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Research Article

ASSESSMENT OF ANTIDIABETIC POTENTIAL OF TRADITIONAL MEDICINAL PLANTS IN HUMAN WHOLE BLOOD SAMPLES

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ABSTRACT

Introduction: Evaluation of antidiabetic studies was conducted on different medicinal plant products (*Trigonella foenum-graecum,* seeds; *Syzygium cumini,* seeds; *Salavadora persica,* leaves; and *Terminalia chebula,* seeds) on human whole blood samples. Since centuries revealed the presence of potent antidiabetic activity in primary and secondary metabolites of medicinal plant products.

Objective: The objective of our study is to screen these plant extracts of four different medicinal plant products on diabetic human whole blood samples.

Methods: In this study, we screened plant extracts pertaining to determine its secondary metabolites qualitatively and also analyzed its activity on diabetic human whole blood samples to determine total cellular content, free hemoglobin in the supernatant and also estimated its glucose content.

Results: The results of these studies claimed that these plant extracts showed antidiabetic effect at lower doses because of decline in total cellular content, free hemoglobin in the supernatant, and glucose content. Overall, this study claimed that all these plant extracts showed antidiabetic activity.

Conclusion: Overall, this study claimed that all these plant extracts showed antidiabetic activity.

Keywords: *Trigonella foenum-graecum, Syzygium cumini, Salavadora persica, Terminalia chebula,* Antidiabetic.

INTRODUCTION

Diabetes mellitus, an endocrine metabolic disorder, is one of the most challenging public health crises in front of the world in the 21st century. An estimated worldwide prevalence of diabetes is 387 million, i.e., approximately 8.3% and is expected to rise to 592 million by 2035 [1]. According to the recent data, the number of people with diabetes in India is around 62.4 million and predicted to be 87 million by the year 2030, making India the "Diabetes Capital" of the world [2]. Although diabetes is non-curable disease, it is controllable using various therapies such as insulin or oral antidiabetic agents. The major drawback of these currently available therapies is their side effects and prohibitive cost. Therefore, efforts are on worldwide to find more potent cost-effective drug with less or no side effects. Medicinal plants are found to have an antidiabetic property without any side effect; most importantly, due to their content of various secondary metabolites such as alkaloids, terpenoids, flavonoids, saponins, and tannins. Medicinal plants also possess the ability to restore the function of pancreatic tissues. Until now, 800 medicinal plants are reported to possess antidiabetic activity [3,4].

Medicinal plants, i.e., *Trigonella foenum-graecum, Syzygium cumini, Salavadora persica,* and *Terminalia chebula* are significant sources and its requirement for drug development. *T. foenum graecum* (fenugreek; family Leguminosae) [5], medicinal plant, especially seeds are useful in various disease therapies including tumors, cholesterolemic, inflammation, ulcer, nervous disorders, and dyspepsia. It is not only useful as a health tonic but also prevents aging, labor pain, and also improve mental function [6]. A lot of biochemical and immunopharmacological studies have already done and showed some medicinal properties as well, i.e., chemopreventive activity, antioxidant activity, anticancer activity, immunomodulatory, and gastroprotective activities [7]. In contrast, estrogenic activity also being reported in seed chloroform extract [8].

S. cumini (Jamun; family Myrtaceae) [9] is another important medicinal plant. Recent studies have demonstrated its several therapeutic roles in the treatment of diarrhea, respiratory diseases, obesity, hemorrhage, and allergic disorders [10]. As per the literature, out of four seed extracts studied, namely, the petroleum ether, chloroform extract, ethanol extract, and aqueous extract, it is found that ethanol seed extract of *S. cumini* Linn. (Jamun) has highest potential to inhibit alpha-amylase activity which is significant in the prevention of postprandial hyperglycemia [11]. *S. persica* popularly known as miswak belonging to family Salvadoraceae is an integral part of oral hygiene, especially among Muslim community. It is used as a chewing stick or toothpaste and as an endodontic irrigation solution in root canal irrigation [12]. Literature has been revealed various medicinal properties of *S. persica* including antifungal activity, antibacterial activity, antimicrobial activity, anti-inflammatory activity, analgesic activity, antihyperlipidemic and antitumor, and antibiofilm activity. Aqueous extract of leaves of *S. persica* has the ability to inhibit alpha-amylase activity potentially [13]. A king of medicine, *T. chebula* Retz. or Haritaki belongs to family Combretaceae and has extraordinary power of healing; it is effectively used to cure heart diseases, bladder diseases, gastrointestinal, and Alzheimer's Disease [14-16]. "Triphala," a polyherbal formulation containing *T. chebula,* as one of its constituents is reported to have immunomodulatory [17] and anticancer effect [18] including hepatoprotective activity [19]. In an effort to search for those medicinal plants that are responsible for antidiabetic effect in infected (diabetic; higher glucose content) human whole blood samples.

