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Evaluation of anti diabetic, antioxidative and proteolytic potentials of *Salacia chinens* organic extracts

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Abstract
Salacia chinens this herb has been reported in Ayurveda as a treatment for Madhumeha (ancient name of Diabetes mellitus) called as saptarangi and is distributed in Sri Lanka, India, China, Malaysia and other countries. in ayurveda is used for treatment of Rheumatism, Itches, Asthma and Ear diseases along with Diabetes and Obesity, scientists have tried to explore the hypoglycemic potential of various extracts. In this research we have identified biological activities important in human health. In this study of phytochemical analysis of *Salacia chines* is which was extracted in water and methanol shows various phytochemical properties. It recognize. Test for phenol, terpenoids, flavonoids, saponins, tannins, reducing sugar, amino acid respectively. The plant shows higher protein concentration in methanolic extract (2.9793mg/ml) than that of water extract (1.350mg/ml). The study of antidiabetic property was calculated by ERBA kit, both the extract of plant (methanolic and water extract) showing antidiabetic activity**.** Protease activity of *Salacia chinens* extract was carried out by milk agar, it involved determining the protease enzyme activity which present in plant shows respective plant consist of proteolytic enzyme.

Keywords: *Salacia chinens*, Diabetes mellitus, phytochemical studies, primary metabolites studies, antidiabetic assay, proteolytic activity

Introduction
Salacia chinens is a species of plant in the family Celastraceae. A climbing shrub, it is also known as Chinese oblongaz, lolly berry, (Jules 2008) [1]. and saptachakra in Ayurveda (Khare *et al.* 2004) [2]. The plant is found widespread in South-East Asia and Australoceania Medicinal plants are great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, terpenoids, flavonoids and phenolic compounds (Priya *et al* 2011) [3]. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. A number of studies have focused on the biological activities of phenolic compounds (Reddy *et al* 2008) [5] established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others (Devasagayam *et al* 2004) [8]. The genus Salacia (Family: Celastraceae) comprises of several medicinally important species (S. oblonga, S. reticulata, S. chinensis, etc.) and is known as 'Saptrangi' in Ayurvedic medicine (Singh *et al* 2009) [10]. This herb has been reported in Ayurveda as a treatment for Madhumeha (ancient name of Diabetes) and is distributed in Sri Lanka, India, China, Malaysia and other countries. The root bark is used by either boiling in oil as a decoction or as powder for the treatment of Rheumatism, Itches, Asthma and Ear diseases along with Diabetes and Obesity (Rao 2010) [9]. Since 1960s, scientists have tried to explore the hypoglycemic potential of various extracts of roots, root bark, and stems of SO in diabetic models (Augusti 1995) [12]. The present study aims to investigate the antioxidant activity, total phenol and total flavonoid content from stem extract of Salacia oblonga.

Collection of plant material *Salacia chinesis* was collected from local area nashik, trambekeswar.

Preparation of extract: The Plant materials were collected and shade dried at room temperature, subjected to get coarse powder through mechanical grinder. The powdered plant was passed through sieve and stored in air tight containers. Each powdered materials were extracted with methanol and water separately, the slurry was allowed to stand closed at room temperature for 72 hours to prevent the solvent from evaporating, then filtered using gauze and Whattman No. 1 filter paper. The filtrate were evaporated under abridged pressure in a rotary evaporator to get the dry extract.

Preliminary phytochemical qualitative analysis: (Patil *et al.* 2016) [13].

Test for alkaloid: Mayer's Test: To 1ml of extract 1ml of mayers reagent (potassium iodide solution) was added. Formation of white yellow or cream colour precipitate indicates presence of alkaloids.

Test for proteins Ninhydrin Test: To the extract, a little of Ninhydrin reagent was added. Appearance of purple colour depicted the presence of proteins.

Test for phenol: Lead Acetate Test: To the 1ml extract, 1ml of lead acetate solution was added and observed for formation of precipitate.

Test for terpenoids Salkovaski Test: A small quantity of chloroform extract was treated with 5ml of concentrated sulphuric acid. The solution changed colour from yellow to red at the junction indicating the presence of terpenoids.

Test for Flavanoids: Alkaline reagent Test: To 1 ml of extract, few drop of dilute ammonium solution and few drop of concentrated hydrochloric acid were added. A presence of yellow colour indicates presence of flavoinoid.

Test for Saponins Froth Test: To ml of extract, 5ml of distilled water was added and shaked vigorously. Formation of froth indicated presence of saponins.

Test for tannins: Lead Acetate Test: To the 1ml extract, 1ml of lead acetate solution was added and observed for formation of white precipitate.

Test for reducing sugar: Fehling's test: To the 1ml of extract equal quantities Fehling's solution A and B and heated on a water bath for few minutes. It was observed for the formation of a red precipitate of cuprous oxide

Test for amino acid: Ninhydrin Test: To the 1 ml of extract, 3 to 4 drop of ninhydrin solution was added and boiled in water bath for 10 minutes. formation of purple or blue colour indicates the presence of amino acid.

