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Phytochemical screening and *in-vitro* antidibetic activity of extracts of some Indian medicinal plants

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Abstract

The present scientific investigation deals with the extraction of some Indian medicinal plants include *Manilkara hexandra* Roxb. stem bark (Sapotaceae), *Strychnous Potatorum* Linn. dried seeds (Loganiaceae), *Salacia reticulata* Wight. stem bark (Celastraceae) and identification of chemical constituents by using preliminary phytochemical tests. The extracts were screened for their potential in-vitro anti-diabetic studies so as to ensure the biological potency of the plant. The study also includes qualitative screening of the phytonutrients, Free radical scavenging activity by DPPH assay method and assessment of total antioxidant activity by phosphor-molybdate assay method were evaluated. From the study we revealed that the all plants contain various classes of secondary metabolites and also possess a moderate anti-diabetic activity it terms of alpha amylase inhibition.

Keywords: *Manilkara hexandra, Strychnous Potatorum, Salacia reticulata*, Anti-diabetic activity, Alpha amylase inhibition.

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1. Introduction

Diabetes mellitus is a principal cause of morbidity and mortality in human. It is a syndrome characterized by hyperglycemia, polydipsia and polyuria and causes complications to the eyes, kidneys, and nerves. It is also associated with an increased incidence of cardiovascular disease. The clinical manifestations and development of diabetes often differ significantly between countries and also between racial groups within a country. This increase can be attributed to many factors, including a stressful lifestyle as well as improper dietary habits. This is of economic concern as the disease requires life-long treatment and is also associated with high morbidity from the resulting complications.[1]

According to the IDF statistics, presently every seven seconds someone is estimated to die from diabetes or its complications, with 50% of those deaths (4 million in total per year) occurring under the age of 60 years. [2] This is against the background of a global diabetes prevalence of 8.8% of the world population in 2017, standardized for the age group 20-79 years. [3]

The prevalence is expected to further increase to 9.9% by the year 2045. In total numbers, this reflects a population of 424.9 million people with diabetes worldwide in 2017 with an estimate of a 48% increase to 628.6 million people for the year 2045. Global umbers of diabetes prevalence have continuously risen from 151 million in 2000, when the IDF Diabetes Atlas first was launched, to 285 million in 2009 and to 382 million in 2013. Disturbingly in this context, some 50% of all individuals with diabetes are undiagnosed, especially in developing countries. [4]

Moreover, it was estimated that the number of adults with diabetes in the world had increased from 108 million in 1980 to 422 million in 2014 (28.5% due to the rise in prevalence, 39.7% due to population growth and ageing, and 31.8% due to interaction of these two factors). Besides the growth and aging of the world population in

general, the global obesity epidemic has turned out to be a key factor for the rise of diabetes incidence together with the immense progress of multifactorial cardiovascular risk management and successful revascularisation therapy of people with diabetes also contributing to the expansion of the worldwide diabetes population. [1-5]

Diabetes more or less equally affects both sexes with men having a small edge over women at younger age groups and women surpassing men at higher age groups. [6]

Depending on age groups, global diabetes prevalence is about 5% for the age group 35-39 years, 10% for the age group 45-49 years, 15% for the age group 55-59 years, and close to 20% starting at age group 65-69 years. [6] Diabetes prevalence numbers are largely determined by people with type2 diabetes who comprise about 90% of the total population. These individuals are characterized by various degrees of relative insulin deficiency in conjunction with a wide spectrum of insulin resistance.

Mortality, though decreasing in the last thirty years, has remained at least twofold increased both in adult type 1 and type 2 diabetes compared with the general population. [6]

The National Diabetes Data Group of the National Institutes of Health recommends the following criteria for diagnosing diabetes:

a. Fasting (overnight) venous plasma glucose concentration greater than or equal to 140 mg/dL on at least two separate occasions.
b. Venous plasma glucose concentration greater than or equal to 200 mg/dL at 2-h post-ingestion of 75 g of glucose and at least one other sample during the 2-h test.

***Manilkara hexandra* (Roxb.)** Family- **Sapotaceae**

M. hexandra is a slow-growing evergreen tree that grows in tropical and temperate forests. It grows 40 to 80 feet tall and 1 to 3 meters in circumference. The bark is greyish black, rough and with smooth branchlets. Leaves are alternately arranged, often closely clustered towards the end of branchlets, with conspicuous scars. Leaf-stalks are 0.8-2 cm. The wood is very hard, heavy, and very durable, weighing 70 pounds per cubic foot. Flowers arise in fascicles in leaf axils. Pedicel is thick, 1-1.8 cm. Sepals are ovate- triangular, 3-4 mm, yellowish gray velvety. Flowers are white or light yellow, about 4 mm. Flowers bisexual or unisexual, usually in sessile axillary clusters, rarely solitary; cluster pedunculate or in raceme-like inflorescence, bracteolate and Flowering in the month of August-December.

