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In vitro anti-diabetic and anti-bacterial activity of unexplored species of Atuna

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ABSTRACT

The present study highlights the Antidiabetic and Antibacterial activity of the various parts of wild plants *Atuna indica* (Bedd.) Kosterm and *Atuna travancorica* (Bedd.) Kosterm belonging to the family Chrysobalanaceae from Western Ghats, India. The leaves and flowers of *Atuna indica* and the leaves of *Atuna travancorica* were collected, shade dried, powdered and extracted in 50% aqueous ethanol by maceration process and concentrated to dryness with the use of rotary vacuum evaporator. The ethanol extracts of the test plant parts of the two species were subjected to the *Invitro* Antidiabetic and Antibacterial activity with the help of α-amylase inhibition and microtiter plate assay methods respectively. The leaves of the two species possess Antidiabetic and Antibacterial activity; in particular leaves of *A.indica* and *A.travancorica* shows prominent Antidiabetic activity when compared with the other parts of the two species examined. In the case of Antibacterial activity the leaves of *A.travancorica* possess significant activity against *Pseudomonas aeruginosa* and *Bacillus subtilis.* The results suggested that the flowers of *A.indica* does not have the said activity. Based on the results obtained from the preliminary screening, it can be concluded that the leaves of *A.travancorica* has the maximum activity when compared with the other parts of the two species.

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INTRODUCTION

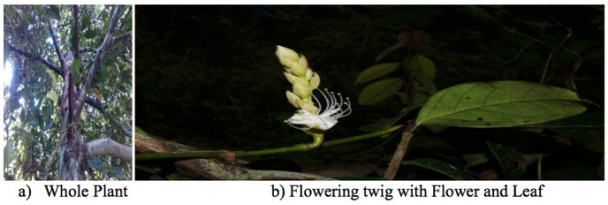
The two species of *Atuna* distributed in the Southern Western Ghats are *Atuna indica (*Bedd.) Kosterm and *Atuna travancorica (*Bedd.) Kosterm. The specimens of *Atuna indica* were collected from Nadukani (Nilambur North Forest Division, Kerala State, India) and it was first collected by Beddomei from Carcoorghats of Wayanad, and it was the rediscovery of this species, about 150 years later. Similarly, *Atuna travancorica* was described based on Hooker's collection, collected from Near the valara water falls, under Munnar Forest Division, Kerala State, India. The taxonomical as well as morphological characterization of the species was clearly done (Sasidharan and Sujanapal, 2011).

Atuna indica, a 20m tall tree with smooth, thin, brown bark having elliptic-oblong or elliptic ovate leaves with 17-21cm long and 5.5-7.5cm wide with white flowers. Similarly, in the case of *Atuna travancorica* 25m tall tree with smooth thin grayish brown bark having alternate, lanceolate leaves with 7-16.5cm long and 1.7-4.5cm wide with pale lavender or white flowers. A unique feature of the family (Chrysobalanaceae) is seen such as they are prominently keeled (Ghillean *et al*., 2003; Flowering Plants, 1989). In the case of *Atuna in-*

dica(Bedd.)Kosterm; Flowering and Fruiting: November to February; Habitat: West Coast tropical evergreen forests (Western Ghats in India) where as in the case of *Atuna travancorica*(Bedd.) Kosterm; Flowering and Fruiting: January to May; Habitat : West Coast tropical evergreen forests, usually riparian (Southern India, Travancore region) Both the species are belonging to the endangered category (Sudhakar*et al*., 2007; SasidharanN, 2002; Malin *et al*., 2015; Jean *et al*., 2003)

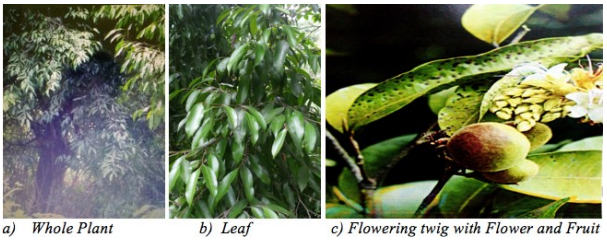
Diabetes is a serious metabolic disorder damaging many of the body systems such as the blood vessels and nerves and is the commonest endocrine disorder that affects approximately more than 100 million people worldwide. It is caused mainly due to the improper functioning of the pancreas with regard to the production of insulin leading to the abnormal blood glucose level.

