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Research Article

Phytochemical Profiling & Antioxidant Activity of *Atuna indica* (Bedd.) Kosterm - An Unexplored Tree Species Reported from Western Ghats, India

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ABSTRACT

The present study is the first report of phytochemical screening of this wild plant, *Atuna indica* (Bedd.) Kosterm from Western Ghats, India. The percentage of total phenols (87.6 ± 5.2 mg 100 g^{-1} gallic acid equiv.) and total flavonoids (67.1 ± 8.1 mg 100 g^{-1} quercetin equiv.), which contribute significantly to the free radical scavenging activity, was high. In addition, Umbelliferine, a powerful antioxidant agent, has been identified and quantified for the first time in this species.

Key words: antioxidant activity, primary metabolite profiling, umbelliferone

INTRODUCTION

The species, *Atuna indica* was collected from northern Kerala, India. This was the rediscovery of this particular species after the type collection about 150 years later. (Sasidharan & Sujanapal 2011). The tree is about 20 m tall with smooth, thin, brown bark. The leaves are elliptic-oblong or elliptic ovate, with 17-21 cm long and 5.5-7.5 cm wide with white flowers. The tree is found in the West coast tropical evergreen forest of Southern Western Ghats region, India (Sasidharan & Sujanapal 2011).

Recent years, there is an increasing interest in finding out antioxidant phytochemicals from plants because they can inhibit the propagation of free radical reactions and protect human body from disease. In this context, *Atuna indica*, requires a special and detailed attention with respect to its primary metabolites and antioxidant potential. The antioxidant activity could be due to total phenolic content. An active coumarin Umbelliferone was found in the bark of this tree species and it was identified for the first time by HPTLC, followed by spectral data comparison with Umbelliferone standard.

Umbelliferone is an active coumarin with a countable number of reported pharmacological activites. It is also a reported antioxidant compound. The antioxidant activity of Umbelliferone in *Eagle marmelos* was reported earlier (Dhalwal *et al.* 2008). Umbelliferone is known to prevent the complications of Type 2 diabetes (Okada *et al.* 1995) and is also known to be used in Cancer Prevention Therapy (Gawron & Glowniak 1987). Treatment with Umbelliferone (60 and 90 mg/kg) caused a marked reduction of cellularity and eosinophil count in asthmatic mice (Juliana *et al.* 2009)**.** Umbelliferone possesses a promising antihyperglycemic effect that is comparable with glibenclamide (Ramesh & Pugalendi 2007). In this context, the high percentage of Umbelliferone in this plant species requires special attention.

MATERIALS AND METHODS

Primary metabolite profiling of plant species: The plant materials (leaf and bark) were identified and collected by the Taxonomy division of Centre for Medicinal Plants Research, Arya Vaidya Sala Kottakkal. The quantification of primary metabolites such as total carbohydrate, starch, reducing sugars, total phenols and total flavonoids was done by using UV-160A Spectrophotometer with wavelength varying from 490-650 nm. The estimation of Total carbohydrate was done by following phenol- sulphuric acid method, while the percentage of starch was calculated by Anthrone method. (Sadasivam & Manickam 1992). The Estimation of reducing sugars was done by Nelson Somgoyi method (Sadasivam & manickam 1992). The above quantifications were done by preparing their corresponding standard solution for optical density measurement. The total phenols (Singelton *et al.* 1999) and total Flavanoids (Marinova *et al.* 2005) present in the leaf and bark tissues were quantified and expressed as Gallic acid and Quercetin equivalents. Five replications of each analysis were carried out for the quantification and their mean along with standard deviations were calculated and showed in the Table1&2.

Antioxidant activity profiling of the plant : Antioxidant activity of different solvent extracts (Ethyl acetate, Methanol and Water) of bark tissue was measured by DPPH scavenging assay (517 nm) and followed by calculating their IC_{50} values. The most commonly used antioxidant method is DPPH free radical method and it does not require any special preparation. One thousand microlitres of various concentrations of the extracts were

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Fig-1 Spectral comparison of Umbelliferone Standard and Sample

