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PHYTOCHEMICAL EVALUATION AND QUANTITATIVE ESTIMATION OF COROSOLIC AND URSOLIC ACID FROM *PSIDIUM GUAJAVA*

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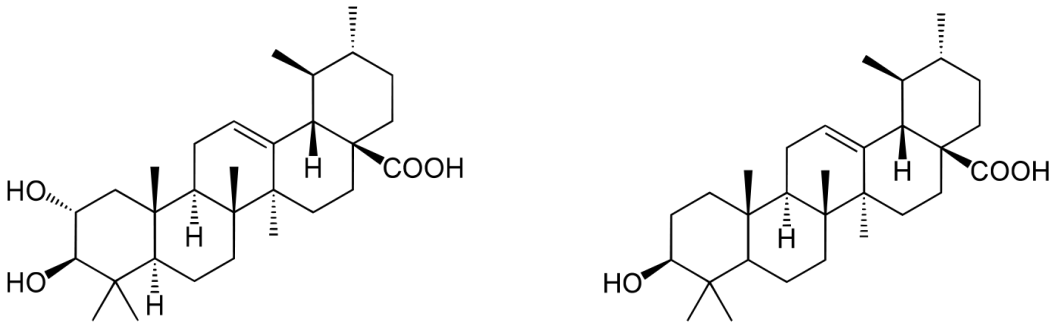
ABSTRACT

Diabetes is one of the major concerns in the twenty first century. Unhealthy lifestyles, strenuous work environments and stress are the major contributors for diabetes. Approximately 387 million people in the world, including 75 million people in India, suffer from diabetes. In addition to allopathy people are turning to traditional medicines for the treatment of diabetes. In traditional medicine there are many plants that are used to control diabetes. Some of the important plant-derived molecules for the treatment of diabetes are D-Pinitol, B-Sitosterol, Urosolic acid and Corosolic acid. Owing to the tremendous therapeutic potential of Corosolic acid and Ursolic acids they play a significant role in treatment of diabetes. *Psidium guajava* is a common fruiting tree cultivated mainly in tropical and subtropical regions of the world. It is used to treat different ailments in traditional medicine. The phytochemical analysis was performed using methanolic extract. HPTLC was used for quantitative estimation. The result reveals that in case of Corosolic acid, the maximum amount is present in the fruit (1.14 mg/g) followed by leaves (0.528mg/g). A comparison of the quantities of Ursolic acid shows that the maximum amount is present in outer bark (5.10mg/g) followed by leaves (5.05mg/g). Ursolic acid was not detected in the seed sample. *Psidium guajava* is potentially a very cost effective source of Corosolic acid and Ursolic acid for the treatment of diabetes.

KEYWORDS: Diabetes, Urosolic acid, Corosolic acid, *Psidium guajava*

INTRODUCTION

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Type 2 diabetes (formerly called non-insulin-dependent or adult-onset) results from the body's ineffective use of insulin. Type 2 diabetes comprises 90% of the occurrence of diabetes around the world, and is largely the result of excess body weight and physical inactivity. [41] There are about 387 million people in the world, including 75 million people in India, suffering from diabetes. A prediction by WHO states that more than 550 million people are likely to suffer from this disorder by 2035.[17] The allopathic mode of treatment is the most common but because of its numerous side-effects it has limited efficiency. Since ancient times, different plant based drugs have been used to treat diabetes. There are many references in Ayurveda of plants which can be efficiently used in the treatment of diabetes, such as *Aegle marmelos*, [21, 24, 32] *Allium sativum*, [11, 29] *Azadirachta indica*, [6, 8, 25] *Gymnema sylvestre*, [30, 34] *Lagerstromia speciosa*. [3, 20] Despite active research, the contribution of Ayurvedic drugs in the international market is not significant as compared to that of allopathic medicine. Using ancient literature from Ayurveda as a reference, researchers are focusing on the standardisation active compounds from different plants. Some of the major plant-derived molecules for diabetic treatment are D-Pinitol, [5, 13] B-Sitosterol, [14, 37] Urosolic acid [27, 43] and Corosolic acid. [39] Corosolic acid, the active ingredient of *Lagerstromia speciosa* extract shows decreased post-challenge plasma glucose level in humans. [12] *L. speciosa* leaves show significant hypoglycaemic activity on experimental diabetic rats through the suppression of gluconeogenesis and the stimulation of glucose oxidation, using the pentose phosphate pathway. [33] A new stearoyl glucoside of Ursolic acid, urs-12-en-3β-ol-28-oic acid 3β-D-glucopyranosyl-4′-octadecanoate showed significant reduction in blood glucose level in streptozotocin-induced diabetic rats. [23] Ursolic acid may be beneficial in preventing diabetic complications by improving the polyol pathway as well as the lipid metabolism by up-regulating glucose utilization and glycogen storage and down-regulating gluconeogenesis in the liver. [18] Ursolic acid exhibits potential anti-diabetic and immunomodulatory properties by increasing insulin levels with preservation of pancreatic β-cells and modulating blood glucose levels. [19]



