioxidant activity. Phenolic compounds are abundantly present in plant derived product and it is responsible for free radical scavenging activity. Phenolic compounds directly contribute the antioxidant actions. Polyphenolic compounds possess the inhibitory potential of carcinogenesis and mutagenesis [29]. The hydroxyl radicals are the most reactive oxygen radical [30] which is formed *via* Fenton's reaction in the living systems [31]. In the oxidative metabolism, the detrimental by product, hydroxyl radical, causes the molecular damage of nerve in the living organism. These radicals have major direct or indirect role in several pathological conditions such as brain ischemia, Parkinson's disease, hepatitis and carcinogenesis. In this assay method, HAETIL scavenged the hydroxyl radical generated from Fe⁺³ ascorbate-EDTA-H₂O₂ system (*Fenton's* reaction). In our study it was observed that percentage of hydroxyl scavenging potential of HAETIL is more at 500 µg/ml than AETIL at same concentration and it was also observed that HAETIL possessing OH radical scavenging potential comparable to

standard ascorbic acid. IC₅₀ values of HAETIL is less comparable to ascorbic acid, so HAETIL is having more capacity to scavenge the OH radicals.

For measurements of the reductive ability, we investigated Fe⁺³ to Fe⁺² transformations in the presence of hydroalcoholic and aqueous extract. The reducing capacity of the extract may serve as a significant indicator of its potential antioxidant activity [32]. In this study ascorbic acid was used as standard antioxidant and its FRAP value is 2 µM. It was observed that at the concentration of 500 µg/ml HAETIL showed FRAP value more than AETIL, so HAETIL is having more ferric reducing capacity.

Nitric oxide (NO) exhibits numerous physiological properties and it is also implicated in several pathological states [33]. However, the specificity of this assay has been questioned since nitrite is one final product of the reaction of nitric oxide with oxygen, through intermediates such as NO₃, N₂O₄ and N₂O₃ [34]. Therefore, the decrease in the nitrite production could also be due to interaction of the extract with other nitrogen oxides. Result from this study it revealed that HAETIL was shown more NO radical scavenging potential than AETIL. IC₅₀ values of HAETIL was comparable to ascorbic acid, so HAETIL possesses more capacity to scavenge the NO radicals.

A major problem in antimicrobial chemotherapy is (now) increasing incidence of resistance to antibiotics, which leads to insufficiency of antimicrobial treatment [35]. Spices and herbs have been safely medicine for ancient times as food and flavoring agents and also herbal medicines are now "generally regarded as safe" Recently there have been considerable emphasis studies involving herbs on inhibiting growth of microbes [36]

T. indica was known to have been used by local people for fever, dysentery and other ailments caused by bacteria. Our results revealed activity against *E. coli*, *P. aeruginosa*, *K. pneumonia* and *P. aeruginosa*. These microorganisms are etiological agents in urinary tract infections (UTI), wounds, and pneumonia and paratyphoid fever [37]. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores [38].

Many plant phenolic have been found to be responsible for several biological properties, including antimicrobial properties [39]. (Dhaniyan SY *et al.*, 2008 and Doughari JH, 2006) also reported that leaf, bark and pulp extract of *T. indica* possess antimicrobial activities [37,9]. Our study also demonstrate that the presence of antimicrobial activity in the leaf extract of *T. indica*. Many extract from medicinal plant have been known to possess antimicrobial effects and used for the purpose of food preservation and medicinal purpose [40].

In this study, the extracts obtained from different solvent showing effective antimicrobial properties against food spoilage and food born pathogen and pathogenic microbes. The result of



antimicrobial assay by disc diffusion method shows that *P. aeruginosa* and *E. coli* were the most sensitive organisms tested, showing the largest zone of inhibition and lowest MIC values. The results in these studies are in conformation with (Doughari JH 2006) [9].

Conclusion

Antioxidant along with the antimicrobial activities important for both food preservation and control of human and animal diseases of microbial origin. Results indicate that *T. indica* leaves possess a good antioxidant activity and also show a broad spectrum of antimicrobial activity. The results of antioxidant activity observed that HAETIL posses more antioxidant activity then AETIL at the same dose level and also showed concentration dependent antioxidant activity. Inhibition of four bacterial stains by hydro alcoholic extract may be attributed to the presence of soluble phenolic and polyphenolic compounds in the extracts.

The antimicrobial activities of *T. indica* leaves extracts were assayed by using disc diffusion method as well as minimum inhibitory concentration (MIC) method. HAETIL and AETIL shown larger inhibition zone diameter (mm) against *Ps. aeruginosa* and *S.*

aureus respectively. Determined MIC values indicated that HAETIL shown lowest against *Ps. aeruginosa* and maximum antimicrobial activity. The results show that *T. indica* leaves extract could be considered as a natural alternative to traditional food preservatives and can be used to enhance food safety and shelf life. *T. indica* can also be used as natural anti-oxidant in metabolic disorder in which level of free radical increased. It posses antimicrobial activity which can be applied as alternative drug for modern system of medicine and need for exploration of its therapeutic value.

Conflict of interest

The authors declare that there is no any conflict of interest.

Acknowledgment

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