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SHORT COMMUNICATION

Phytochemical and Ethnomedicinal Assessment of Medicinal Plant *Gymnema sylvestre*

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Abstract

Background: Diabetes is one of the major disorders around the world. In recent times mortality rate increased due to diabetes which is comparatively low in tribal habitations. **Aim:** The objective of the present investigation was to identify the phytochemical components of *Gymnema sylvestre* (Retz.) R. Br. ex Sm, an ethnomedical plant used to treat diabetes, in the ethno-habituated hills of the Eastern Ghats of India. Based on the research design, work was taken up to find bioactive compound percentage. **Methods:** Semi-structured interviews were conducted to collect ethnomedicinal data from 20 tribal healers. Leaf extracts using appropriate solvent solutions were examined in order to determine the active components. A Soxhlet apparatus configured for continuous hot extraction using 90% methanol, was used to extract gymnemic acid from the defatted plant parts using appropriate solvents. Conventional analytical methods were used to measure gymnemic acid, and preparative chromatographic methods were used to purify it. Phytochemical analysis was used to characterize the species. **Results:** Very little work has been documented in East Godavari. The highest concentration of gymnemic acid was found in the leaves of the ecotype plant generated from Maredumilli-1 (40.3%). Notwithstanding the inconsistent findings, the current study shows that the content of gymnemic acid is considerably high. Bioactive substances such as alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, quinones, anthraquinones, glycosides, *etc*., have been identified by phytochemical analysis. **Conclusion:** The study reveals that the most commonly utilized ethnomedicinal plant in the study area for diabetes treatment is *G. sylvestre*. Numerous bioactive components were found in the phytochemical data. Gymnemic acid concentration in wild species is the highest % ever recorded. It is advised that *in vivo* anti-diabetic benefits be studied and tested further.

Major Findings: Gymnemic acid is more abundant in plant species found in tribal areas, according to a phytochemical analysis of the current study. The research area's high-altitude highland regions contain highly enriched compositions.

Keywords: Diabetes, Ethnobotany, Gymnemic Acid, Medicinal Plant, Phytochemical

1. Introduction

Diabetes is one of the major disorders around the world. In recent times mortality rate increased due to diabetes which is comparatively low in tribal habitations[1]. Medicinal plants and herbs are good sources of alternative and complementary medicine, with a substantial role in illness treatment[2]. Recent studies have revealed that *G. sylvestre* extract can help manage blood sugar levels and treat Type II diabetes. It boosts insulin-producing β-cells in the pancreas and inhibits sugar absorption after digestion. It has also been shown to have anti-haemolytic, antibacterial, and anti-cancer properties. It has also demonstrated hepatotoxic properties[3].

Gymnema sylvestre (Family: *Asclepiadaceae*), is locally called as *podapatri* in Alluri Sitharama Raju (erstwhile East Godavari) district is also known as *Gurmar* or 'sugar destroyer', is a woody, climbing traditional medicinal herb found in central and peninsular India with numerous therapeutic applications in the *Ayurvedic* school of medicine[4]. It is regarded as a plant with significant medicinal characteristics and is utilized in folk, ayurveda, and

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homeopathic medical systems. It is also used to treat asthma, coughs, eye problems, inflammations, family planning, and snake bites. Furthermore, it has antibacterial, diuretic, stomach-ache, anti-hyper cholesterolemic, hepato protective, and anti-saccharine properties[5,6].

The leaves of *G. sylvestre* contain triterpene saponins, which are classified as dammarane and oleanane. Dammarane saponins are gymnemosides, while oleanane saponins comprise gymnemic acids and gymnema saponins. Flavones, anthraquinones, hentriacontane, pentatriacontane, α and β-chlorophyll, phytin, resins, d-quercitol, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin glycosides, and stigmasterol are additional plant chemicals. Alkaloids were also detected in the plant extract. Gymnemic acids, one of *G. sylvestre's* bioactive ingredients, exhibit anti-inflammatory, anti-saccharine, and anti-diabetic properties[7-9]. Because it contains phytochemicals including gurmarin, gymnemic acid, and gymnema saponins, it has the ability to reduce blood sugar levels. Antioxidant, antibacterial, anti-inflammatory, antiviral, gastro and hepatoprotective, anticancer, and lipid-lowering qualities have also been demonstrated for *G. sylvestre*[10-11].