Strychnos Potatorum* Linn. Family- *Loganiaceae

S. potatorum is a moderate sized tree found in southern and central parts of India, Sri Lanka and Burma. The plant is largely used in some parts of India for clearing muddy water. The fruit is also employed by the native practitioners, under the name of *nirmali,* as an *emetic* and in *dysentery*. The tree, which grows to a very large size, produces a shining, black, one-seeded berry. The seeds are broadly lenticular, about half an inch in diameter and a quarter of an inch in thickness, of a dirty whitish-gray color, and covered with a thick coating of delicate appressed hairs. The seeds contain a large quantity of an albuminous principle, upon which their virtues probably depend.

In traditional system of medicine the seeds are used for the treatment of various ailments like jaundice, bronchitis, diabetes, conjunctivitis, chronic diarrhea, dysentery etc. They are also used to clear muddy water by its coagulant action.

***Salacia reticulata* Wight. Family- Celastraceae**

Salacia reticulata is a large woody climbing, perennial, woody shrub naturally found in Sri Lanka and Southern region of India.. The plant has dichotomous branching pattern. Bark is smooth, greenish grey in colour, thin, and white internally. Leaves are opposite and elliptic-oblong. Leaf-base is acute, apex abruptly acuminate, margin are toothed with minute rounded teeth. Flowers are bisexual and arranged as 2-8 clustered in leaf axils. They are greenish-white to greenish-yellow in color. Fruit is a drupe which is globose and tubercular. The drupe assumes pinkish-orange color on ripening. Seeds are 1to 4 in number and resembles with almond.

Different species of *Salacia* have medicinal principles with a high pharmacological significance. In traditional system of medicine, different species of the genus, Salacia are being used as acrid, bitter, termogenic, urinary and as liver tonic. The aerial parts and roots of Salacia are extensively used in Ayurvedic and Unani system of medicine for treating diabetes, gonorrhoea, rheumatism, itching, asthma, ear diseases, leukaemia and inflammations.

2. Materials and methods

2.1 Materials

a) Plant material

The bark of *Manilkara hexandra,* seeds of ***Strychnous Potatorum*** *and* bark of *Salacia reticulata* were collected from an area around local forest area in Gondia district in Maharashtra and were shade dried. The identification of the plant parts was confirmed by Dr. S. M. Biskute, a botanist in the Department of Botony, Nagpur University.

b) Chemicals and reagents

Organic solvents used for extraction of the plant material all purchased from SARCHEM. These solvents were of analytical grade. Enzymes (glucose-6-phosphatase, and α-amylase and α-glucosidase) and substrates were purchased from Sigma Chemical (India). All other chemicals used were of analytical grade.

2.2 Determination of phytochemicals

a) Qualitative screening of phytochemicals

A phytochemical screening of phenolics, alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, cardiac glycosides, glycosides/reducing sugars, and phlobatannins present in plant extracts was performed using standard methods.

Phenolics were determined as per the method given by Krishnaiah *et al*., 2009., Saponins indicates its presence as per methods given by Houghton and Raman, 1998 and Jigna and Sumitra, 2007. The terpenoids were screened by method of Trease and Evans, 2002. Cardiac glycosides were screened in the plant material by Krishnaiah *et al*., 2009 method, for the presence of phlobatannins the method given by Trease and Evans, 2002 was utilized.

***2.3 In vitro* antioxidant activities of the plant extracts**

a) Free radical scavenging activity by DPPH assay:

The free radical scavenging activities of the medicinal plants was determined by 11-diphenyl-2-picrylhydrazyl (DPPH) method according to Brand-Williams *et al*. 1995. The scavenging activity was calculated by using the formula:

$$\%\ \text{Scaving activity} = \frac{\text{Absorbance of the blank} - \text{Absorbance of the sample}}{\text{Absorbance of the blank}} \times 100$$

The blank contained all reagents except the medicinal plant extract. Ascorbic acid at a concentration of 1 mg/ml was used as reference.

b) Total antioxidant activity by phosphomolybdate assay:

To carry out phosphomolybdate assay, the procedure described by Umamaheswari and Chatterjee, 2008 was used. The results were expressed as µg of ascorbic acid equivalent (AAE) per mg of the dried weight of the sample as determined from the equation of the standard calibration curve and the following expression was used to calculate the AAE:

$$\text{Ascorbic acid equivalent} = \frac{\text{Absorbance at 765nm}}{0.0034}\ (\mu g/\text{mg of dried matter})$$

2.4 Determination of the moisture content:

The percent moisture content was calculated by the following formula:

$$\%\ \text{Moisture} = 1 - \frac{\text{Weight dry sample}}{\text{Weight wet sample}} \times 100$$

2.5 Determination of ash content

Percent ash was calculated by following formula:

$$\%\ \text{Ash} = \frac{\text{Difference in weight of ash}}{\text{Weight of medicinal plant powder}} \times 100$$

Difference in weight of ash = W3 - W1

Weight of empty crucible was noted (W1). One gram of each of the medicinal plant powder was taken in crucible (W2). After ignition crucible was cooled and weighed (W3).

2.6 Determination of crude fiber content

The percent crude fiber was calculated as follows:

$$\%\ \text{Crude fibres} = \frac{W1 - W2 \times 100}{W0}$$

The sample was dried in an oven at 150°C for 1 hour and then allowed to cool in a desiccator and weighed (W1). The samples were kept in crucibles in muffle furnace at 55°C for 3-4 hours. weighed again (W2). medicinal plant material was weighed (W0 of 0.153 g)

***2.7 In vitro* methods employed in anti-diabetic studies**

2.7.1 Inhibition assay for α-amylase activity

Four concentrations of plant extract were prepared by dissolving in double distilled water. These were 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml. A total of 500µl of plant extract and 500 µl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α-amylase solution (0.5mg/ml) were incubated for 10 minutes at 25°C. After pre-incubation,500 µl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was added to each tube at 5s intervals. This reaction mixture was then incubated for 10 minutes at 25°C.1 ml of DNSA colour reagent was added to stop the reaction. These test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Finally this reaction mixture was again diluted by adding 10ml distilled water following which absorbance was measured at 540nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

3. Results

For all three plant materials were extracted by soxhlation method by using ethyl acetate and water. The yields were calculated on dry weight basis.

Table 1: Yield of crude plants extracts (mg/g dry weight)

Plant	Solvent	Yield mg/g
Manilkara hexandra Roxb.	Ethyl acetate	132.84
	Water	176.92
Strychnous Potatorum Linn.	Ethyl acetate	278
	Water	186
Salacia reticulata Wight.	Ethyl acetate	215.7
	Water	168

3.1 Proximate composition of all three plants

As depicted in table 2, the powder of *Manilkara hexandra Roxb.*, *Strychnous Potatorum Linn.,* and *Salacia reticulata* Wight. content was compared.

Table 2: Proximate composition of the dry medicinal plants

Medicinal plants	Proximate composition				
		Dry matter	Moisture	Total Ash	Crude fiber
M. hexandra	Stem bark	91.54±0.24	8.46±0.42	5.33±0.15	19.11±0.89
S. Potatorum	Dried seeds	91.44±0.46	8.56±0.80	6.60±0.06	30.99±0.89
S. reticulata	Dried bark	87.05±0.15	12.95±0.26	14.41±0.38	16.90±2.01

3.2 Phytochemical present in the plants leaves and stem barks powders

The phytochemicals detected in the powder of plants were tannins, phenolics, saponins, phylobatannins, terpenoids, flavonoids, steroids, cardiac glycosides (in trace amounts) and alkaloids.

Table 3: Qualitative screening of the phytonutrients in the medicinal plants powders

	Dry medicinal plants powders		
Phytochemicals	*M. hexandra*	*S. Potatorum*	*S. reticulata*
	Bark	Seeds	Bark
Tannins	++	++	++
Phenols	++	++	++
Saponins	++	++	++
Phylobatannins	++	++	++
Terpenoids	++	++	++
Flavonoids	++	Trace	+++
Steroids	++++	++	++
Cardiac glycosides	++++	+ trace	+
Glycosides/ reducing sugar	-	++	+
Alkaloids	+	+	+

3.3 Free radical scavenging activity by DPPH assay of powders

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. As demonstrated in table 3, *Salacia reticulata* powders had the highest radical scavenging activity of 90.93±0.40% which was higher than other medicinal plants extracts. The observed activities indicate that the plant powders have a strong proton-donating ability and could serve as free radical scavengers, acting perhaps as primary antioxidants.