Quantitative estimation protein by Lowry's method: 1. Standard BSA (1mg/ml) was pipette in 3 test tubes in increasing volumes and in test samples (0.1 ml) in remaining tubes. The volume was adjusted to 1 ml with 4 ml of complex forming reagent (alkaline copper sulphate) was added, mixed and kept at room temperature for 10 min. 1 ml of Folin-Ciocalteu reagent was added, mixed thoroughly and incubated in dark for 30 min.Optical density was read on spectrophotometer at 660 nm and recorded the readings. Calibration curve was plotted by taking optical density reading on Y axis against standard protein concentration (µl) on X axis. Value x was recorded from the graph corresponding to the optical density reading for the test sample. The protein concentration was calculated using the following equation calculated based on graph: y = mx+c.

Glucose adsorption assay: (Patel *et al.*) 10mg of extract was added to 50 ml glucose solution of five different concentrations (5, 10, 15, 20, 25 and 30mM), Each of this mixture was mixed well, stirred and incubated in a shaker water bath at 37 °C for 6 hours, respectively. After incubation, the mixture was centrifuged at 4800 rpm for 20 min and finally the glucose content was determined in the supernatant by using Glucose Oxidase peroxidase diagnostic Erba kit. The absorbance was measured at 505nm by spectrophotometer (Bionate) and the glucose bound was calculated using the following formula. Formula Glucose adsorbed = [(G1- G6) / (Weight of the sample)] * Volume of the solution Where, GI represents the glucose concentration of original solution, while G6 represents the glucose concentration after 6hours.

Assay procedure

Table 1: glucose adsorption assay

Pipette into tubes marked	Blank	Standard	Test
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Test	-	-	10µl

Agar was prepared along with 1% (w/v) casein, skimmed milk, gelatin and poured in petri dishes. The plates were solidified for 30 min and then after crude extract of saptarangi (methanol and distilled water) 100 µl in well. After incubation at overnight the plate were observed for percent inhibition.

Result and discussion

Preliminary phytochemical analysis

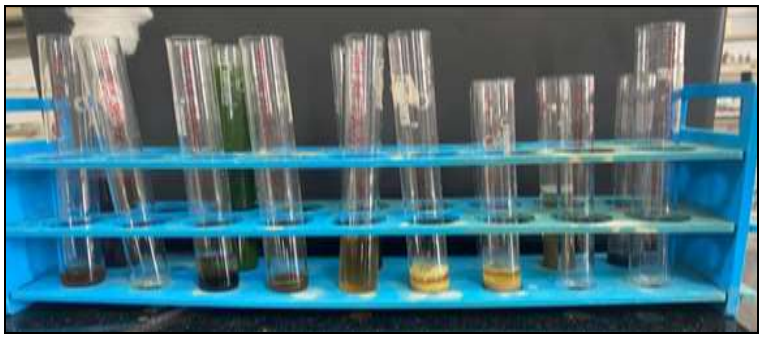


Fig 1: Qualitative Phytochemical analysis of methanol and water extract of *salacia chinesis*.

Table 2: Qualitative Phytochemical analysis of methanol and water extract of *Salacia chinesis*.

Phytochemical Qualitative Test	*Salacia chinesis* extracts	
	Water extract	Methanol extract
Alkaloids (Mayer's Test)	-	-
Protein (Ninhydrin Test)	+	+
Phenol (Lead Acetate Test)	+	+
terpenoids SalkovaskiTest	-	+
Flavanoids (Alkaline reagent Test)	+	+
Saponins (Froth Test)	+	-
Tannins (Lead Acetate Test)	-	+
Test for reducing sugar Fehling's test)	+	-
Test for amino acid (Ninhydrin Test)	+	+

(Positive +) (Negative-)

Phytochemical are non-nutritive, chemical compounds occurs naturally on plants during metabolic processes and they have diverse proactive properties, In present study of phytochemical analysis of *Salacia chinesis* which was extracted in water and methanol shows various phytochemical properties.it recognized with help of mayers test, ninhydrin test, lead acetate test, salkovaski test, alkaline reagent test, froth test, lead acetate test, fehlings test, ninhydrin test for the protein, phenol, terpenoids, flavonoids, saponins, tannins, reducing sugar, amino acid respectively.