METHODS

Collection of plant material

Seeds of Fenugreek and *T. chebula* were purchased from the local market of Ahmednagar, whereas *S. cumini* seeds and *S. persica* leaves were collected from a region of Ahmednagar, Maharashtra, India. All samples were authenticated by the Department of Botany, Padmashri

Vikhe Patil College, Pravaranagar, Loni, Rahata, Ahmednagar, Maharashtra, India.

Preparation of extracts

Plant materials (*T. foenum graecum, S. cumini, S. persica,* and *T. chebula*) were ground in a grinder. Finely ground powder was then mixed with organic solvents (chloroform, ethanol, and petroleum ether; Himedia), especially for seeds of *T. foenum graecum, S. cumini,* and *T. chebula* and for the leaves of *S. persica* (Table 1). All these extracts were subjected to mechanical shaking for 3 days at room temperature in an orbital shaker. The filtrate was next filtered through Whatman No. 41 filter paper and was concentrated in a rotary evaporator. For aqueous extracts of *S. persica*, the filtrate was frozen at −77°C and finally lyophilized at −46°C. [20].

Qualitative phytochemical estimation of metabolites

The phytochemical investigation of these medicinal plants, i.e., *T. foenum graecum, S. cumini, S. persica,* and *T. chebula* was studied qualitatively using standard methods [20] as shown in Table 2. These studies revealed the presence of alkaloids, saponins, and steroids in these medicinal plants. In addition, flavonoids, phlobatannins, terpenoids, coumarins, terpenoids, anthocyanins, leucoanthocyanins, and quinones are absent in *T. foenum graecum;* phlobatannins, leucoanthocyanins, and quinones in *S. cumini;* tannins, phlobatannins, terpenoids, fatty acids, anthocyanins, and leucoanthocyanins in *S. persica,* whereas in *T. chebula,* phenolics, phlobatannins, coumarins, anthocyanins, leucoanthocyanins, and quinones are absent.

Estimation of total cellular content and free hemoglobin content

Infected (diabetic; high glucose content; n=10) human whole blood samples were collected from Mangal pathology lab. Lysed human whole blood samples (100 µl cells containing 10^5 cells/well) were cultured in 24-well flat bottom tissue culture plate for 48 hr incubation along with variable doses of test material, i.e., *T. foenum graecum, S. cumini, S. persica,* and *T. chebula*. Huminsulin 50/50 was used as a standard for these studies. Performed these studies in two different set of experiments. In first set, centrifuge the samples at 15000 rpm after 48 hr incubation and then collect the supernatant to determine the total cellular or protein content [21] using NanoDrop 1000 A280 module. In NanoDrop, Beer-Lambert equation (A=E*b*c) is applied and used for all protein calculations to correlate absorbance with concentration. In the second set of experiment, lysed diabetic human whole blood samples were treated with test material and incubate for 48 hrs and then washed with phosphate-buffered saline pertaining to observed the free hemoglobin in the supernatant. Finally, samples were analyzed through ultraviolet (UV) visible spectrophotometer at 570 nm [21].