Table 5: Percentage free radical scavenging activity by DPPH assay of the studied medicinal plants

Plants studied	Free radical scavenging activity by DPPH assay
Manilkara hexandra	90.85±0.40
Strychnous Potatorum	88.98±0.001
Salacia reticulata	90.93±0.03

Results are expressed as Mean ± Standard Deviation (SD) powders of the studied in the medicinal plants.

3.4 Total antioxidant activity by phosphomolybdate assay of the powders

The phosphomolybdate assay is based on the reduction of Mo (VI) to Mo (V) by the plants extracts and the ascorbic acid fractions and subsequent formation of a green phosphate Mo(V) complex at the acid pH. As depicted in table 6, the powders of *Strychnous Potatorum* were found to contain the highest amount of ascorbic acid equivalent (206.81±0.02 µg/mg), followed by *Manilkara hexandra* (115.38±0.09 µg/mg) and then *Salacia reticulata* (60.08±0.11 µg/mg).

Table 6: Total antioxidant activity by phosphomolybdate assay expressed as ascorbic acid equivalent in µg/mg of dried plant powder

Plants studied	Total antioxidant activity
Manilkara hexandra	115.38±0.09
Strychnous Potatorum	206.81±0.02
Salacia reticulata	60.08±0.11

Results are expressed as Mean ± Standard Deviation (SD)

3.5 Inhibition assay for α-amylase activity

(DNSA): The results of the DNSA study are summarized in Figure 1 and Table 1. All the above plants showed varying effect on glucose utilization. At all concentrations, *Manilkara hexandra* showed maximum inhibition of the enzyme with the highest value of 84% seen at 100mg/ml concentration of plant extract. *Salacia reticulata* showed the next highest value of 71.71% seen at 100mg/ml concentration of plant extract and *Strychnous Potatorum* showed the third highest value of 65.78% seen at 100mg/ml concentration of the plant extract.

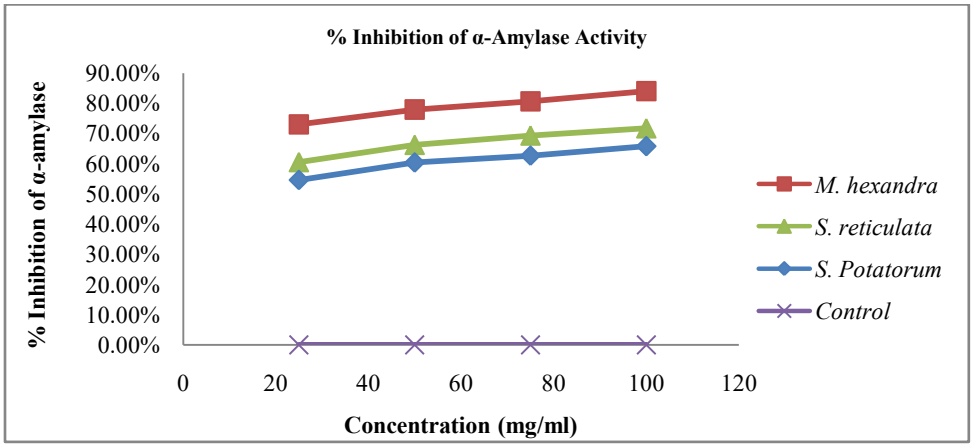

Figure 1: % Inhibition of α-amylase

Table 7: % Inhibition of α-amylase enzyme brought about by extracts of varying concentrations of *Manilkara hexandra*, *Salacia reticulate* and *Strychnous Potatorum* as compared to an aqueous control

Concentration (mg/ml)	Control %*	*M. hexandra*	*S. reticulata*	*S. Potatorum*
25	**0**	73.02%	60.52%	54.60%
50	**0**	77.92%	66.23%	60.39%
75	**0**	80.66%	69.33%	62.66%
100	**0**	84%	71.71%	65.78%

4. Discussion and Conclusions

Plants serve as an excellent source of various therapeutic agents. One of the major advantages of using plants is that they seldom show the deleterious side effects commonly associated with other allopathic drugs. This study investigated the ability of *Manilkara hexandra*, *Salacia reticulata* and *Strychnous Potatorum* to serve as effective anti-diabetic agents. The current study appears to be the first to investigate the inhibitory effects of all extracts on the activity of diabetes related carbohydrate metabolizing enzymes. Thus, further studies are needed to confirm the result of the current study and to evaluate the effect of these extracts on insulin signaling and action.

All the plants used in this study are of Indian origin and are well known by herbal pharmacologists for their medicinal properties. This study provides scientific evidence of their anti-diabetic effect. These plants are very affordable to the common man and can be easily incorporated in their daily diets. They can be further analysed to develop anti-diabetic drugs free from harmful side effects.

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