Quantitative estimation protein by Lowry's

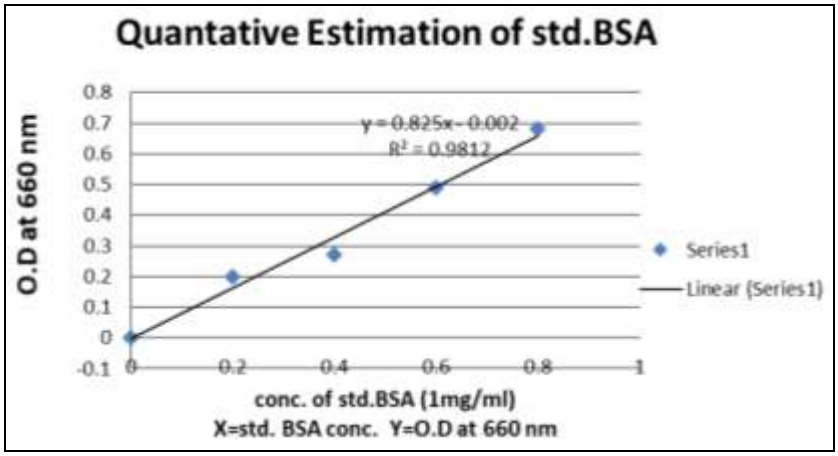


Plate 1: standard graphical representation of quantitative analysis of protein by lowery method.

The present step involved the quantative estimation of enzyme through the lowery method. The plant solvent which was previously extracted in water and methanol. It was calculated by taken the standard BSA concentraction (1mg/ml). The *Salacia chinesis* plant showing higher protein concentration in methanolic extract (2.9793 mg/ml) than that of water extract (1.350mg/ml).

Glucose adsorption assay

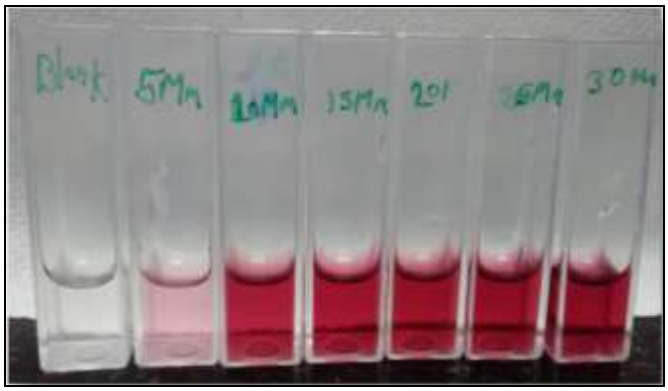
Observation Table

1) *Salacia chinesisi* Aqueous extract

Before the incubation optical density was taken at 505 nm.

Table 2: Initial concentration of glucose

Concentration in Mm	O.D at 505 nm			
	Before incubation Aqueous extract	After incubation Aqueous extract	Before incubation Methanol extract	After incubation Methanol extract
Blank	0.0	0.00	0.0	0.00
Standard	1.921	1.921	1.912	1.912
5mm	0.291	0.451	0.372	0.401
10mm	1.394	1.957	1.892	1.974
15mm	1.982	2.103	2.132	2.742
20mm	2.010	2.292	2.147	2.871
25mm	2.094	2.502	2.267	2.921
30mm	2.123	2.752	2.370	2.981



generally the drugs used in diabetes mellitus are by lowering glucose level in the the blood, in the present study antidiabetic property was calculated by ERBA kit. both the extract of plant (methanolic and water extract) showing antidibateic activity.

Determination of protease activity

Before incubation After incubation

Fig 1: protease inhibition on milk agar plate determination of enzyme

protease activity of saptarangi extract was carried out by milk agar, generally agar was prepared along with 1% (w/v) casein, skimmed milk, gelatin and poured in petri dishes. The saptarangi plant extract was expose in petriplate. it involved determining the protease enzyme activity which present in plant. Due to its photolytic activity incubation at 37 ºCy it degrade the milk agar contains casein. The zone of inhibition is 0.91 cm its show respected plant consist of proteolytic enzyme.

Conclusion

Phytochemical are non-nutritive, chemical compounds occurs naturally on plants during metabolic processes and they have diverse proactive properties. In present study of phytochemical analysis of *Salacia chinesis* which was extracted in water and methanol shows various phytochemical properties. It recognized with help of Mayer's test, ninhydrin test, lead acetate test, salkovaski test, alkaline reagent test, froth test, lead acetate test, fehlings test, ninhydrin test for the protein, phenol, terpenoids, flavonoids, saponins, tannins, reducing sugar, amino acid respectively. The quantitave estimation of protein involved the quantative estimation of enzyme through the lowery method. The plant solvent which was previously extracted in water and methanol. It was calculated by taken the standard BSA concentraction (1mg/ml). The *Salacia chinesis* plant showing higher protein concentration in methanolic extract (2.9793mg/ml) than that of water extract (1.350mg/ml).generally the drugs used in diabetes mellitus are by lowering glucose level in the the blood, in the present study antidiabetic property was calculated by ERBA kit, both the extract of plant (methanolic and water extract) showing antidiabetic activity**.** protease activity of saptarangi extract was carried out by milk agar, generally agar was prepared along with 1% (w/v) casein, skimmed milk, gelatin and poured in petri dishes. The saptarangi plant extract was expose in petriplate. it involved determining the protease enzyme activity which present in plant. Due to its proteolytic activity it degrade the milk agar contains casein. The zone of inhibition is 0.91 cm its respective plant consist of proteolytic enzyme.

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