Corosolic acid **Ursolic acid**

Fig.1 Chemical structures of Corosolic acid and Ursolic acid

Psidium guajava is cultivated in tropical and subtropical regions belonging to family myrtaceae. Different parts of plant are used to treat different aliments. Due to its tremendous medicinal and nutritional potential it is also called the poor man's apple. Guava contains many major phytoconstituents like pentacyclic triterpenoid Guajanoic acid, β-sitosterol, Uvaol, Oleanolic acid, and Ursolic acid, [31] alkaloids, glycosides, steroids, flavanoids, tannins, saponins. [9]

MATERIALS AND METHODS

Collection of plant material

The plant material was collected from a healthy plant in the Mumbai, Ghatkopar region. The Plant material was air dried in the shade and pulverized to a coarse powder and sieved (180 µm) and was stored in air tight containers until further use.

Preparation of methanolic and aqueous extracts

The coarse powder (3 g) of each plant part, leaves, outer bark, fruit and seed were refluxed in a soxhlet apparatus twice with 200 mL of methanol and water for 8 hrs. All the extracts were collected and vacuum dried under reduced pressure. The dried extracts were weighed in glass bottles the extract was reconstituted with ten times the volume of solvent (extract: solvent 1:10 w/v). The diluted extract was used for the preliminary analysis of phytoconstituents and quantification of Ursolic acid and Corosolic acid using HPTLC.

Preliminary phytochemical screening [7, 36, 40]

Test for Alkaloids: Methanolic and aqueous extract were warmed with 2% H_2SO_4 for two min. The extracts were filtered and few drops of Mayer's reagent were added. Formation of a yellowish precipitate indicated the presence of Alkaloids.

Test for Flavonoids: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which became colourless on the addition of dilute acid, indicated the presence of flavonoids.

Test for Phenolic compounds: The extract was diluted with 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

Test for Terpenoids: The extract was mixed with 2 mL of chloroform and concentrated H_2SO_4 (3 mL) was carefully added to form a layer. A reddish brown coloration was formed at the interface to show the presence of terpenoids.

Test for Tannins: A small volume of each extract was boiled with 5 mL of 45% (V/V) ethanol for 5min. The mixture was cooled and filtered. 1 mL of filtrate was diluted with distilled water and two drops of 10% ferric chloride were added. A transient greenish to black colour indicated the presence of Tannins.

Test for Steroids: To the test solution, 3-4 drops of acetic anhydride was added, the solution was boiled cooled and conc. Sulphuric acid (3 drops) was added. A brown ring appears at the junction of the two layers. The upper layer turns green showing the presence of steroids.

Test for Saponins: 1mL of extract was diluted with distilled water to 20 mL and shaken in a graduated cylinder for 15 min. Development of stable foam suggests the presence of saponins.

Detection of glycosides: Extracts were treated with 1 mL glacial acetic acid + $FeCl_3$ + concentrated H_2SO_4 formation of green–blue colour indicates the presence of cardiac glycoside.

Quantification of Ursolic acid and Corosolic acid by HPTLC

The samples were spotted with a Camag microliter syringe on a 100µl pre-coated silicagel aluminium plates 60 F – 254 (10 cm x 10 cm) with 250 µm thickness, (E. Merck, Darm Stadt, Germany) using a Camag Linomat IV (Camag, Switzerland) applicator. Linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (Camag, Switzerland) using mobile phase consisting of Toluene: Ethyl acetate: Glacial acetic acid (11:5:0.5). The length of the chromatogram run was 8 cm. the plate was air dried and sprayed

with 10% ethanolic sulphuric acid and was developed in a hot air oven at 110 °C for 5 min. The TLC plates were scanned using Camag TLC scanner at 540 nm, controlled by winCATS software Version 4.03. Evaluation was performed using peak areas with linear regression.

Calibration curve of standard Corosolic acid and Ursolic acid

Standard solution of Corosolic acid and Ursolic acid (1mg/mL each) prepared in a methanol (stock solution). Standard working solutions were prepared by diluting standard stock solution with methanol in the concentration range 50–200 µg/mL (Corosolic acid) and 100–1000 µg/mL. Each working standard solution was spotted on the TLC plate to obtain final concentration range 0.25–2.5 µg/spot (Corosolic acid) and 2–10µg spot (Ursolic acid). Calibration curves were generated by linear regression based on the peak areas.

