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Research Article

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In vitro antibacterial, antioxidant and α-amylase inhibition activity of medicinal plants

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ABSTRACT

Diabetes has emerged as a major healthcare problem in India. Traditional medicines are practiced worldwide for treatment of type II diabetes mellitus since ancient times. Herbal remedies typically are part of traditional and folk healing methods with long histories of use. WHO suggests plants house a vast potential that can be harnessed to control diabetes. Rauvolfia tetraphylla and Shorea robusta showed presence of biologically active compounds such as alkaloids, flavonoids, phenolics, tannin, glycosidase, reducing sugars and terpenoids. The antimicrobial properties of plants have been proven effective against selected human pathogens such as Staphylococcus aureus and Klebsiella pneumoniae. Antioxidant activities of plants were performed using DPPH free radical scavenging assay, for different concentration of plant extracts. R.tetraphylla has produced higher efficiency of about 53.22% than S.robusta. Plant methanolic leaf extracts of R.tetraphylla was subjected to Alpha-amylase inhibition assay and the results were 87.47% inhibition whereas S.robusta showed low inhibition potential of 44.93%. This reveals that R.tetraphylla was very effective and significant in treating diseases. This confirms that the plant might protect cells from oxidative damage, resulting in certain diseases. Further studies would reveal the novel compound responsible for anti-diabetic activity of the plant.

Keywords: α-amylase inhibition assay, antioxidant activity, antimicrobial, phytochemicals, free radical scavenging activity.

INTRODUCTION

Diabetes Mellitus is a public health problem worldwide. Defective insulin secretion and action therefore leads to multiple metabolic abnormalities in type 2 diabetes, including hyperglycemia due to impaired insulin-stimulated glucose uptake and uncontrolled hepatic glucose production, and dyslipidaemia, which includes perturbed homeostasis of fatty acids, triglycerides and lipoproteins. These chronic increases in circulating glucose and lipid levels can further impair insulin secretion and action and cause other forms of damage [3, 9]. *Rauvolfia tetraphylla* contains a number of bioactive phytochemicals and is mainly known for its phytochemical reserpine, which was widely used as an antihypertensive drug. But very least research has been published on the leaves of plant *Rauvolfia tetraphylla* [15]. *Shorea robusta* is widely distributed in India, Nepal and Bhutan. In India, the species is distributed from Himachal Pradesh to Assam, Tripura, West Bengal, Bihar and Orissa, Eastern districts of Madhya Pradesh extending further to the Eastern Ghats of Andhra Pradesh [7, 14]. In recent years, secondary plant metabolites or phytochemicals, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [2]. There were many reports on the antimicrobial activity of plant extracts against human pathogenic bacteria [4, 13]. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism against predation by many microorganism, insects and herbivores [1].

The antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalyzed by free radical. Therefore, a search for natural antioxidant has greatly been increased in the recent scenario [5]. Specific enzyme inhibitors are biochemical tools that have potential utility in the treatment of diseases. It has become evident that actinomycetes are the potential producers of various enzyme inhibitors. α-amylase and α-glycosidase inhibitors are drug targets for the treatment of diabetes, obesity and hyperlipidaemia. Inhibitors inhibit the action of these enzymes results in reduction in starch hydrolysis which shows beneficial effects on glycemic index. These inhibitors can retard the liberation of glucose, resulting in reduced postprandial plasma glucose levels and suppress postprandial hyperglycemia [6].

Since several adverse effects on available modern treatment system. In this regard, plants provide the best option for search of desired safe and effective medication. Medicinal plants are used in the treatment of diabetes mellitus, especially in the developing countries due to their cost effectiveness, the present study on antioxidant and α-amylase inhibition activity of plant sample might have support and help the research scientific community to study and encourage the use of medicinal plants for the treatment of diseases.

EXPERIMENTAL SECTION

Collection and processing of plant sample

Healthy, uninfected leaves of *Rauvolfia tetraphylla* and *Shorea robusta* were collected from the natural locations in Kelambakkam forest, Chennai, Tamilnadu.