Table 1: Extraction solvent for medicinal plant products

S. No	Plant name	Part used	Extraction solvent
1	*Trigonella foenum graecum* Linn.	Seeds	Chloroform extract
2	*Syzygium cumini* Linn.	Seeds	Ethanol extract
3	*Salavadora persica*	Leaves	Aqueous extract
4	*Terminalia chebula* Retz.	Seeds	Petroleum ether extract

Table 2: Qualitative estimation of metabolites

Phytochemicals	*Trigonella foenum graecum*	*Syzygium cumini*	*Salavadora persica*	*Terminalia chebula*
Alkaloids	+	+	+	+
Phenolics	+	+	+	-
Flavonoids	-	+	+	+
Saponins	+	+	+	+
Tannins	+	+	-	+
Phlobatannins	-	-	-	-
Terpenoids	-	+	-	+
Steroids	+	+	+	+
Fatty acids	+	+	-	+
Coumarins		+	+	-
Anthocyanins	-	+	-	-
Leucoanthocyanins	-	-	-	-
Quinones	-	-	+	-

Estimation of glucose content

Non-infected lysed human whole blood samples were cultured for 48 hrs incubation along with variable doses of glucose content. After incubation, centrifuge the samples at 2500 rpm and estimate free glucose content in the supernatant. All these readings were determined and prepared standard curve pertaining to determine the glucose content in human diabetic blood samples which is determined through nanodrop method.

Statistical analysis

The difference between control and treated group of medicinal plant products is controlled by one-way ANOVA test (Bonferroni multiple comparison test). *p<0.05, **p<0.01, and ***p<0.001.

RESULTS

Estimation of total cellular content

The effect of these plant extracts on total cellular content and free hemoglobin content as shown in Table 3. The results of these studies claimed that these plant extracts showed enhancement in total cellular content at higher doses as compared to control. The maximum effect of these plant extracts at lower doses as compared to Huminsulin 50/50.

Estimation of free hemoglobin content

In addition, similar results also observed in free hemoglobin in the supernatant containing plant extracts (Fig. 1). Overall, the results

Table 3: Effect of variable doses of medicinal plant products on total cellular content

Treatment	Doses (µg)	Total cellular content (mg/ml)	% suppression/ stimulation
Control		0.198±0.004	-
Fenugreek	25	0.097±0.001***	51.01 ↓
	50	0.158±0.002**	20.20 ↓
	100	0.284±0.012	43.43 ↑
	200	0.329±0.010	66.16 ↑
Control	-	0.198±0.004	
Syzygium cumini	25	0.059±0.001***	70.20 ↓
	50	0.147±0.001**	25.75 ↓
	100	0.228±0.002	15.15 ↑
	200	0.243±0.002	22.72 ↑
Control	-	0.198±0.004	
Salvadora persica	25	0.173±0.003**	12.62 ↓
	50	0.189±0.003*	4.54 ↓
	100	0.464±0.001	134.34 ↓
	200	0.549±0.016	177.27 ↑
Control	-	0.198±0.004	
Terminalia chebula	25	0.187±0.001*	5.55 ↓
	50	0.258±0.001	30.30 ↑
	100	0.387±0.002	95.45 ↑
	200	0.434±0.004	119.19 ↑
Huminsulin 50/50	10	0.176±0.001**	11.11 ↓

Lysed diabetic human whole blood (high glucose content) were cultured with variable doses of medicinal plant products. Total cellular content was measured after high-speed centrifugation and collect supernatant for the estimation of total cellular content. Values are expressed as mean±SE. The difference between control and variable doses of medicinal plant products is controlled by one-way ANOVA test (Bonferroni multiple comparison test). *p<0.05, **p<0.01, and ***p<0.001. SE: Standard error

claimed that these plant extracts at lower doses showed antidiabetic effect because of decline in free hemoglobin in the supernatant.

Estimation of glucose content

The effect of these test materials on glucose content in human whole blood samples as shown in Table 4. The results of these studies showed that these plant extracts showed decline in glucose content at lower doses as compared to Huminsulin 50/50.

DISCUSSION

Use of blood glucose-lowering agents is the most common strategies applied for the treatment of diabetes mellitus. Natural products being no adverse effect are the fundamental source of lead candidate that can prove as an effective antidiabetic agent. These natural products obtained from herbal preparations are easily available with lower cost [22]. Recently, Rashid *et al*., 2013, reported total 108 plant species belonging to 56 families with antidiabetic activity [23]. As per literature survey, various medicinal plants including *Gynura procumbens* (Lour.) Merr, *Abies balsamea, Alnus incana, and Vaccinium vitis-idaea* L. *V. vitis* shows antidiabetic properties by various mechanisms of action. Aqueous extract of *G. procumbens* shows the hypoglycemic effect by promoting glucose uptake by muscles cells [24]. Ethanolic extract of bark *A. balsamea* enhances insulin-stimulated glucose uptake in the skeletal muscle cells and adipocytes, whereas *A. incana* Subsp. *Rugosa* and *V. vitis-idaea* L. *V. vitis* found to inhibit glucose-6-phosphatase activity in hepatocytes [25].