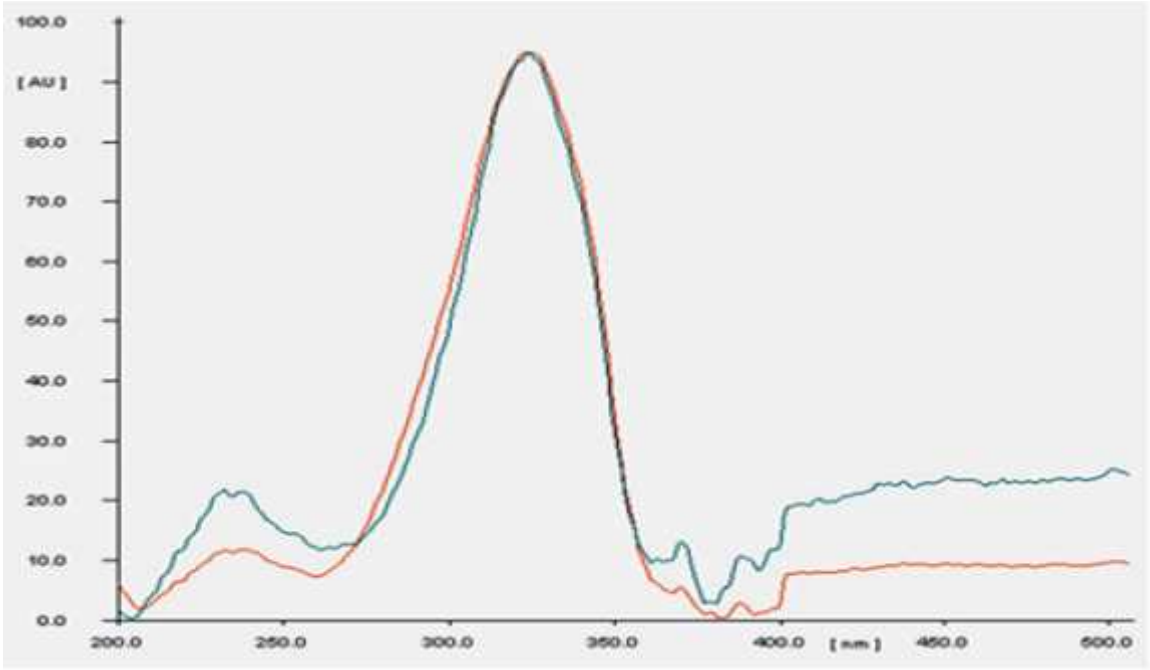

Fig-2 TLC comparison of Plant extract with Standard Umbelliferone

added to 4 ml of 0.004% methanol solution of DPPH. After 60 minute incubation period at room temperature, the absorbance was read against a blank at 517 nm. The inhibition of free radical by DPPH was calculated by the following way.

Inhibition (I) % = (A(b) – (A(s) / A(b))) x 100,

Where A(b)- Absorbance of blank, A(s)- Absorbance of Sample and I is the inhibition percentage. Statistical analysis (mean & standard deviation) were also done.

Identification and quantification of Umbelliferone by HPTLC: A HPTLC procedure was optimized with a view to identify and quantify the presence of Umbelliferone in plant extract. For TLC analysis of plant material (5g) was refluxed with methanol (50 X 3) ml on soxhlet apparatus for complete extraction. Excess solvent was evaporated under reduced pressure and made up to 10 ml. Initially toluene: ethyl acetate: formic acid in varying ratios was tried. Well-defined spots were obtained when the chamber was saturated with mobile phase for 20 min at room temperature. TLC of the methanolic extract and HPTLC assay of Umbelliferone was done by using stationary phase: Pre-coated silica gel plates Merck 60 F_{254} (0.2 mm thickness) Mobile phase: Toluene: Ethyl acetate: Formic acid (8: 2: 0.2, v/v/v), application mode: CAMAG ATS IV and developed in ADC II. The plate after development was scanned under wavelength 325 nm by using scanner: CAMAG TLC Scanner 3.

A photograph of a TLC plate after chromatography of Umbelliferone standard and a methanol extract of the dried bark of *A. indica* are shown (Fig. 2). The identity of the Umbelliferone bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution (Figure 1 & 2) and by comparing retention factors of Umbelliferone from sample and standard solutions. It was then confirmed and quantified based on spectral matching and linearity of Umbelliferone standard from sigma chemicals. (Fig. 2 & Fig. 3).

Fig-3 Linearity curve obtained for quantification of Umbelliferone

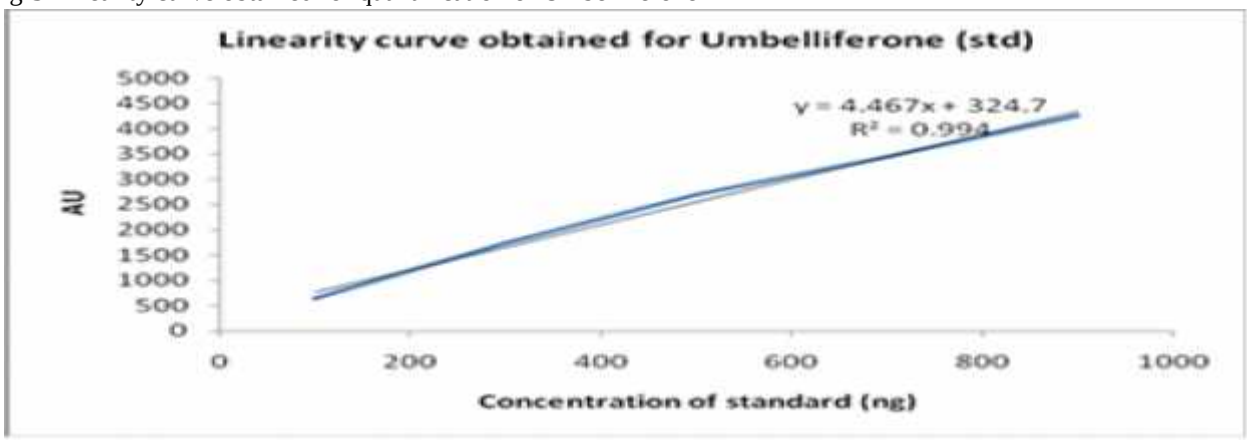

Table 1-Primary metabolite profiling of the leaf and bark tissue of *Atuna indica*