Recently, hydroxyl free radical scavenging activity and considerable antioxidative potential of this plant against DPPH (2,2-diphenyl-1-picrylhydrazyl) were reported[12]. Both antioxidant and antidiabetic functions are beneficial in treating Type 2 diabetes. Other antioxidants such asterpenoid, flavonoid, cinnamic acid, folic acid, tannin and phenol, have both antioxidant and antidiabetic properties[13-18]. Antidiabetic components of *G. sylvestre* exhibit antioxidant activity and antioxidants exhibit antidiabetic potential and the literature citations are depicted in Table 6[19-24].

Gymnema sylvestre activates the pancreatic β cells of Langerhans[25,26]. *G. sylvestre* however, might be detrimental to the liver. Using *G. sylvestre* without a doctor's supervision may be dangerous[27]. The use of *Gymnema*-based food supplements in addition to (or instead of) an approved antidiabetic medication is recommended due to the uncertainties surrounding the composition of different *Gymnema* preparations, possible herb-drug interactions, and worries about glucose-lowering or hypoglycemic effects. Information about the effectiveness of *G. sylvestre*, a traditional medicine plant with varying gymnemic acid levels, in treating diabetes in tribal areas of Andhra Pradesh, India's East Godavari District is scarce. As a result, we make an effort to partially address the problem in this work.

2. Materials and Methods

2.1 Study Area

The study locations are 15 in number in hilly tribal areas of East Godavari district (Figure 1). The study area is rich in wild herbs, and many of the peoples of this region have rich traditional knowledge. Many of the local peoples have significant traditional knowledge. To treat a variety of human ailments, the majority of people in the hilly tribal area employ traditional medical practices that involve indigenous medicinal plants.

2.2 Data Collection

The present study primarily focused on a single plant *G. sylvestre* (Figure 2) is commonly used to cure diabetes. A somewhat structured questionnaire about medicinal plants used to treat diabetes was employed for the study. Demographic profile of the informants, plants used for curing diabetes, methods of acquiring the knowledge, cure percentage *etc*. With the help of local translators, a series of questions were posed in their mother tongues (Koya and Telugu). The questions were broken down into the following categories: The informants demographic profile, diabetes-curing plants, information acquisition strategies, cure rate, *etc*.

2.3 Demography of Informants

A total of 20 key informants (16 men and 4 women) were interviewed. The ages of the respondents ranged from 32 to 72 years (Table 1). Most of the respondents were men compared with women in this area. In the study area, there are about 5 tribal groups residing in surrounding villages. In some of the tribal communities, the recipe is considered proprietary.

2.4 Plant Identification

Gymnema sylvestre belongs to *Asclepiadaceae* family and locally called Podapatri. *G. sylvestre* (Figure 2) large woody perennial vine-like climber, climbs on tops of shrubs and trees. Stems are 8m, sparsely lenticellate;

Table 1. Demographic profile of the informants of the tribal hill areas

S. No.	Name of the Tribal Physician	Gender	Age (Years)	Community
1	T. Subba Rao	Male	68	*Konda kammara*
2	N. Raja Rao	Male	42	*Konda reddy*
3	Kanem Thamma Rao	Male	54	*Konda dora*
4	Saramkota Basava Reddi	Male	59	*Konda reddy*
5	GorleVinasa Rao	Male	51	*Valmiki*
6	Ganta Chinna Rao	Male	55	*Konda kammara*
7	Vetla Lacchi Reddi	Male	56	*Konda reddy*
8	Kattula Rami Reddi	Male	72	*Konda reddy*
9	Vajjam Baludora	Male	45	*Koya dora*
10	Koyyala Muniya	Female	48	*Konda kammara*
11	Vasam Satyavathi	Female	55	*Valmiki*
12	Shambhoji Mysareddy	Male	48	*Konda reddy*
13	Jammala Kannayya	Male	55	*Konda reddy*
14	Para Bullemma	Female	72	*Konda kapu*
15	Vanka Muthayya	Male	52	*Konda kapu*
16	Kuram Laxmayya	Male	32	*Konda kammara*
17	Madakam Ramanna Dora	Male	39	*Koya dora*
18	Mirthivada Latchi Reddy	Male	39	*Konda reddy*
19	V. Venkateswara Rao	Male	43	*Valmiki*
20	Kangala Ramana	Female	38	*Koya dora*