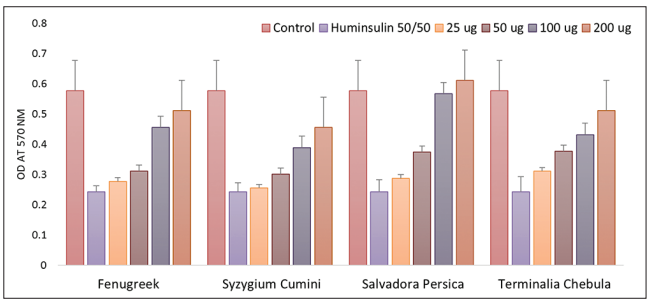

Fig. 1: Estimation of free hemoglobin content in the supernatant of diabetic human whole blood samples containing plant extracts. Lysed diabetic human whole blood (high glucose content) samples were cultured with variable doses of medicinal plant products (as described in materials and methods section). Values are expressed as mean±standard error. The difference between control and variable doses of medicinal plant products is controlled by one-way ANOVA test (Bonferroni multiple comparison test). *p<0.05, **p<0.01, and ***p<0.001

Table 4: Effect of variable doses of medicinal plant products on total glucose content in human diabetic blood samples

Treatment	Doses (µg)	Total glucose content (mg/ml)	% suppression/ stimulation
Control		1.24±0.004	-
Fenugreek	25	0.62±0.08**	50.0 ↓
	50	0.90±0.06*	27.41 ↓
	100	1.38±0.18	11.29 ↑
	200	2.50±0.44	101.6 ↑
Control	-	1.24±0.004	
Syzygium cumini	25	0.52±0.02***	58.06 ↓
	50	1.07±0.08	13.70 ↓
	100	1.63±0.10	31.45 ↑
	200	2.37±0.12	91.12 ↑
Control	-	1.24±0.004	
Salvadora persica	25	0.64±0.003**	48.38 ↓
	50	1.09±0.003	12.09 ↓
	100	1.63±0.001	31.45 ↑
	200	2.93±0.016	136.29 ↑
Control	-	1.24±0.004	
Terminalia chebula	25	0.68±0.04**	45.16 ↓
	50	1.48±0.14	19.35 ↑
	100	2.09±0.22	68.54 ↑
	200	2.67±0.24	115.32 ↑
Huminsulin 50/50	10	0.70±0.02**	43.54 ↓

Lysed diabetic human whole blood (high glucose content) were cultured with variable doses of medicinal plant products. Total cellular content was measured after high-speed centrifugation and collect supernatant for estimation of total cellular content. Values are expressed as mean±SE. The difference between control and variable doses of medicinal plant products is controlled by one-way ANOVA test (Bonferroni multiple comparison test). *p<0.05, **p<0.01, and ***p<0.001. SE: Standard error

Glycated hemoglobin (hemoglobin exposure to plasma glucose) is formed in a non-enzymatic glycation pathway. In general, the average amount of glucose content increases in diabetic person and also showed enhancement in the fraction of glycated hemoglobin (i.e., free hemoglobin in plasma) in a predictable way. In this study, treatment of variable doses of test candidates on diabetic human whole blood samples and determined total cellular content (i.e., proteins) and free hemoglobin in the supernatant through UV-visible spectrophotometer. The results showed that these test candidates declined in the total cellular content and free hemoglobin in the supernatant at lower doses as compared to Huminsulin 50/50. Overall, the results of test candidates showed antidiabetic activity in human whole blood samples.

CONCLUSION

These results provide evidence for the antidiabetic activities of four medicinal plant products. Further experimental and clinical trial studies are now warranted to investigate the benefit of these extracts for the treatment of diabetic patients.

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