The fresh leaves were washed carefully with tap water, then with distilled water and allowed to shade dry for two weeks at room temperature. The shade dried samples were pulverized into coarse powder using commercial blender and stored in air tight container for further use.

Preparation of plant extract

The shade dried plant powders were successively extracted using solvent methanol by cold percolation method [10]. The leaves coarse powder were soaked in methanol and kept in dark for 72hrs at room temperature in a temperature controlled incubator. The crude extracts were filtered using Whatmann No: 1 filter paper. Then the crude extracts were condensed to dryness at room temperature.

Phytochemical analysis

Plants constitute a wide array of bioactive compounds that have potential health beneficial properties. Similarly, the leaves are suspected to be rich in phytochemicals. To confirm, leaves of *Rauvolfia tetraphylla* and *Shorea robusta* were subjected to a qualitative analysis [12]. Test for reducing sugars, flavonoid, alkaloids, terpenoids, phenols, tannin and glycosides were performed according to the procedure.

Determination of Antibacterial activity

Agar well diffusion assay

Antibacterial activity was determined by agar well diffusion method [8]. Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify. 24h overnight bacterial cultures *Staphylococcus aureus* and *Klebsiella pneumoniae* were swabbed separately on it using sterile cotton buds. Then, five wells (8mm diameter) were made by using a sterile cork borer. The four different concentrations (250µg/mL, 500µg/mL, 750µg/mL and 1000µg/mL) of the test sample were loaded in the wells. Tetracycline was used as the positive control. The plates were then incubated at 37ºC for 24h. After incubation the diameter of zone of inhibition was measured.

Antioxidant assay, free radical scavenging activity

The Radical Scavenging Activity of different extracts was determined by using DPPH assay [11] with small modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2960 µL of 0.1 mM ethanolic DPPH solutions mixed with 40µL of 20 to 100 µg/mL of plant extract and vortexes thoroughly. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. The ability of the sample extract to scavenge DPPH radical was calculated by the following equation:

$$\%\ \text{of DPPH Radical Scavenging Activity (\% RSA)} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

Abs. control is the absorbance of DPPH radical + ethanol; Abs. sample is the absorbance of DPPH radical + sample extract.

α-Amylase Inhibition assay

The α-Amylase Inhibition Assay of the plant extracts were performed by following the method described by KR Suthindhiran (2009) [6] with slight modification. 500µL of extract and 500µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution (0.5 mg/mL) were incubated for 10 min at 25ºC. After pre-incubation, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5 sec intervals. The reaction mixtures were then incubated at 25ºC for 10min. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 10 mL distilled water and absorbance was measured at 540 nm in UV/ VIS spectrophotometer.

$$\% \text{ inhibition} = \frac{A_{540}\text{control} - A_{540}\text{ extract}}{A_{540}\text{control}} \times 100$$

RESULTS AND DISCUSSION

Qualitative analysis of phytochemicals

The qualitative analysis of phytochemicals or secondary metabolites of *Rauvolfia tetraphylla* and *Shorea robusta* methanolic leaf extract was presented in table 1. The results revealed that, both the plant extract contains reducing sugars, flavonoids, alkaloid and terpenoids. *Rauvolfia tetraphylla* contains tannin which is absent in *Shorea robusta* whereas *S.robusta* contains the glycosidase, it is absent in *R.tetraphylla*. Both the plants show negative results for the presence of phenols.

Table 1: Qualitative analysis of phytochemicals of plant extracts

Parameters/ phytochemical tests	*Rauvolfia tetraphylla* methanol extract	*Shorea robusta* methanol extract
Reducing sugars	+++	+++
Glycosides	---	+++
Tannins	+++	---
Flavonoids	+++	+++
Phenols	---	---
Alkaloids	+++	+++
Terpenoids	+++	+++

Legend :(+++) indicates the presence and (---) indicates the absence.

Evaluation of Antibacterial activity

The antibacterial activity of methanolic extract of *R.tetraphylla* against *S.aureus* and *K.pneumoniae* was carried out in increase dose dependent manner from 250-1000µg/ml was represented in table 2 and figure 1,in which *S.aureus* are more susceptible to *R.tetraphylla* with highest activity (23mm) for concentration of 1000 µg/ml which is nearly equal and/or significant to synthetic antibiotic, Tetracycline. Similar effect was showed in *K.pneumoniae* with higher zone of inhibition about 24mm for 1000µg/ml of plant extract. This result has confirmed the efficiency of the plant extract against human pathogen.