Table 2. Sample collection areas in Southern Eastern Ghats hills of East Godavari district

S. No.	Different Regions	Sample Collection Location with Coordinates		
		Place	Latitude (N)º	Longitude (E)º
1	Addateegala-1(GS-1)	Kottampalem	17º 27.98′	82º 07.270′
2	Addateegala-2(GS-2)	Vetamamidi	17º 28.698′	82º 00.011′
3	Addateegala-3(GS-3)	Addateegala	17º 28.813′	82º 01.498′
4	Maredumilli-1(GS-4)	Maredumilli forest	17º 35.547′	81º 42.876′
5	Maredumilli-2(GS-5)	Vamuleru	17º 38.023′	81º 38.133′
6	Maredumilli-3(GS-6)	GMValasa	17º 31.323′	81º 43.438′
7	Rampachodavaram-1 (GS-7)	Rampachodavaram forest	17º 25.983′	81º 46.662′
8	Rampachodavaram-2 (GS-8)	Folkspeta	17º 18.804′	81º 50.401′
9	Rampachodavaram-3 (GS-9)	Komaravaram	17º 20.663′	81º 49.207′
10	Y-Ramavaram-1 (GS-10)	Chavididibbala	17º 37.092′	81º 59.025′
11	Y-Ramavaram-2 (GS-11)	Dalipadu	17º 36.361′	81º 51.379′
12	Y-Ramavaram-3 (GS-12)	Chirumanuveedhi	17º 39.500′	81º 56.956′
13	Rajavommangi-1 (GS-13)	Rajavommangi forest	17º 32.691′	82º 14.133′
14	Rajavommangi-2 (GS-14)	Vattigadda	17º 30.782′	82º 11.587′
15	Rajavommangi-3 (GS-15)	Jaddangi	17º 28.766′	82º 09.387′

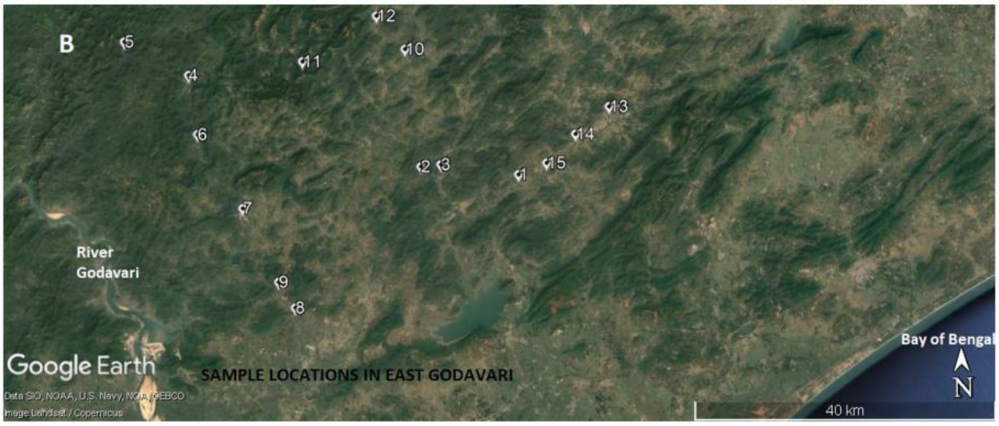


Figure 1. Map showing locations of sample collection (Table 2).

young branchlets are pubescent. Leaves ovate, elliptic or ovate-lanceolate. Petiole 3-12 mm; leaf blade obovate to ovate, 3-8.5 × 1.5-5.5 cm, thick, adaxially pubescent to glabrous except for groove of midvein, lateral veins 4 or 5 pairs, convergent. Flowers minute, in axillary pedunculate cymes. Cymes are much shorter than leaves, pubescent; pedunculate, and rachis with scars. Sepals 5. Corolla greenish white, united, 5. Ovary bicarpellary, stigma head exserted. Follicles single-beaked at apex, mostly solitary, broadly lanceolate in outline, 5-9 × ca. 2 cm. Flowering and fruiting: September-December.