Plants containing natural antioxidants such as flavonoids, tannins, C & E Vitamins stimulate the beta cells of pancreas thus reducing the blood glucose level. Also generally it is noticed that medicinal plants are cost effective devoid of side effects.(Kooti W *et al*., 2016; Bhoyar P *et al*., 2012). Plants are a rich source of secondary metabolites such as flavonoids, alkaloids, tannins and terpenoids which have been found in vitro to have antimicrobial properties and their activity is probably due to their ability to complex with extracellular and soluble proteins and the ability to complex with bacterial cell walls.(Cowan MM, 1999; Palombo EA, 2011). In the species of *Atuna indica*, shows the presence of Umbelliferone, an active coumarin having a number of reported pharmacological activities found to possess therapeutically better antioxidant activity as reported (Asish *et al*., 2013). In this context, the search to find the Antidiabetic and Antibacterial activities present in this wild species, *Atuna indica* and *Atuna travancorica* has been taken as the main objective of this study.



a) Whole Plant b) Flowering twig with Flower and Leaf

Figure 1: Habit of *Atuna indica* (Bedd.) Kosterm



a) Whole Plant b) Leaf c) Flowering twig with Flower and Fruit

Figure 2: Habit of *Atuna travancorica* (Bedd.) Kosterm

MATERIALS AND METHODS

Plant Materials

leaves and flowers of *Atuna indica* and leaves of *Atuna travancorica* were collected from Western Ghats of Kerala; *Atuna indica* collected from Nadukani (Nilambur North Forest Division, Kerala State, India) and *Atuna travancorica* collected from Near the valara waterfalls, under Munnar Forest Division, Kerala State, India.

Extraction of Plant Parts

The collected parts viz., leaves and flowers of the two species were chopped into suitable smaller size and shade dried, powdered in a mixer and stored in suitable air tight containers. Weighed a quantity of 10 gram in three separate stoppered conical flasks with proper labeling on it. To that added 100 ml of 50% aqueous ethanol solution and kept aside with occasional shaking for a period of 24 Hours. After a period of 24 hours the supernatant solvent replaced with fresh 100 ml 50% aqueous ethanol solution and the mixture again kept for a period of 24 Hours.(Molnar *et al.,* 2017). After the completion of the prescribed time, both the supernatant liquids mixed together and concentrated for the complete removal of the solvent using rotary vacuum evaporator. The extracts obtained from leaves and flowers of each species properly labeled and transferred to air tight vials.

Antidiabetic activity by α-Amylase Inhibition Assay

α-amylase inhibition activity was performed according to the modified method described by (Giancarlo *et al*., 2006), Reagents Required: 0.5% (w/v) soluble starch solution, Colour reagent solution: 96mM 3.5 dinitro salicylic acid (20ml), 5.31M sodium potassium tartarate in 2M sodium hydroxide (8ml) and deionized water(12ml), α- amylase solution (0.5unit/ml) 0.001g enzyme in 100ml of 20mM sodium phosphate buffer(pH-6.9).

Method: 25µL, 50µL, 100µL of extract and 1000µL of α-amylase enzyme were mixed and incubated at 25°C for 30 minutes. To all the test tubes added 1000µL of starch solution and the tube incubated at 25°C for 3 minutes. Then, 1ml of the color reagent was added and the tubes were placed in water bath at 85°C. After 15min, the reaction mixture was removed from the water bath and cooled thereafter; diluted with 9ml distilled water and absorbance value was determined at 540nm. The inhibition percentage of α-amylase was assessed by the formula given below:

$$\text{Inhibition\%} = \frac{\text{AC540} - \text{AT540X100}}{\text{AC540}}$$

Where AC is Absorbance of control and AT is Absorbance of Test.

