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In Vivo Antidiabetic Activity and GC-MS Analysis of Ethanolic Extracts of *Rabdosia Rugosa* (Wal. ex Benth.) H. Hara.

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ABSTRACT

The current study examined the effects of an ethanolic extract of *R. rugosa* (*Rabdosia rugosa* (Wall. ex Benth.) H. Hara) on alloxan-induced diabetes rats, detailing its hypoglycemic potential and using gas chromatography-mass spectrometry to analyze its phytoconstituents. The FTIR spectrum verified the presence of various functional groups in the active ingredients. This research opted to use an extract from fresh leaves and flowers. Chichiri is the local name for the shrub *R. rugosa*, which belongs to the sage family (Labiatae) and is used as traditional medicine. To determine the hypoglycemic activity of plant extracts the rats were randomly assigned to one of four treatment groups for two weeks of experiments: a normal control group that received no treatment, a diabetic control group that received only alloxan (150 mg/kg BW), a drug control group that received glibenclamide (5mg/kg BW), and a treatment group that received *R. rugosa* extract (50 mg/kg BW). Our results demonstrated that the extract and medication group saw statistically significant improvement ($p \leq 0.001$) in body weight, blood glucose levels, lipid profile, liver and renal parameters. The GCMS analysis showed that numerous active phytoconstituents were present. Phenols, alkanes, alcohols, and other compounds were detected in the FTIR spectrum. After examining the data, we determined that the leaves and inflorescences of *R. rugosa* have hypoglycemic potential. Continued study of the naturally separated chemicals can aid in the creation of organic medications for diabetic treatment.

Keywords: Antidiabetic; *Rabdosia rugosa*; Alloxan monohydrate; GCMS; FTIR

1. INTRODUCTION

Diabetes mellitus (DM) has always been a great concern of global health issues. It is a long-term, complicated severe disorder with diverse and complex aetiology, which often causes devastating outcomes. As per the latest report of the IDF 2021 (International Diabetes Federation), Over 537 million people worldwide between the ages of 20 and 79 would have been impacted by DM. By 2030, the population will increase to over 643 million, and by 2045, it will be almost 783 million (IDF Atlas tenth edition 2021). According to the IDF Report 2022, people with diabetes had a higher rate of hospitalization and death due to COVID-19 infections as compared to non–diabetic patients.

DM is a persistent insulin resistance syndrome described by hyperglycemia with the dysfunction of carbohydrate, fat, and protein metabolism. An aberrant concentration of glucose in the blood plasma causes insulin insufficiency or diminished insulin action. Insulin is an indispensable hormone that stabilizes the metabolism of the human body. It is secreted by the pancreatic beta cells, which aid in restraining irregular spikes of glucose in our bloodstream[2]. An increased risk of diabetes is primarily caused by adopting destructive lifestyles, including smoking, eating junk food, and engaging in less physical exercise. If treatment is delayed, it will negatively impact the physiological processes of different organs, including the heart, liver, pancreas, kidneys, nervous system, etc. It can be treated using various pharmacological synthetic drugs, such as insulin and other antidiabetic drugs. These recommended allopathic medications could control diabetes but can also cause severe side effects, such as hypoglycemia, gastrointestinal disorder, and other obstacles. Due to these disruptive side effects, alternative treatments are required with minimum side effects. Indeed, herbal medicines are the most suitable alternative to synthetic ones as they are more suitable to human physiological functions, have easy availability, and have few side effects.

Considering the tremendous benefits of natural remedies, the focus on folk medicinal plants with antihyperglycemic activity is enhanced. Different phytoconstituents are found in medicinal plants with numerous bioactive compounds with magnificent antioxidants, antidiabetic, anticancer, antiaging, and other medicinal aids. These elements synergistically interact with the body. If taken as recommended by a licensed health professional, they seldom give any negative side effects.

R. rugosa is a bushy shrub with a pleasant aroma indigenous to the Himalayas in Nepal, India, Pakistan,

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Afghanistan, southeast Arabia, and southwest China. It is dominated in the community on dry mountainous slopes at lower altitudes ranging between 1500 – 3000 m. Previous research mainly focused on the essential oil of leaves and inflorescence. The occurrence of monoterpenoids and triterpenoids, beta-sitosterol, ursolic acid, oleanolic acid, botulin, and hexacosanol in leaf extracts was reported in previous investigations. In a previous study, *in vitro* investigation of the essential oil of *R. rugosa* revealed that it had anti-inflammatory, analgesic, antipyretic, myorelaxant, antibacterial, and antifungal action.