2.5 Plant Sample Collection, Locations and Identification

Gymnema sylvestre leaves were harvested in hilly tribal areas listed in Table 2 by indigenous herbal practitioners in zip lock polythene bags from August to December, 2023 and brought to the lab for phytochemical analysis. The geographical parameters of the study region are measured using a Garmin GPS instrument (model: Oregon 550). The plant was recognized using floras and authenticated by the Department of Botany at Government Autonomous College, Rajamahendravaram, AP, India[28,29].

Details of the study area are shown in the maps below. Based on the coordinates, sample collection map was prepared.

2.6 Phytochemical Evaluation

2.6.1 Sample Processing and Extraction

Fresh *G. sylvestre* leaves were dried in the air for 15 days in the shade at room temperature. An electrical grinder is used to pulverize the dried material into a coarse powder.



Figure 2. *G. sylvestre* plant.

The dried leaf powder (1000g) was extracted using a Soxhlet apparatus separately with n-hexane, ethyl acetate, chloroform, and methanol solvents in their pure form for approximately 12-15 hours at room temperature. Finally, crude extracts of various solvents were concentrated in a vacuum rotary evaporator (Buchi Labortech Ag, model l, R-215) using reduced pressure. The concentrates of various solvent extracts were refrigerated at 4-5 °C until usage. Phenols, flavonoids, terpenoids, steroids, tannins, saponins, quinones, anthraquinones, glycosides, alkaloids, and xanthoproteins were all measured after the dried *G. sylvestre* leaves from all the locations were combined, processed, and examined (Table 3). Individual leaf samples taken from different locations were used to determine gymnemic acid (Table 3).

2.6.2 Preliminary Phytochemical Screening

Using standard methods and testing techniques, as enumerated in literature, like phenolic test, steroid test,

glycoside test, the dried extracts of different solvents, including hexane, chloroform, ethyl acetate, and methanol, were first screened[30-45].

2.6.3 Extraction and Conformation of Gymnemic Acid (GA)

Hoopers's and other standard procedures were followed for the extraction of gymnemic acid as enumerated in latest literatures[46-52].

2.6.3.1 Extraction of Gymnemic Acid by Hooper's Method

- **Step1:** Extraction with petroleum ether: 1 kg of dry leaf powder was packed into a clean Soxhlet extraction unit. Seven liters of petroleum ether (60-80^0 C) was added and extracted for 24-36 hours till all the components are soluble in petroleum. Petroleum extract is collected and distilled in a distillation unit. Then a net weight of 250 gm of petroleum ether extracts was obtained. Petroleum ether extraction was used for defatting dried leaf powder.
- **Step 2:** Extraction with 90% methanol: The plant material is then extracted with 90% methanol. 90% methanol was added and the extraction was carried out for 24-36 hours till the total methanol soluble extract was obtained. The methanol soluble extract was distilled and finally 175 gm of the thick paste were obtained.
- **Step 3:** Isolation of pure gymnemic acid from methanol extract: 175gm thick paste of methanol soluble extract was dissolved in 1% aqueous KOH solution on continuously stirring for 45 min to 1 hour. The solution is then filtered through filter paper to separate the un-dissolved particles. Diluted HCl was added slowly under constant stirring, during which the gymnemic acids were precipitated. Precipitated solution was filtered under suction and precipitate was dried. The pure gymnemic acid was obtained.

2.6.4 The Following Color Tests were Used to Confirm Gymnemic Acid

2.6.4.1 Phenolic Test

Two milliliters of methanol were added to a clean test tube along with a pinch of gymnemic acid. After adding a few drops of 1% alcoholic ferric chloride, a dark blue color develops, indicating a positive phenolic test result.

2.6.4.2 Steroid Test

A solution of 2 ml of chloroform and 1 ml of acetic anhydride was mixed with a pinch of gymnemic acid.