Estimation of Corosolic acid and Ursolic acid

To estimate the amount of Corosolic acid and Ursolic acid from *Psidium guajava*, 4 µl of extracts for Leaf, Seed, Fruit, Outer bark, standard Ursolic acid and Corosolic acid were spotted on the TLC plates (band length 8mm). The plate was run using solvent system(Toluene : Ethyl Acetate: Glacial Acetic acid in ratio 11: 9: 0.5) till 8cm after then it was dried and sprayed with 10% Ethanolic sulphuric acid reagent and was developed at 110 °C for 5 min. The plate was scanned at 540 nm wavelength of light using CAMAG TLC scanner. The amount of Corosolic and Ursolic acid was calculated according to the calibration curve.

RESULTS

Table 1 (Phytochemical analysis of *Psidium guajava)*

Test	Leaves		Outer bark		Seed		Fruit	
	Methanol	Water	Methanol	Water	Methanol	Water	Methanol	Water
Alkaloid	+	+	+	-	+	+	+	-
Tannins	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	-	+	-	+
Steroids	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	-	+	-
Phenolics	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	-	+	+	+	-
Glycosides	+	+	+	+	+	+	+	+

Table 2 Quantitative estimation Of Corsolic acid and Ursolic acid From *Psidium guajava*

Plant part	Corosolic acid(mg/g)	Ursolic acid (mg/g)
Leaf	0.528	5.050
Seed	0.420	ND
Fruit	1.140	0.171
Outer bark	0.404	5.100

The total amounts of Corosolic acid and Ursolic acid were calculated using their respective calibration curves. The results reveal that in case of Corosolic acid the maximum amount was present in the fruit (1.14 mg/g) followed by leaves (0.528mg/g). In the case of Ursolic acid, the maximum amount was present in the outer bark (5.10mg/g) followed by leaves (5.05mg/g). Ursolic acid was not detected in seed sample. The detailed result is shown in (Table no 2.).

DISCUSSION

The occurrence of diabetes is alarmingly widespread in India, and it is likely to increase in the coming years. [15] Diabetes has been shown to occur across various sections of the population and across all economic classes. [2] There are several allopathic formulations, which are available in the market for the treatment of diabetes. However, on account of the side effects of allopathic treatments such as hypoglycaemia, weight gain, lactic acidosis etc. [10] alternative sources of medicine like Ayurveda, which is an ancient plant-based therapy, are being explored. Ursolic acid and Corosolic acid are the two major plant-derived active ingredients which can be successfully used in the treatment of diabetes. Corosolic acid shows decreased post-challenge plasma glucose level in humans. [12] They also show significant hypoglycaemic activity on experimental diabetic rats through the suppression of gluconeogenesis and the stimulation of glucose oxidation, using the pentose phosphate pathway. [33] A new stearoyl glucoside of Ursolic acid, urs-12-en-3β-ol-28-oic acid 3β-D-glucopyranosyl-4′-octadecanoate, showed a significant reduction in the blood glucose level in streptozotocin-induced diabetic rats. [23] Ursolic acid may be beneficial in preventing diabetic complications by improving the polyol pathway as well as the lipid metabolism by up-regulating glucose utilization and glycogen storage and down-regulating gluconeogenesis in the liver. [18] Ursolic acid exhibits potential anti-diabetic and immunomodulatory properties by increasing insulin levels with the preservation of pancreatic β-cells and modulating blood glucose levels. [19] There are many plants from which Corosolic and Ursolic acid are extracted such as *Vaccinium macrocarpon*,[26] *Ugni molinae*,[1] *Eriobotrya japonica*,[16]

Perilla frutescens,[4] *Weigela subsessilis*,[38]*Glechoma longituba*,[42] *Potentilla chinensis*,[35] *Rubus biflorus*,[22] and *Phlomis umbrosa*.[28] A majority of the plants listed above are not native to India, and many of them are not available in India. Guava, in India, is easily available and is considered among the relatively inexpensive fruits. The present study shows that considerable amounts of Corosolic acid and Ursolic acid are present in the fruit (1.140/ 0.171 mg/g), leaf (0.528/ 5.050 mg/g), seed (0.420/ ND mg/g) and outer bark (0.404/ 5.100 mg/g). The significance of the present study lies in the fact, that it quantifies the amounts of Corosolic acid and Ursolic acid that are present in different parts of the guava plant whose fruits have been traditionally used to manage diabetes. The present study justifies the use of a traditional method to manage diabetes through the means that are acceptable to modern medicine using the standard methods applicable to modern medicine.

CONCLUSION

The results indicate that *Psidium guajava* is a potentially rich source of Corosolic acid and Ursolic acid. Considering that the number of patients suffering from diabetes is showing tremendous growth across economic groups. [2] *Psidium guajava* has the potential to become a low cost alternative to expensive medicines. The very wide spread, easily available and inexpensive resources like *Psidium guajava* can make a significant contribution to the cost effective management of diabetics.

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