WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 1, 672-681. Research Article ISSN 2277– 7105

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ETHANOL BARK EXTRACT FROM TAMARINDUS INDICA LINN

Gidhamari Srinivas* and Dr. Raja Kumar R.

Post Graduate and Research Department of Biotechnology, Marudupandiyar College, Thanjavur-613 403, Tamil Nadu, India.

Article Received on
03 Nov. 2017,

Revised on 24 Nov. 2017,
Accepted on 15 Dec. 2017

DOI: 10.20959/wjpr20181-10439

***Corresponding Author**
Gidhamari Srinivas
Post Graduate and Research Department of Biotechnology, Marudupandiyar College, Thanjavur-613 403, Tamil Nadu, India.

ABSTRACT

This study focused on the biomedical significance of the ethanol bark extract of *Tamarindus indica* L. The dried bark was extracted with ethanol; rotary evaporated and lyophilized which was used for the evaluations of antioxidant and antimicrobial properties. Antioxidant activities of bark extract observed using nitric oxide radical scavenging assay showed a most appreciable value of 87.9%, followed by DPPH photometric assay which showed the next appreciable activity of 78.89%, moreover hydrogen peroxide scavenging assay revealed 43.8% activity and superoxide radical scavenging assay showed the least activity of 21.32% among the tested procedures. Further, the extract also revealed promising antimicrobial activity against three human pathogens viz., *Streptococcus pneumoniae*, *Escherichia coli* and *Salmonella paratyphi*. All these observations showed the ethanol bark extract from *Tamarindus indica* showed significant properties of antioxidant and antimicrobial activities which proved to be the excellent sources of applications in the field of biomedicine.

KEYWORDS: *Tamarindus indica*; Ethanol extraction; Bark; Antimicrobial; Antioxidant.

INTRODUCTION

Traditional medicine, on the contrary of pharmacotherapy, can be easily accessible and ready to use especially in tropical countries, so it has an important role in first line approach. For example, in Burkina Faso, 90% of people prefer to use traditional medicine.[1] Plants are the basic elements of traditional medicine and preferred as a treatment choice in an increasing amount than synthetic medicines.[2] *Tamarindus indica* is evergreen tree that can reach 24 m height and 7 m girth that has pale yellow and pink flowers.[3] *T. indica* commonly known as

tamarind is one of the most important dietary plants in which all parts have some nutritional or medicinal value. *T. indica* is a monotypic genus and belongs to the subfamily Caesalpinioideae of the family Leguminosae (Fabaceae). There are different varieties of *Tamarindus* and they can be divided into acidic and sweet fruit. The sweet and sour at the same time in the fruit is unique and it is used popularly in cooking.[4]

T. indica needs dry climate so the region it is commonly seen extends Africa to Senegal in west, Sudan and Ethiopia in east, Mozambique and Madagascar in south. It is also thought that the plant came to India from Africa. Thailand, Bangladesh, Indonesia in Asia; Mexico, Costa Rica in America are some of the countries in which this plant is mostly encountered.[5] *Tamarindus indica* possesses great potential to address various nutritional, health, socioeconomic and environmental constraints.[6] Every part of *T. indica* plant luke root, body, fruit, leaves etc, not only has rich nutritional value and broad usage area in medicine but also has industrial and economic importance. Tamarind can be the most acidic and sweet fruit according to its growing season.[7] According to World Health Organization report, tamarind fruit is an ideal source of all essential amino acids except tryptophan (82%).[8] It's seeds also have similar properties so it becomes an important, accessible protein source especially in countries where protein malnutrition is a common problem.

Pharmacological investigations on *T. indica* extracts reported from fruit, seeds, leaves and flowers of these plants have been screened for various activities including diabetes and oxidative stress.[9] They were reported for their unique antibacterial, antifungal, hypoglycaemic, cholesterolemic, cytotoxic, anti-inflammatory, gastrointestinal, hypolipomic and antioxidant activities.[10] Despite the fact that lesser reports have been found on the stem barks, also not much literature was available on studies showing the effect on antioxidant and antimicrobial activities, hence the present study concentrated on both the above pharmacological activities.