Table 3. Ecotype evaluation of *G. sylvestre*

Eco types	Plant Height (cm)	No. of Laterals-1vine	No. of Leaves -1lateral	Leaf Length (cm)	Leaf Breath (cm)	Leaf Area (cm^2)	Single Leaf Weight (gm)	100 Leaves Weight (gm)	Fresh Leaf Yield -1plant (kg)	Dry Leaf Yield -1plant (kg)
GS-1	114.05	19.55	47.05	2.85	2.40	4.00	0.19	20.03	853.1	586.10
GS- 2	133.35	29.28	46.44	4.95	4.21	8.80	0.31	31.70	894.6	588.46
GS-3	148.14	32.07	74.61	5.79	4.92	10.72	0.36	36.74	1016.7	644.94
GS-4	158.28	41.14	80.87	5.58	4.74	10.24	0.32	32.96	1186.2	830.82
GS-5	168.42	47.42	65.22	4.94	4.02	8.32	0.30	30.44	1140.4	809.04
GS-6	153.49	46.37	62.09	4.53	3.84	7.84	0.29	29.18	925.18	802.08
GS-7	118.56	35.56	58.96	4.32	3.66	7.36	0.37	27.92	909.92	595.52
GS-8	113.63	44.63	55.83	5.16	4.38	9.76	0.34	34.22	970.96	623.26
GS-9	118.70	34.21	77.74	4.41	3.48	6.88	0.26	26.66	848.88	597.88
GS-10	133.21	23.70	52.70	3.90	3.30	6.40	0.25	25.40	879.40	588.45
GS-11	103.22	23.37	49.57	3.69	3.12	5.92	0.94	24.14	864.14	578.67
GS-12	143.87	28.35	68.35	5.37	4.56	9.28	0.35	35.48	1001.4	637.88
GS-13	118.84	21.84	71.48	3.48	2.94	5.44	0.22	22.80	955.70	616.70
GS-14	113.91	22.91	43.31	3.27	2.76	4.96	0.20	20.36	844.62	580.72
GS-15	96.78	29.48	40.48	3.16	3.28	4.58	0.21	21.62	838.36	575.46

Concentrated sulfuric acid was introduced in little drips *via* the tube walls. A ring of pink or red color forms, indicating a positive test result for the presence of steroids in the gymnemic acid.

2.6.4.3 Glycoside Test

In a dry test tube, a pinch of gymnemic acid was dissolved in 2ml of methanol. 1 ml of α-naphthol alcoholic solution was poured from the test tube edges. The presence of glycoside is shown by the formation of a bluish red ring at the intersection of the two layers.

2.6.5 Thin Layer Chromatography (TLC)

TLC was utilized to identify and separate the constituents detected in various *G. sylvestre* preparations. After cooling and drying the chromatograms to get rid of the solvent, the detecting reagents were sprayed on them. Spots developed on the dried plates. Studies using thin-layer chromatography were conducted using different solvent systems. Each gymnemic acid sample produced spots with various R_f values (Table 5). In comparison to the other solvent systems, the Chloroform: Methanol (6:5) solvent system produced superior results. According to TLC investigations, the profiles are comparable to those of normal gymnemic acid, which has an R_f of 0.71.

3. Results

A total 20 informants were interviewed. All the respondents (local tribes) expressed that *G. sylvestre* is the well-known plant species and available source to treat diabetes. However, the native healers preferred to keep the details to the minimum possible and conveyed that the composition of the medicine is proprietary to them. Many of the respondents

Table 4. Phytochemical screening of powdered leaves of *G. sylvestre* in different solvents

S. No.	Chemical Species	Methanol	Chloroform	Hexane	Aqueous
1	Phenols	+	+	+	+
2	Flavonoids	+	-	-	-
3	Terpenoids	-	+	+	-
4	Steroids	-	+	-	-
5	Tannin	-	-	+	+
6	Saponin	-	-	-	+
7	Quinones	-	+	-	-
8	Anthraquinones	+	-	-	-
9	Glycosides	+	-	-	-
10	Alkaloids	+	+	-	+
11	Xanthoproteins	+	-	-	-

Note: (+) - presence; (-) - absence

Table 5. R_f values with corresponding percentage of gymnemic acid from different ecotypes

S. No.	Ecotype Name	Sample Location	R_f Value	Gymnemic Acid %
1	Addateegala-1	Kottampalem	0.69	39.2
2	Addateegala-2	Vetamamidi	0.72	38.3
3	Addateegala-3	Addateegala	0.71	37.1
4	Maredumilli-1	Maredumilli forest	0.72	40.3
5	Maredumilli-2	Vamuleru	0.68	40.1
6	Maredumilli-3	GMValasa	0.67	40.2
7	Rampachodavaram-1	Rampachodavaram forest	0.69	31.3
8	Rampachodavaram-2	Folkspeta	0.70	36.7
9	Rampachodavaram-3	Komaravaram	0.70	34.8
10	Y-Ramavaram-1	Chavididibbala	0.71	28.7
11	Y-Ramavaram-2	Dalipadu	0.67	32.8
12	Y-Ramavaram-3	Chirumanuveedhi	0.74	23.9
13	Rajavommangi-1	Rajavommangi forest	0.74	32.8
14	Rajavommangi-2	Vattigadda	0.72	38.7
15	Rajavommangi-3	Jaddangi	0.67	39.9