Table 2: Antibacterial activity of *R. tetraphylla* methanolic leaf extract against *S.aureus* and *K.pneumoniae*

Organism	Diameter of Zone of Inhibition(mm)				
	Control	250µg/mL	500µg/mL	750µg/mL	1000µg/mL
S.aureus	25	21	21	22	23
K.pneumoniae	27	17	22	23	24

The antibacterial activity of methanolic extract of *S.robusta* against *S.aureus* and *K.pneumoniae* was carried out**,** the result were given in table 3 and figure 2. The results revealed that the methanolic extracts of *S.robusta* presented less inhibition activity against *S.aureus* and it doesn't show any antibacterial activity against *K.pneumoniae*. The result confirms that this plant has less potential against selected pathogen.

Table 3: Antibacterial activity of *S. robusta* methanolic leaf extract against *S.aureus* and *K.pneumoniae*

Organism	Diameter of Zone of Inhibition(mm)				
	Control	250µg/mL	500µg/mL	750µg/mL	1000µg/mL
S.aureus	25	-	-	12	14
K.pneumoniae	27	-	-	-	-

Figure1: Representation of Antibacterial activity of methanolic extract of *Rauvolfia tetraphylla* against *S.aureus* and *K.pneumoniae*

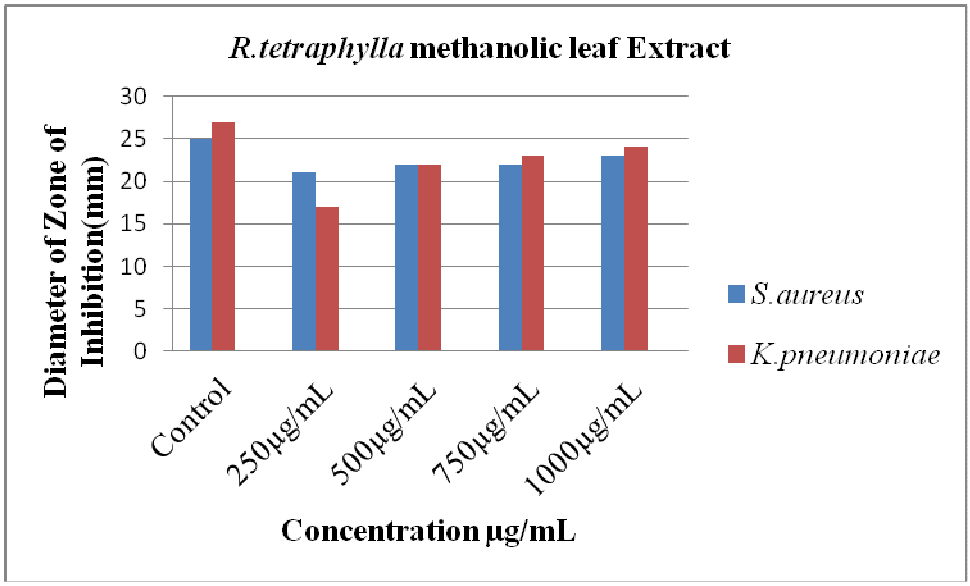
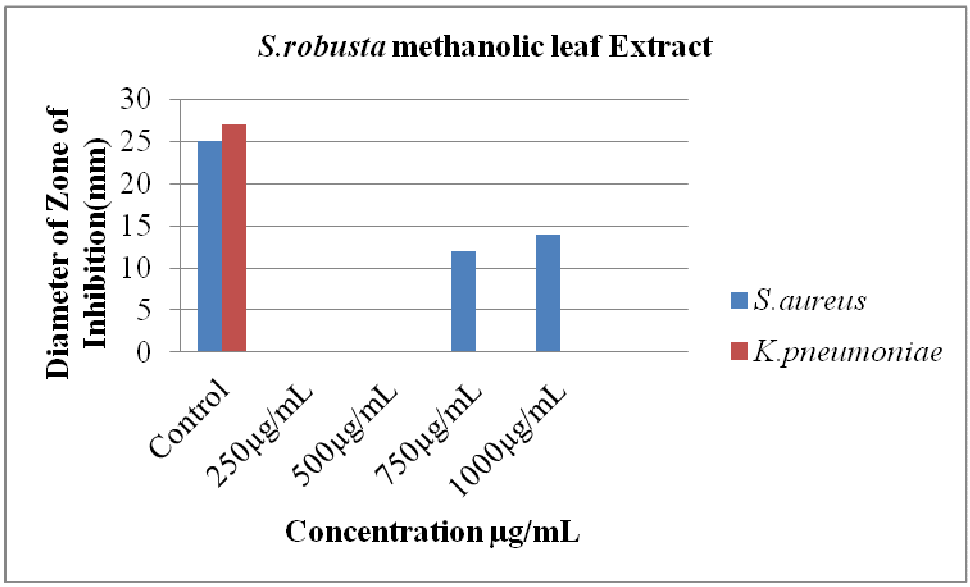


