THE IN VITRO ANTI-OXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF FLAVONOID LUTEOLIN AND TAMARINDUS INDICA POD EXTRACT AND ITS METHANOL FRACTION


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ABSTRACT

Antioxidant potential of Luteolin (L), Crude (hydroalcoholic - HAE) extract and methanol fraction (MF) of Tamarindus indica pod was measured using different in vitro assays. Ascorbic acid was used as the standard. DPPH radical scavenging activity and reducing power of the extracts was observed to be greater in Luteolin > Ascorbic acid > MF > HAE. The total phenolic content of MF was found to be 17.23±1.26 µg of luteolin/mg of extract and that of HAE was 10.40 ±0.71 µg of luteolin/mg of extract respectively.

KEYWORDS: Tamarindus indica L, Luteolin, antioxidant, hydroalcoholic extract, methanol fraction.

1. INTRODUCTION

Living cells may generate free radicals and other reactive oxygen species as a result of physiological and biochemical processes. These free radicals can cause oxidative damage to the biomolecules such as lipids, proteins and DNA which eventually leads to many chronic diseases such as cancer, diabetes, aging and other degenerative diseases in humans[1]. Plants possess rich phytoconstituents such as phenolic acids, terpenoids, vitamins, flavonoids and many other metabolites which are capable of scavenging the free radicals due to their rich
antioxidant activities.\textsuperscript{[2,3]} Studies have shown that plant extracts exhibited varieties of pharmacological activities like anti-inflammatory, anti-bacterial, anti-tumor, anti-carcinogenic, anti-mutagenic, and antiviral activities.\textsuperscript{[4,5]} Living cells possess a protective system of antioxidants which prevents excessive formation and enables the inactivation of reactive oxygen species. Antioxidants protect from the potentially damaging oxidative stress, which is the result of an imbalance between the formation of ROS and the body antioxidant defense. Antioxidants have also been used in the food industry to prevent deterioration, nutritional losses and off-flavoring in various foods, especially those containing polyunsaturated fatty acids. Recently, interest has increased considerably in finding natural antioxidants for use in foods because of their potential, in health promotion and disease prevention, and their high safety and consumer acceptability.\textsuperscript{[6]} Therefore study of plants, as a resource medicine has become more important in the context of present global trade scenario where oxidative stress is found to be one of the major causes of health hazards. Traditional system of medicine such as Ayurveda, Unani and Siddha have identified 1500 medicinal plants of which 500 species have been used mostly as healing agents for various disorders. \textit{T. indica} is an important medicinal plant native to tropical Africa, and was so long ago introduced into and adopted in India that it has often been reported as indigenous there also. \textit{T. indica} is used in Indian medicine since ancient times. \textit{Tamarindus indica} belongs to the family Leguminosae. It is a slow growing, long lived massive fruit tree of the tropics. It is used as antiscorbutic, applied to heal inflammations and sore throat, mixed with salt to treat rheumatism, administered to alleviate sunstroke, daisine poisoning and alcoholic intoxication in Southeast Asia.\textsuperscript{[7]} It is used to treat hypertension, diabetes, worm infection, jaundice, asthma, biliousness, dysentery, vaginal and uterine complaints, burning sensation, and cardioprotective activity.\textsuperscript{[8]} Seed and peri-carp of Tamarind contain polyphenolic compounds. Soxhlet extraction with methanol, the total yield of polyphenolic compounds in seeds was 6.54g/Kg and in pericarp it was found to be 2.82g/kg. The Polyphenolics in the pericarp were dominated by proantho-cyanidins (73.4\%) in the form of catechin (2\%), procyanidin B2 (8.2\%), epicatechin (9.4\%), procyanidin trimmers till procyanidin hexamers and the flavonoids-taxifolin, apigenin, eriody-ctyol, luteolin, and naringenin.\textsuperscript{[9]} Luteolin is widely distributed in the plant kingdom and is known to possess good antioxidant activity. Hence we have used it for comparison with the plant extracts. Aim of this study is to determine the total phenolic content and to evaluate the antioxidant activities of Luteolin, HAE and MF of \textit{T. indica} pod using different \textit{in-vitro} assays.
2. MATERIALS