Table 6. Reported chemical constituents and pharmacological activities of *G. sylvestre*

S. No.	Chemical Entity	Reported Biological Activity
1	Terpenoid	Cytotoxic and anti-tumour activity[12]
2	Saponins	Anti-diabetic activity[11]
3	Gurmarin	Sweet-taste and suppressing activity[3]
4	Sterols	Reduction of cholesterol and anti-cancer activity[14]
5	Triterpene, β- amyrin related glycosides, Saponins	Anti-viral activity[4]
6	Anthraquinones	Anti-inflammatory and antinociceptive activity[10]
7	Lupeols	Anti-cancer and anti-inflammatory activity[13]
8	Quercitol	Antiviral activity[15]
9	Flavanol glycosides	Anti-cancer and anti-inflammatory activity[12]
10	Triterpene Saponins	Cytotoxic activity, anti-microbial activity[10]
11	Dammarene Saponins	Anti-tumour activity.[16]

conveyed that they acquire knowledge from their ancestor family members.

Preliminary phytochemical screening of aqueous and solvent extracts of *G. sylvestre* leaves revealed the presence of various secondary metabolites such as alkaloids, glycosides, steroids, lipids, saponins, phenols, and flavonoids (Table 4). The documented evidence on *G. sylvestre* metabolites and extracts shows their efficacy against several human ailments (Table-6). The phytochemical study of several organic extracts of *G. sylvestre* reveals fatty acids, secondary alcohols, diketones, esters, and phenols. The majority of the secondary metabolites found in methanol extracts of the plant are physiologically active.

The extraction with 90% methanol produced the highest quantity of gymnemic acid. Gymnemic acid yields from fifteen ecotypes were determined and presented in Table 5.

4. Discussion

Gymnemic acid level in all of these ecotypes ranges between 23.9 and 40.3%, according to studies. Ecotypes from the Maredumilli forest area, which is within the Maredumilli mandal of the East Godavari district, had the greatest proportion of gymnemic acid: Maredumilli - 1 had 40.3%, Maredumilli - 3 had 40.2%, and Maredumilli - 2 had 40.1%. Examining the existing research in this region, relatively little study has been conducted. A similar investigation published elsewhere revealed a gymnemic acid content ranging from 23% to 40%[50]. When the mean of all ecotypes is evaluated, the current study's results are superior. The research area's high-altitude hill areas contain highly enriched content. Other reasons like, wild nature, superior soil nutrients available, high precipitation, longevity in life cycle, would be the reasons behind high chemical constituents.

5. Conclusion

Documentary evidence on *G. sylvestre* metabolites and extracts showed their efficacy against various human diseases, so the knowledge can be used to control or cure diseases. The phytochemical screening of *G. sylvestre* leaves from the study area revealed the presence of various secondary metabolites. Studies have shown that the levels of gymnemic acid in each of these ecotypes range from 23.9% to 40.3%. The highest percentage of gymnemic acid was found in ecotypes from the Maredumilli forest region, which is located within the Maredumilli mandal of the East Godavari district: 40.3% for Maredumilli-1, 40.2% for Maredumilli-3, and 40.1% for Maredumilli-2.

A similar study published elsewhere found that the gymnemic acid content ranged between 23% and 40%. When the mean of all ecological types is evaluated, the current study's results are superior. The research area's high-altitude highland regions contain highly enriched compositions. Gymnemic acid is more abundant in plant species found in tribal areas, according to a phytochemical analysis of the current study. As a

result, many indigenous peoples use this plant as an effective ethnomedical treatment for diabetes. Further research and testing on *in vivo* anti-diabetic benefits is recommended.

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7. Author Contributions

Venkata Rao Basa – Sample collection, investigation, experimentation, and writing the original maunscript; Srinivasa Rao Tirunagari – Reviewing, editing, conceptualisation, experimentation, and supervision. All authors have read and agreed to the published version of the manuscript.

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