MATERIALS AND METHODS

Collection and preparation of bark extract from *T.* indica

The bark of *T. indica* was collected from the Thanjavur rural regions, Tamil Nadu, India. Collected samples were washed by distilled water and they were sliced into small pieces. The sliced sample was allowed for excess water drained off and shade dried for seven days. The shade dried bark was finely powdered using grinding machine and the powdered sample were extracted three times repeatedly with 99.9% ethanol and the insoluble contents during the

sample extraction were separated using centrifugation at 3000 rpm for 15 min. The resulting supernatant was rotary vacuum evaporated at 40°C and further dried under lyophilization. The dried sample content was stored under dark dried conditions and further sample preparation procedures were applied with respect to individual analysis carried out below. All the experimental values were represented from the mean of triplicate experimental procedures.

Antioxidant activities

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. In this study, the antioxidant activities have been evaluated using the below mentioned procedures.

Hydrogen peroxide scavenging activity

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4) solution. Different sample volumes were added to a hydrogen peroxide solution (0.6 mL, 40mM).[11] Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging activity of the positive standard activity was determined using 1mM ascorbic acid was calculated

% Scavenged $[H_2O_2] = [(A_C – A_S)/A_C] \times 100$

Where A_C is the absorbance of the control and A_S is the absorbance of the sample.

DPPH photometric assay

A total of 1 ml of a 0.3 mM DPPH ethanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature (30°C). After 30 min. the absorbance (Ab) values were measured at 518 nm and converted into the percentage antioxidant activity using the following equation:[12]

Scavenging capacity% = 100 – [(Ab of sample - Ab of blank) × 100/Ab of control]

Ethanol (1.0 ml) plus extract solution (2.5 ml) was used as a blank, while DPPH solution plus ethanol was used as a negative control. The positive controls were DPPH solution plus each 1 mM Morin (flavonol).

Nitric oxide radical scavenging assay

Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different sample concentrations and incubated at 25°C for 180 min. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The colour tubes contained ethanol extracts at the same concentrations with no sodium nitroprusside. A volume of 150 µL of the reaction mixture was transferred to tubes. The absorbance was measured at 546 nm OD spectrum. The percentage inhibition of the extract and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the ethanol extracts and gallic acid were calculated using the following formula:[13]

% nitrite radical scavenging activity = $[(A_C - A_S)/A_C] \times 100$

Whereas, A_C is the absorbance of control sample and A_S is the absorbance in the presence of the samples from extracts or standards. The standard of positive activity was done using 1mM gallic acid.

Superoxide radical scavenging assay

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT).[14] Briefly, each 3.0 ml reaction mixture contained 0.05 M PBS (pH 7.8), 13 mM methionine, 2 M riboflavin, 100 M EDTA, NBT (75 M) and 1.0 ml of different test sample solutions and 1 mM rutin as the positive control. The tubes were kept in front of a fluorescent light (725 lumens, 34 W) and absorbance was read at 560 nm after 20 min. The entire reaction assembly was enclosed in a box lined with aluminium foil. The inhibition of superoxide anion was estimated by the equation:

Inhibition % = $[(A_O - A_S)/A_O] \times 100$

Where, A_0 is the absorbance of the control, and A_S is the absorbance of the tested sample.

Antimicrobial activities

The antimicrobial activity of the ethanol bark extract was studied on Muller-Hinton agar (MHA) plates as per the earlier procedure followed by Mani et al.[15] A list of human

bacterial pathogens was used; they were *Escherichia coli*, *Salmonella paratyphi*, *Klebsiella pneumoniae*, *Vibrio cholerae*, and *Streptococcus pneumoniae* which were kindly gifted by SIMPPA (SIRO Intitution), Thanjavur, Tamilnadu, India. These strains were cultured on nutrient broth at 37°C and the OD of these broth cultures were adjusted to 0.1 equivalent to an inoculum concentration of 10^8 cfu/mL (according to McFarland turbidity standard). MHA plates were swab cultured with 100µL of individual pathogenic strains and the wells were impregnated with 50µL of purified biosurfactant dissolved in phosphate buffer (pH 7) at different concentrations (1–128 µg/mL) to obtain the minimum inhibitory concentration (MIC) whereas phosphate buffer solution was used as control. After incubation for 24 h at 37°C, the plates were examined for zone diameter of inhibition using an antibiotic zone scale.

RESULTS AND DISCUSSION

Antioxidant activities

Recently, there has been growing interest in research into the role of plant-derived antioxidants in medical and food applications as well as other fields also need antioxidants, such as in polymerization control in the manufacture of rubber, plastics and paint and for the protection of clear plastics against ultraviolet light, or in the design of better automobile fuels and lubricating oils. However, medical applications have given most priority than others.[16] In this study, a moderate antioxidant as well as a concentration dependent hydrogen peroxide scavenging activity was observed with the ethanol bark extract of *T. indica* which was highest at the maximum sample concentration of 1000µg/ml with 43.80% and showed least value at the lowest tested concentration (100µg/ml) with 4.39%. Further, negative control using distilled water showed 0% activity and positive control with ascorbic acid revealed 98.90% activity (Fig. 1).