A recent research work was carried out on ethanolic extract (leaf and inflorescence) of *R. rugosa*. We chose frequently prescribed plant parts for the treatment of diabetes based on traditional knowledge obtained from local healers. They used a decoction of leaves and inflorescence twice a day to cure diabetes. The current study is a follow-up to our earlier work, in which we described the *in vitro* antioxidant and antidiabetic evaluation of *R. rugosa* ethanolic extract. Hence, in the current research, we have assessed the plant sample's in vivo hypoglycemic potential and identified the bioactive compounds by the GCMS method and a significant class of the compounds by FTIR spectrum.

2. METHODOLOGY

2.1 Utilized Chemicals

Alloxan monohydrate of Sigma-Aldrich, glucose solution, and sodium chloride was purchased from GK enterprises (Chandigarh, India), commercial kits (for lipid, renal, and liver parameters) by Reckon private limited diagnostics.

2.2 GC-MS Analysis

Phytoconstituents in *R. rugosa* were identified using the GC-MS method. The sample was introduced employing a glass injector with helium gas functioning in split form. Shimadzu QP 2010 ultra-mass spectrometer analysis was done with Rtx - 5MS, length 30M, and diameter 0.25 mm column. For the analysis, a column flow rate: of 1.00 ml/min, a total rate of flow: of 14.0 ml/min, a flow pressure: of 61.3 kPa, an injector 280 ℃ of temperature, and 230 ℃ of an ion source temperature were used for analysis.

2.3 Fourier Transform Infrared Scrutiny

The FTIR spectra were performed by using pellets of potassium bromide. The spectrometer Perkin Elmer Spectrum 400 was used to identify the various functional groups. The adsorption spectrum was between 400 to 4000 cm-1.

2.4 Collection

The sample was collected in Ralli village in Himachal Pradesh's Kinnaur region in September. The plant sample was identified by comparing it to a previously existing specimen in the Panjab University, Chandigarh herbarium (PAN), and submitted under the accession number 22397. The material was collected, washed properly, and dried naturally in the shade for a week.

2.5 Extraction Procedure

The dried plant material is processed into powder in a grinder. 20 g of powder was dissolved in 50 ml of 90 % ethanol and shaken for 24 hours (%Yield obtained 25 %). Using Whatman number 1 filter paper, the extract was filtered, and the filtrate was partially covered for 5-7 days at room temperature. Then, it was placed in a refrigerator at 4 °C for future use.

2.6 Animal Model

For this experiment, a total of twenty-four Wistar male rats weighing in the range of 230 and 280 g were employed, with six rats placed in each group. The University's Central Animal Ethics Committee approved all trial protocols. The rats were housed in clean polypropylene cages under standard settings (12-hour light/dark cycle, 23±2 °C).

2.7 Toxicity Test

The acute oral toxicity test was conducted in accordance with OECD guideline 420. Several doses of extracts (5, 50, 300, and 2,000 mg/Kg) were administered via oral gavage feeding. During the next 72 hours, anomalies in behavior, including agitation, anger, convulsions, diarrhea, touch sensitivity, sleep, and an increased mortality rate, were observed.

2.8 Induction of Diabetes

One dosage of 150 mg/Kg alloxan in 154 mM saline was administered intraperitoneally. After three hours, 5% glucose was added to drinking water for 24 hours to treat hypoglycemia shock, and they could eat and drink. Following 72 hours, tail vein blood sugar was tested using Code free glucometer, and rats with blood sugar readings over 200 mg/dl were noted.

2.9 Experimental Design

The rats were divided into four groups (six rats in each group) for two weeks and treated accordingly. Oral gavage fed the medication and ethanolic plant extract. The experimental work followed the conventional methodology with few modifications. Rats were divided into four groups:

(1) Normal control (non-diabetic group)
(2) Diabetic control (untreated alloxan-induced diabetic group)
(3) Diabetic rats treated with glibenclamide (5 mg/kg BW).
(4) Diabetic rats treated with R.rugosa ethanolic extract (5 mg/kg BW).