2.1 T. indica pods were collected from Horticulture Dept garden, Shimoga.

2.2 Flavonoid luteolin - purchased from sigma Aldrich.

2.3 Chemicals: 1,1-Diphenyl, 2-picryl hydrazine (DPPH), Potassium ferricyanide-K[Fe(CN)₆], Ferric chloride (FeCl₃), Sodium dihydrogen phosphate (NaH₂PO₄), Disodium hydrogen phosphate (Na₂HPO₄), Trichloroacetic acid (TCA), Folin-ciocalteau reagent, Sodium carbonate (Na₂CO₃), methanol, ascorbic acid. All chemicals used were of analytical grade – Viz. MERCK, Ranbaxy, SD-fine chemicals.

3. METHODOLOGY

3.1 Collection and authentication of plant species

T. indica pods were collected in the month of February 2012 from Horticulture Dept garden, Shimoga, Karnataka. Later the plant material was identified and authenticated by an expert Taxonomist Dr Sridhar at Jnanabharathi, Bangalore University, Bangalore, India (Acce no- Ti/ SAN 001) and a voucher specimen was also deposited there for future reference.

3.2 Extraction & Fractionation

Extraction is carried out according to Nandini et al.[17] and fractionation as per the standard procedures reported by Yamin et al.[10]

3.4 DPPH assay

DPPH activity was measured using DPPH free radical test, by employing the method of Wong et al. [11] Different concentrations of each of the extracts were prepared in methanol and were added to 3ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30min at room temperature in dark. Changes in absorbance of samples were measured at 517nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid was used as the standard. Using the standard curve, the DPPH scavenging activity of the extracts was calculated and is expressed as vit C equivalence.

3.5 Reducing Power Assay

The reducing power of the extracts was evaluated according to Oyaizu.[12] Different amounts of extracts were mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6), and 2.5ml of 1% K₃Fe(CN)₆. This mixture was incubated at 50°C for 20 min. After incubation, 2.5ml of 10% TCA was added to terminate the reaction and was centrifuged at 3000 rpm for 10 min. The upper
layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and 0.5ml FeCl₃ solution (0.1%) was added to it and the absorbance was measured at 700nm. Increase in absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as the standard. Using the standard curve, the reducing power of the extracts were calculated and expressed as vit C equivalence.

3.6 Determination of Total Phenolic Content

The total phenolic content was estimated according to the method of Makkar et al.[13] The aliquots of the extract was taken in a test tube and made up to the volume of 1ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) was added. After mixing, the solution was incubated at 90°C for one minute and the absorbance was recorded at 725nm against the reagent blank. Using luteolin, a standard curve was prepared, and the total phenolic content was calculated which was expressed as luteolin equivalent in µg/mg of extract.

3.7 Statistical analysis: All the assays were carried out in triplicates. The results were expressed as mean±standard deviation.

4. RESULTS

4.1 DPPH assay The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of L, HAE and MF are given in Fig-1B. A standard graph was plotted with ascorbic acid (Fig-1A). DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, it gets decolorized, which can be quantitatively measured from the changes in absorbance. DPPH scavenging activity of 1 µg of Vit C is equivalent to 0.97 µg of Luteolin, 40.9 µg of HAE and 24.77 µg of MF. DPPH scavenging activity of both Vit C and luteolin are almost equal. Among the plant extracts MF is found to have significant scavenging activity when compared to HAE.
Table. 1: Anti-oxidant activities of L, HAE and MF by DPPH and reducing power assay. Values are expressed as Mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Luteolin</th>
<th>HAE</th>
<th>MF</th>
</tr>
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<tbody>
<tr>
<td>DPPH</td>
<td>0.97±0.91µg vit C equiv</td>
<td>40.9±2.1µg vit C equiv</td>
<td>24.77±1.92µg vit C equiv</td>
</tr>
<tr>
<td>Reducing</td>
<td>0.32±0.51µg vit C equiv</td>
<td>30.8±2.8µg vit C equiv</td>
<td>9.83±1.4µg vit C equiv</td>
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Fig. 1: DPPH radical scavenging activity of standard ascorbic acid (1A), Comparison of anti-oxidant activity of L, HAE and MF by DPPH assay (1B).