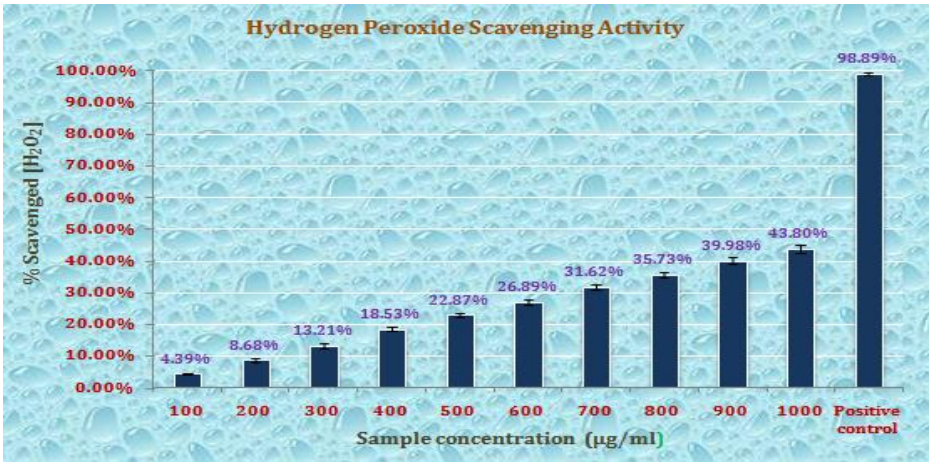

Fig.1: Hydrogen peroxide scavenging assay of ethanol bark extract from *T. indica.*

An appreciable DPPH scavenging activity was observed in ethanol bark extract of *T. indica* compared to the results of hydrogen peroxide scavenging activity. This activity is also dependent on sample concentration and a peak value of 78.89% was recorded with 1000µg/ml and lowest value of 10.98 was observed in 100µg/ml (Fig. 2). Moreover, the sensitivity of the test was predicted with distilled water as negative control with 0% activity and morin as the positive control with 99% activity.

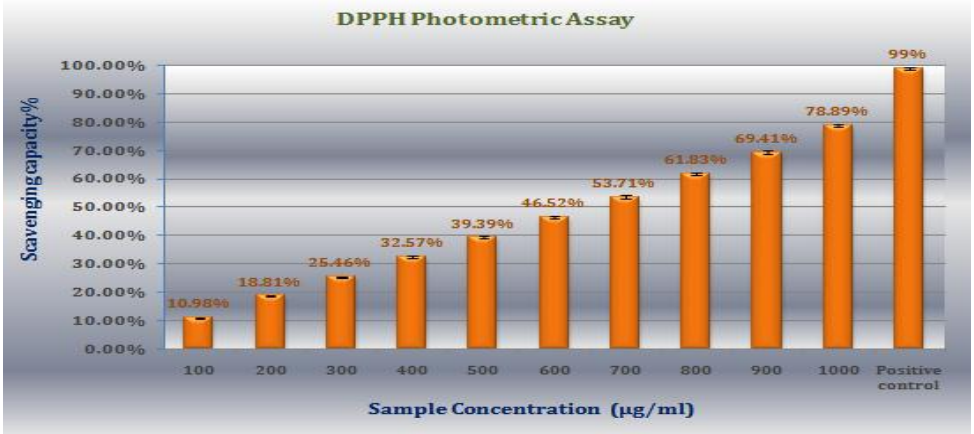

Fig. 2: The DPPH photometric assay of ethanol bark extract from *T. indica.*

The most appreciable antioxidant activity was predicted using the nitric oxide radical scavenging activity which was also depicted with concentration dependent activities. The highest nitric oxide scavenging activity of 87.90% was revealed in the ethanol bark extract at the highest tested concentration (1000µg/ml) and lowest activity of 11.82% was recorded with 100µg/ml. Further, the negative control (distilled water) showed no activity and positive control, gallic acid showed 98.91% scavenging activity.