On the 1st, 7th, and 14th day of the experiment weight and blood sugar levels were measured. After a 12-hour fast, animals were killed via cervical dislocation

on the last day of the experiment. Collected blood samples in Eppendorf tubes. The biochemical analysis of the blood samples and the histopathological study of the liver, pancreas, and kidneys were also examined.

3. BIOCHEMICAL ANALYSIS

To separate the serum from the blood, the collected blood was centrifuged for 10 minutes at 4 °C at 4000 rpm. Estimating lipid profile, liver, and renal parameters were detected using commercial kits manufactured by Reckon Private limited diagnostics.

3.1 Histopathological Studies

The pancreas, liver, and kidneys were removed after sacrificing the animal. For histopathological studies, specimens were dehydrated and blocked, 5 µm thick sections were cut and made to hematoxylin and eosin staining, and then inspected with a light microscope.

3.2 Statistical Analysis Software

SPSS16.0 was used for all of the analysis. The data is presented as the mean±SEM (n=6). A one-way analysis of variance was used for data analysis, followed by Turkey post hoc test with the least significant difference. If $p \leq 0.05$, the result is thought to be statistically significant.

3.3 Results

3.3.1 GC-MS Analysis

R. rugosa's GC-MS study disclosed 111 peaks, of which 106 compounds were recognized and accounted for 95.05 per cent of the total area. Using the NIST library database to validate chemical names displays the name of the chemical, peak area percentage, retention time, retention index, molecular formula, and biological activity of the discovered chemicals. A detailed description is given in Table 1 and Fig. 1.

3.3.2 FTIR Results

Considering the peak value (cm^{-1}) in the IR region, the class of phytoconstituents was identified using FTIR spectrophotometry, as displayed in Fig. 2. The identification and functionality of the compounds were known by matching the frequency range with the reference of the Sigma-Aldrich table and chart database. Our analysis

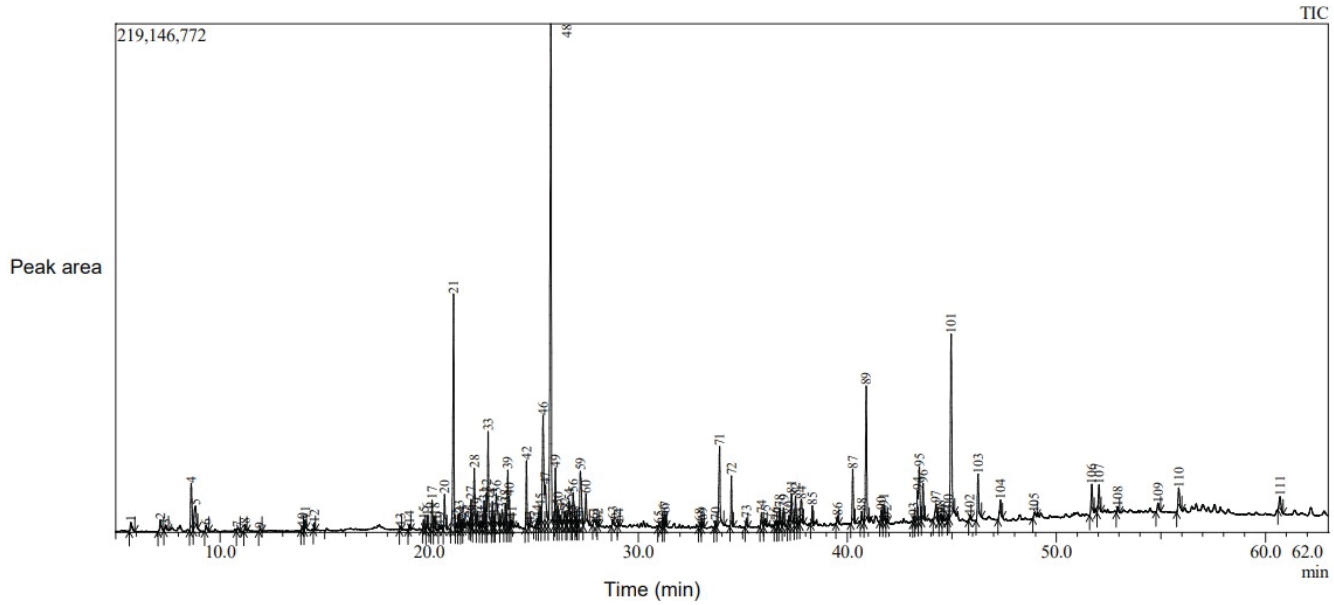

Figure 1. Total ionic chromatography of ethanolic extract of *R. rugosa* by GCMS.