4.2 Ferric ion reducing power assay (FRAP)

In ferric ion reducing power (FRAP) assay, the reductive ability was investigated from the transformation of Fe³⁺ to Fe²⁺ in the presence of samples (extracts). Here Vit C is used to plot the standard graph (Fig-2A). Our results revealed that the reducing power exhibited by 1 µg of Vit C is equivalent to 0.32 µg of Luteolin, 30.8µg of HAE and 9.84 µg of MF. The reducing power of the extracts, compound and standard ascorbic acid were exhibited in the following order: Luteolin >Ascorbic acid > MF > HAE (Fig-2B). The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain or by donating a hydrogen atom. Here luteolin is 3 times more potent than vit C. Among the plant extracts MF is found to have good reducing power than HAE.
4.3 Determination of Total Phenolic Content

The total phenolic content of the extract and fraction was determined using the calibration curve. Absorbance values of Luteolin was measured at 700nm and the standard curve was drawn (Fig-3A). The total phenolic compounds in the extracts were calculated using the calibration curve and the results were expressed as the number of equivalents of Luteolin (µg/mg of extract). From the results it is found that total phenolic content of HAE is 10.40 ± 0.71 µg of luteolin/mg of extract and that of MF is 17.23±1.26 µg of luteolin/mg of extract Respectively. Results showed that MF contains greater phenolic content when compared to HAE (3B).

Fig. 3: Standard luteolin curve for total phenolic content (3A). Comparison of total phenolic content of HAE and MF (3B).
5. DISCUSSION

In traditional societies nutrition and healthcare are strongly interconnected and many plants have been consumed both as food and for medicinal purposes. In the recent years, there has been a growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations. ROS produced in vivo includes superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hypochlorous acid (HOCl). H$_2$O$_2$ and O$_2$ can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical.$^{[14]}$ Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals.$^{[15,16]}$ Literature review confirms the presence of alkaloids, phenols, flavonoids, phytosterols, tannins, saponins, carbohydrates and diterpenes.$^{[17]}$ Presence of these phytoconstituents in the plant extracts may be responsible for their antioxidant properties. Our studies have shown that Luteolin and *T.indica* are known to possess strong antioxidant activity which was evaluated by DPPH & reducing power assay which supports the earlier findings reported by Oraunch et al.$^{[18]}$ According to Shridhar et al total phenolic content of Tamarind pulp powder was 59.45 - 131.33 mg of GAE / 100g.$^{[19]}$ But our study has shown much higher phenolic content in HAE and MF of *T. indica* i.e. 10.40 µg of luteolin/mg of extract and 17.23 µg of luteolin/mg of extract respectively. Indeed, phenolic compounds found in vegetables, fruits or medicinal plants are known for their antioxidant potential and their role in prevention of human diseases. Number of papers highlighted a positive correlation between the antioxidant activity and the total phenolic content.$^{[20]}$ From the above results and discussion it can be concluded that the hydroalcoholic extract and methanolic fraction of *T. indica* possesses the potent antioxidant substances which may be responsible for its anti-inflammatory mechanism as well as justify the basis of using this plant’s extract as folkloric remedies.

6. CONCLUSION

On the basis of our present study, it is concluded that hydroalcoholic extract and methanol fraction of *T. indica* contains a large amount of flavonoids and polyphenolic compounds which may be the reason for its high antioxidant and free radical scavenging activities. It has shown a good reducing power too. These in vitro assays indicate that this plant is a significant source of natural antioxidant, which might be responsible to ameliorate the damage caused by oxidative stress and thus justified its diverse use in treating various ailments in traditional medicine.
7. SCOPE FOR FURTHER STUDY
The components responsible for the antioxidative activity are currently unclear, hence further investigation is needed to isolate and identify the antioxidative compounds present in the plant extract. Furthermore, the in vivo antioxidant activity of this extract needs to be assessed prior to clinical use.

8. REFERENCES