Table 1: Antidiabetic activity of various extracts of *Atuna indica* (Bedd.) Kosterm and *Atuna travancorica* (Bedd.) Kosterm

Name of the part	Sample No	Concentration (mg)	OD at 518nm	% of inhibition
Leaves of Atuna indica	1	0.5	0.328	18.78
		1	0.338	22.75
		5	0.341	**34.13**
Flowers of Atuna indica	2	0.5	0.308	20.90
		1	0.319	23.81
		5	0.324	28.57
Leaves of Atuna travancorica	3	0.5	0.299	26.46
		1	0.304	32.28
		5	0.313	**43.39**
Control	Nil	**0.378**	**Nil**	**Control**

Table 2: Antibacterial activity of various extracts of *A. indica* and *A. travancorica*

Name of the Plant Part	Name of the Bacteria	% Inhibition					
		Control OD	Concentration (100mg/ml)				
			T1(10µL)	T2(15µL)	T3(20µL)	T4(25µL)	T5(50µL)
Leaves of Atuna indica	*E. coli*	1.288	18.40	21.51	23.52	30.12	31.75
	Pseudomonas aeruginosa	1.45	11.38	25.31	28.34	29.93	39.31
	Bacillus subtilis	1.547	28.64	30.25	33.68	37.10	38.20
	Staphylococcus aureus	1.259	8.02	11.91	17.95	23.19	29.07
Flowers of Atuna indica	*E. coli*	1.288	23.99	30.36	31.21	34.01	37.34
	Pseudomonas aeruginosa	1.45	5.86	9.52	10.90	13.52	15.45
	Bacillus subtilis	1.547	14.35	22.43	24.76	26.96	28.25
	Staphylococcus aureus	1.259	3.73	9.45	12.15	15.25	19.62
Leaves of Atuna travancorica	*E. coli*	1.288	26.48	33.39	40.61	46.20	50.16
	Pseudomonas aeruginosa	1.45	20.76	27.86	39.86	49.52	**55.24**
	Bacillus subtilis	1.547	24.95	29.09	33.81	38.66	**54.75**
	Staphylococcus aureus	1.259	17.79	19.70	23.59	30.02	37.97

Antibacterial assay using Microtiter Plate Method

Preparation of microbial culture: Using aseptic tech niques, a single pure colony was transferred into a 10 ml of nutrient broth/potato dextrose broth, capped and placed in incubator overnight at 37°C. After incubation, using aseptic preparation, turbidity of suspensions was calculated and adjusted using McFarland standards as a reference. (Wiegand *et al.,* 2008)

Preparation of the microtiter plate plates: Microtitre plates were prepared under aseptic conditions. A sterile 96 well plate was labeled. A volume of10µl, 50µl, and 100µl of test material was pipetted into the wells. 100µl of nutrient broth/potato dextrose broth was added to each well. Finally, 100µl of microbial suspension was added to each well. Control dilutions of test material were also kept. Plate was wrapped loosely with cling film to ensure that organism did not become dehydrated. Each plate had a set of controls: a column with all solutions except the test compound, and a column

with all solutions except the organism adding 100µl of nutrient broth/potato dextrose broth instead. The plates were incubated at 37°C for 24 hours and OD reading was taken(OD600) after sufficient incubation. Optical density(OD) was obtained from subtracting the control OD from the sample OD.

The % of inhibition was calculated from the following equation:

$$\% \text{ of inhibition } \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

In the present study, each of the three samples weighed a quantity of amount 10gram in three separate and clearly labeled stoppered 250ml conical flask contains the powdered form of leaves and flowers of *Atuna indica* and leaves of *Atuna travancorica* in 100ml of 50% aqueous ethanol solution as solvent used for the extraction. From each sample, ~2gram extracts were collected.

Antidiabetic activity: In the case of Antidiabetic activity studies, *Atuna travancorica* leaves showed comparatively better activity with a percentage of alpha amylase inhibition of 43.39% at a concentration of 5mg whereas *Atuna indica* leaves showed an inhibition of 34.13% in the same concentration.

Antibacterial activity: In the case of Antibacterial activity studies, *Atuna travancorica* leaves showed comparatively better activity against both strains of bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis* with a percentage inhibition of 55.24% and 54.75% respectively.

CONCLUSION

The current study concluded that there is a remarkable Antidiabetic and Antibacterial activities in both the endangered tree species of Atuna. Based on the observation of the results, it can be appreciated that the Antidiabetic activity is prominent in the leaves of *Atuna indica* and *Atuna travancorica* and the Antibacterial activity is pronounced in the leaves of *Atuna travancorica*. Overall results is the leaves of *A. travancorica* shows prominent Antidiabetic and Antibacterial activities when compared with the other parts of the two species examined. From this studies, it is observed that there is an immense requirement for a detailed and authoritative phytochemical screening about the phytoconstituents present in the leaves of *Atuna indica* and *Atuna travancorica* by using modern analytical instruments to find out the mechanism based activity.

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