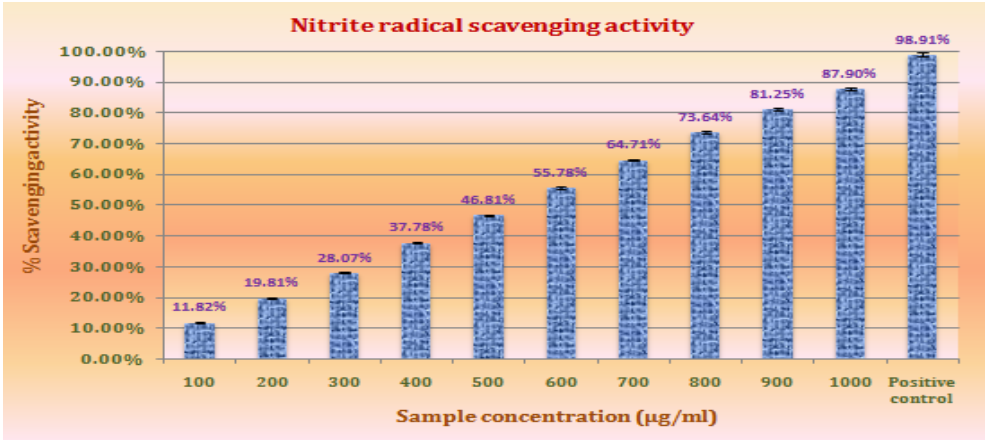

Fig. 3: Nitric oxide radical scavenging activity of ethanol bark extract at different sample concentrations from *T. indica.*

Likely to the above predictions, the superoxide radical scavenging test also showed a concentration dependent activity but revealed a least antioxidant results than the other procedures. The ethanol bark extract of *T. indica* showed highest superoxide radical scavenging activity of 21.32% with 1000 µg/ml sample concentration and lowest activity of 1.89% which was observed with 100 µg/ml. Moreover, the negative control (distilled water), showed no activity and positive control, rutin observed 98.79 % activity. Similar to the present study, an earlier study investigation predicted appreciable antioxidant activities from ethanol extract of leaves from *T. indica* using Hydrogen peroxide scavenging and DPPH photometric assays.[17]

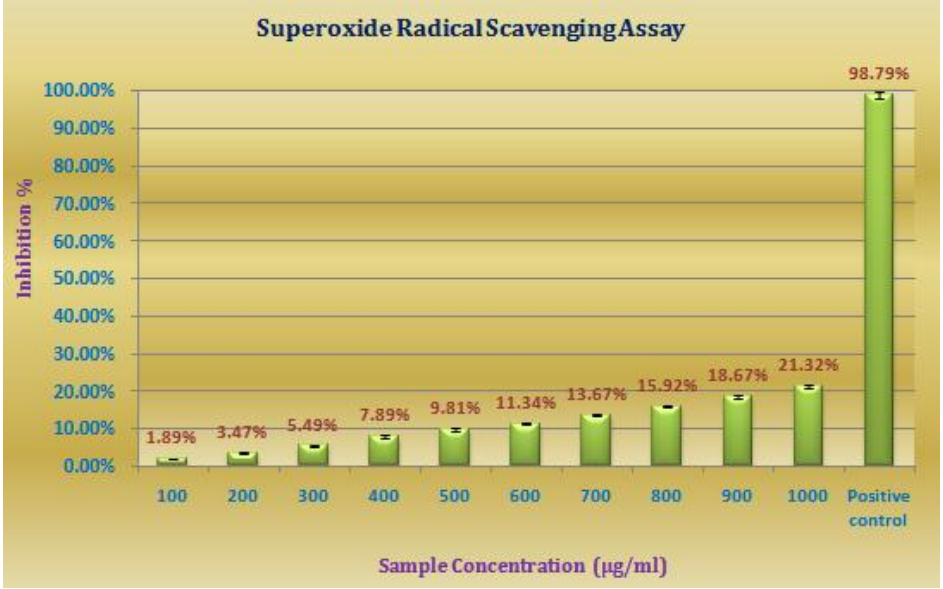

Fig. 4: The superoxide radical scavenging activity of ethanol barks extract at different sample concentrations from *T. indica.*

Antimicrobial activities

Among the five tested bacterial human pathogens, the ethanol bark extract of *T. indica* showed antimicrobial activity against three strains. The maximum zone of inhibition and the respective minimum inhibitory concentration (MIC) values recorded were *Streptococcus pneumoniae* (13mm, 98µg/mL) followed by *Escherichia coli* (11mm, 112µg/mL) and *Salmonella paratyphi* (11mm, 124µg/mL). Moreover, the bark extract showed no antimicrobial activity against *Klebsiella pneumoniae and Vibrio cholerae*. Similar to this investigation, an earlier study recorded methanol extracts of Tamarind fruit showed appreciable antimicrobial activity against many food borne pathogens.[18]