Parameters	Leaf (dry) [a]	Bark (dry) [a]
Total Carbohydrate	8.23 ± 0.2	14.32 ± 0.15
Starch	6.31 ± 0.22	10.02 ± 0.2
Reducing Sugar	2.21 ± 0.2	4.26 ± 0.25

[a] *Mean Percentage value of phytochemicals in w/w are shown, SD was always less than 20%*

Table 2 -Quantification of Total phenols and Total Flavonoids and Antioxidant activity (IC 50) of bark extracts.

Parameters	Bark (dry)[a]
Total Phenolics (Gallic acid Eqv.)	87.6 ± 5.2
Total Flavanoids (Quercetin Eqv.)	67.1 ± 8.1
Antioxidant activity[b] (DPPH assay) of *Atuna indica* bark extracts (517 nm)	
IC_{50} -Methanol extract	5 ± 0.5
IC_{50} - Ethyl acetate extract	15 ± 0.75
IC_{50} - water extract	40 ± 0.5

[a] *mg 100g^{-1} are shown, SD was less than 20%,* [b] *µg ml^{-1} half maximal inhibitory concentration*

RESULTS AND DISCUSSION

Primary metabolite profiling of dry leaf and bark tissues of *Atuna indica:* The primary metabolite profiling of the dry leaf & bark tissues of this plant (Table 1) was screened and the percentage values of total carbohydrate was found high in bark tissue (14.32 ± 0.15%) followed by leaf tissue (8.2 3± 0.2%). The percentages of starch (10.02 ± 0.2%) and reducing sugar (4.26 ± 0.25%) were also found high in dry bark tissues of this plant. A study conducted by Scherling *et al.* 2010) demonstrates that metabolite profiling is a strong diagnostic tool to assess individual metabolic phenotypes in response to inter and intra species variability and ecophysiological adjustment.

In order to screen the medicinal potential of this particular species, the bark tissue was subjected to soxhlet extraction using different organic solvents (methanol, ethyl acetate and water) and these extracts were used for the quantification of total phenolics and total flavonoids

Quantification of Total phenols and Total Flavanoids and Antioxidant Assay: Methanolic extracts of *Atuna indica* were analyzed for its Phytoconstituents. The quantitative estimation of the phytochemical constituents of this species showed that this particular species is rich in total phenols and total flavonoids (Table2). It is well known that the plant phenols and flavonoids in general, are highly effective free radical scavenging and antioxidant agents. The higher percentages of total phenols and total flavonoids (87.6±5.2 mg GAE/100g and 67.1±8.1 mg QUR./100g) as shown in Table 2 with gallic acid and Quercetin as standards, are further subjected to evaluate their antioxidant potential.

Antioxidant activity of the solvent extracts of the plant: DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of these extracts. Free radical scavenging capacities of these extracts, measured by DPPH scavenging assay (Table2).

As mentioned above the IC_{50} is a parameter representing the herb concentration, able to inhibit 50% of the used DPPH amount. In the present study, different extracts (methanol, ethyl acetate and water) of this particular species showed significant antioxidant activity and the scavenging capacity was found high (5 ± 0.5 µg/ml) in methanol extracts. Because of its high antioxidant potential, it was further subjected for compound wise screening and detected the presence of Umbelliferone in its bark extract. The presence of Umbelliferone was then confirmed by spectral matching at 325 nm. The percentage of Umbelliferone in the extract was then quantified by checking the linearity of Umbelliferone

with different concentrations and it was found as 0.055 + 0.2% w/w.

Identification and quantification of Umbelliferone in the plant: The TLC procedure was optimized with a view to quantify Umbelliferone in plant extract. Initially toluene: ethyl acetate: formic acid in varying ratios was tried. Finally, the mobile phase consisting of Toluene: Ethyl acetate: Formic acid (8: 2: 0.2, v/v/v) gave a sharp and well-defined peak at R_f -0.40 (**Fig. 2**). The TLC plate was visualized under UV light at 366 nm. The identity of the Umbelliferone bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution and by comparing retention factors and spectra of Umbelliferone from sample and standard solutions. The peak corresponding to Umbelliferone from the sample solution had same retention factor as that from the Umbelliferone standard (Rf 0.40) and both spectrum were matching (**Fig- 1**). Densitometric scanning was performed at 325 nm for quantifying Umbelliferone. Calibration curve obtained Curve was found to be linear in the range of 100 ng to 900 ng. Equation for the calibration curve is Y = 324.773 + 4.467 X (**Fig. 3**). The correlation coefficient was found to be 0.9969. The amount of Umbelliferone calculated in sample was 0.055 + 0.2% w/w.

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