Figure 2: Representation of antibacterial activity of methanolic extract of *S.robuta* against *K.pneumoniae* and *S.aureus*



Determination of Antioxidant activity of *R.tetraphylla* and *S.robusta*

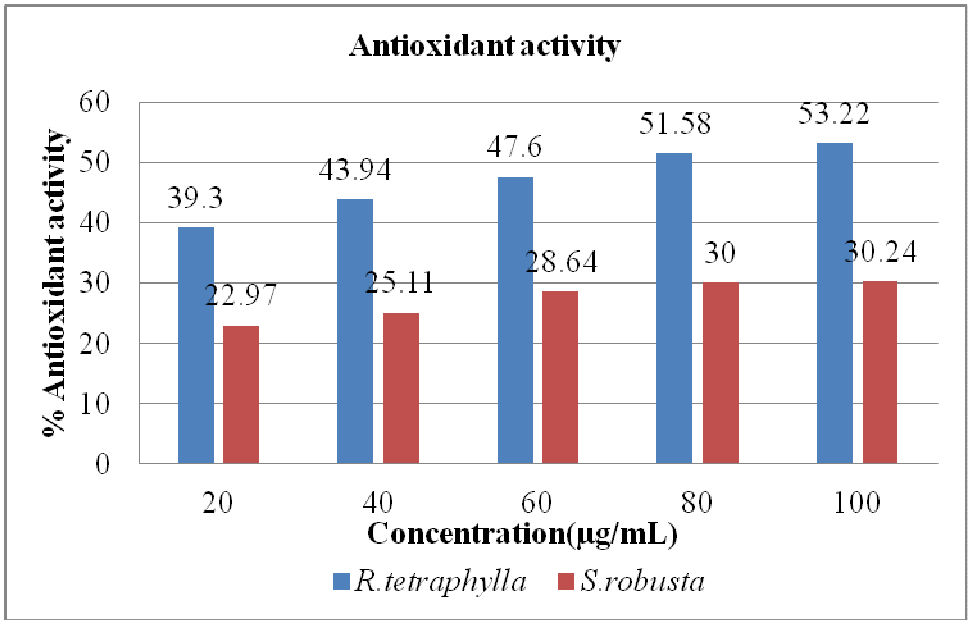
% Free radical scavenging activity

Reactive oxidative substrate produced as by product play a key role in cell signaling. However, biomolecule oxidation produced excessive ROS which caused major damage to cell structure and resulted to different kinds of diseases such as cancer, stroke, and diabetes. Antioxidants are key inhibitors in protection but also as a defense mechanism of living cells against oxidative damage. The effect of the methanolic leaf extracts of two plants at various concentrations against DPPH is presented in table 4. All the tested plants extract exhibited promising antiradical activity. *Rauvolfia tetraphylla* has showed potential radical scavenging of 53.22% whereas *Shorea robusta* showed low antioxidant activities of 30.24% at the concentration of 100µg/mL were given in figure 3.