Fig. 1: Antimicrobial activity of ethanol barks extract from *T. indica* against the human pathogen, *Streptococcus pneumonia.*

CONCLUSION

In the present study, the ethanol bark extract from *T. indica* showed significant properties of antioxidant and antimicrobial activities. The appreciable antibacterial activities of this extract against the clinical human pathogens evidenced its importance in the field of therapeutics. Moreover, this extract exhibited dual functions such as antioxidant and antimicrobial properties which proved its promising role in many biomedical applications.

ACKNOWLEDGEMENT: None.

Conflict of Interest: The authors declare that they have no conflict of interest.

REFERENCES

1. Havinga RM, Hartl A, Putscher J, Prehsler S, Buchmann C, Vogl CR. *Tamarindus indica* L. (Fabaceae): Patterns of use in traditional African medicine. J Ethnopharmacol, 2010; 127: 573-588.
2. Bhadoriya SS, Ganeshpurkar A, Narwaria J, Rai G, Jain AP. *Tamarindus indica*: extent of explored potential. Pharmacogn Rev, 2011; 5: 73-81.
3. De Caluw E, Halamov K, Van Damme P. *Tamarindus indica* L.: A review of traditional uses, phytochemistry and pharmacology. Afrika Focus, 2010; 23: 53-83.
4. Yusuf AA, Mofia BM, Ahmed AB. Proximate and mineral composition of *Tamarindus indica* Linn seeds. Sci World J, 2007; 2: 1-4.

5. Barminas JT, Charles M, Emmanuel D. Mineral composition of non-conventional leafy vegetables. Plant Foods Human Nutri, 1998; 53: 29–36.
6. Arinathan VVR, Mohan D, De Britto AJ. Chemical composition of certain tribal pulses in South India. Inter J Food Sci Nutri, 2003; 54: 209-217.
7. Bhadoriya SS, Mishra V, Raut S, Ganeshpurkar A, Jain SK. Anti-inflammatory and antinociceptive activities of a hydroethanolic extract of *Tamarindus indica* leaves. Sci Pharm, 2012; 80: 685-700.
8. Al-Fatimi M, Wurster M, Schroder G, Lindequist U. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J Ethnopharmacol, 2007; 111: 657-666.
9. Ghoneim AI, Eldahshan OA. Anti-apoptotic effects of tamarind leaves against ethanol-induced rat liver injury. J Pharm Pharmacol, 2012; 64: 430-438.
10. Kalra P, Sharma S, Suman, Kumar S. Antiulcer effect of the methanolic extract of *Tamarindus indica* seeds in different experimental models. J Pharm Bioallied Sci, 2011; 3: 236-241.
11. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 1989; 10: 1003-1008.
12. Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, Leitao SG. Screening of brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, Phytother Res, 2001; 15: 127-130.
13. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. Biochem Biophys Res Commun, 1994; 15: 748– 755.
14. Awah FM, Uzoegwu PN, Oyugi JO, Rutherford J, Ifeonu P, Yao X, Fowke KR, Eze MO. Free radical scavenging activity and immunomodulatory effect of *Stachytarpheta angustifolia* leaf extract. Food Chem, 2010; 119: 1409–1416.
15. Mani P, Dineshkumar G, Jayaseelan T, Deepalakshmi K, Kumar CG, Balan SS. Antimicrobial activities of a promising glycolipid biosurfactant from a novel marine *Staphylococcus saprophyticus* SBPS 15.3 Biotech, 2016; 6: 163.
16. Das SS, Dey M, Ghosh AK. Determination of anthelmintic activity of the leaf and bark extract of *Tamarindus indica* Linn. Indian J Pharm Sci, 2011; 73: 104-107.

17. Imam S, Azhar I, Hasan MM, Ali MS, Ahmed SW. Two triterpenes lupanone and lupeol isolated and identified from *Tamarindus indica* Linn. Pak J Pharm Sci, 2007; 20: 125-127.
18. Escalona-Arranz JC, Péres-Roses R, Urdaneta-Laffita I, Camacho-Pozo MI, Rodriguez-Amado J, Licea-Jimenez I. Antimicrobial activity of extracts from *Tamarindus indica* L. leaves. Pharmacogn Mag, 2010; 6: 242-247.