Table 4: Determination of antioxidant properties of *R.tetraphylla* and *S.robusta*

Concentration(µg/mL)	% Antioxidant activity of *R.tetraphylla*	% Antioxidant activity of *S.robusta*
20	39.30	22.97
40	43.94	25.11
60	47.60	28.64
80	51.58	30.00
100	53.22	30.24

Figure 3: Representation of antioxidant activity of *R.tetraphylla* and *S.robusta*



α- Amylase Inhibition Activity

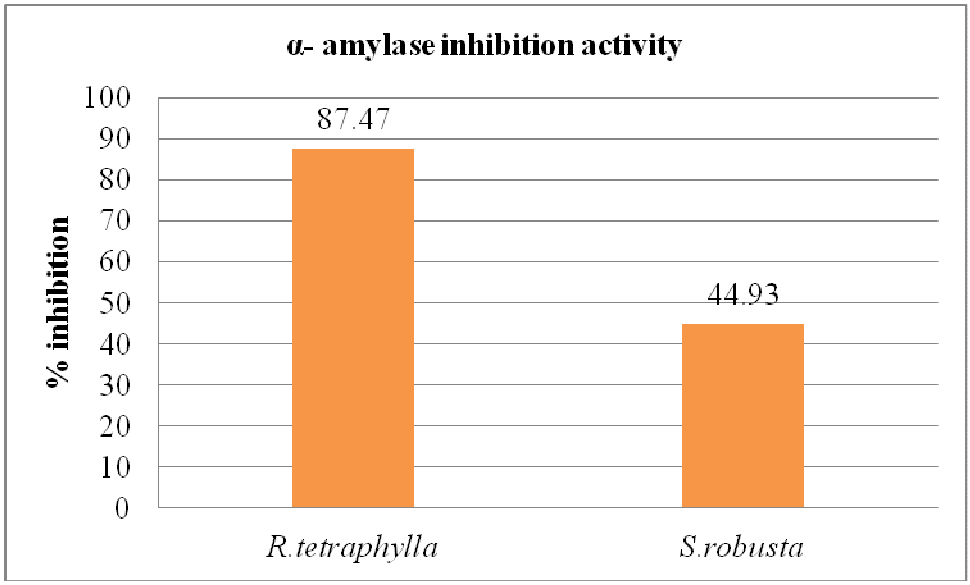
α-amylase inhibitors are drug targets for the treatment of diabetes, obesity and hyperlipidaemia. Inhibitors inhibit the action of these enzymes results in reduction in starch hydrolysis which shows beneficial effects on glycemic index. These inhibitors can retard the liberation of glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppress postprandial hyperglycemia [6]

The % inhibition of α- amylase of *Rauvolfia tetraphylla* and *Shorea robusta* methanolic leaf extract was given in the table 5. The results represent that *Rauvolfia tetraphylla* is highly effective and showed enzyme inhibition of 87.47% whereas *Shorea robusta* showed very low inhibition of 44.93% and where graphically shown in figure 4. Since *R.tetraphylla* has showed higher inhibitory activity it may suppress hyperglycemia. It might have effects on obesity and hyperlipidaemia.

Table 5: Determination of α- amylase inhibition activity of methanolic extract of *R.tetraphylla* and *S.robusta*

Plant sample	% Inhibition
Rauvolfia tetraphylla	87.47
Shorea robusta	44.93

Figure 4: Representation of α- amylase inhibition activity of methanolic extract of *R.tetraphylla* and *S.robusta*



CONCLUSION

Phytochemicals found in leaves of *Rauvolfia tetraphylla* and *Shorea robusta* indicates their potential as a source of principles that may supply novel medicines. The above results clearly demonstrate that *Rauvolfia tetraphylla* has effective and significant antibacterial activity against selected pathogens than *Shorea robusta*. The plant *Rauvolfia tetraphylla* has potent free radical scavenging activity hence it may turn out to be highly beneficial in solving health issues. The results of α-amylase inhibition assay have confirmed that the plant has effect in treating diabetes. The current finding directed to isolate new, rare, and novel bio active molecules from the leaves of *Rauvolfia tetraphylla* for treating diseases.

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