Table 1. Biological activities of the identified compounds

S. No.	Compound name	Retention time	Retention index	% area	Molecular formula	Biological activities
1	α – Pinene	5.732	948	0.38	$C_{10}H_{16}$	Antibacterial, antifungal, antiviral, antitumour, insecticidal.
2	1-Octen-3-ol	7.141	969	0.48	$C_8H_{16}O$	Antimicrobial, antibacterial.
3	β- Myrcene	7.390	958	0.20	$C_{10}H_{16}$	Antioxidant, antiaging, anti-inflammatory, analgesic.
4	o-Cymene	8.619	1042	1.72	$C_{10}H_{14}$	Antibacterial, antifungal, antioxidant, antimicrobial

5	Linalool	11.201	1082	0.18	$C_{10}H_{18}O$	Antioxidant, antimicrobial, antitumour, analgesic and anti–inflammatory
6	Terpinene-4-ol	13.933	1137	0.09	$C_{10}H_{18}O$	Antimicrobial, antibacterial and antioxidant.
7	Naphthalene	14.070	1231	0.23	$C_{10}H_{8}$	Antidiabetic, anticancer, antimicrobial, anti-inflammatory.
8	2-Acetyl-5 methyl furan	14.506	967	0.14	$C_{7}H_{8}O_{2}$	Antimicrobial, antibacterial .
9	γ.-Elemene	18.623	1431	0.08	$C_{15}H_{24}$	Antileishmanial activity.
10	α.-Cubebene	19.056	1344	0.11	$C_{15}H_{24}$	Antimicrobial
11	Copaene	19.901	1221	0.34	$C_{15}H_{24}$	Anticancerous
12	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	20.736	1494	1.06	$C_{15}H_{24}$	Antimicrobial
13	Caryophyllene	20.167	1494	6.19	$C_{15}H_{24}$	Anticancer, antioxidant, and antimicrobial
14	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	22.005	1507	0.58	$C_{15}H_{24}$	Antioxidant, anticancer, antimicrobial, anti-inflammatory
15	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7] decane-rel-	22.819	1216	2.53	$C_{15}H_{24}$	Antioxidant
16	(-)-Spathulenol	25.314	1536	0.52	$C_{15}H_{24}O$	Antioxidant, anti-inflammatory, antimicrobial, Insecticide
17	Diethyl Phthalate	25.448	1639	4.20	$C_{12}H_{14}O_{4}$	Antimicrobial
18	Ledol	25.820	1530	16.83	$C_{15}H_{26}O$	Antimicrobial, anti-inflammatory
19	Quinidine	26.807	2694	0.90	$C_{20}H_{24}N_{2}O_{2}$	Antimalarial
20	.tau.-Cadinol	26.887	1580	1.21	$C_{15}H_{26}O$	Antimicrobial
21	α.-Bisabolol	27.873	1625	0.24	$C_{15}H_{26}O$	Analgesic, anticancer, antimicrobial
22	Eicosen-1-ol, cis-9-	31.194	2260	0.22	$C_{20}H_{40}O$	Antimalarial, antifungal, antioxidant.
23	2-Pentadecanone, 6,10,14-trimethyl-	31.295	1754	0.27	$C_{18}H_{36}O$	Antibacterial, anti-inflammatory
24	7-Hexadecenal, (Z)-	32.949	1808	0.11	$C_{16}H_{30}O$	Antiviral
25	Hexadecanoic acid, methyl ester	33.067	1878	0.19	$C_{17}H_{34}O_{2}$	Antibacterial
26	Dibutyl phthalate	33.691	2037	0.17	$C_{16}H_{22}O_{4}$	Antibacterial, anticancer
27	n-Hexadecanoic acid	33.894	1968	2.73	$C_{16}H_{32}O_{2}$	Anti-inflammatory, antioxidant
28	Hexadecanoic acid, ethyl ester	34.458	1978	1.15	$C_{18}H_{36}O_{2}$	Antioxidant, anti-androgenic
29	Phytol	36.773	2045	0.56	$C_{20}H_{40}O$	Antioxidant, anti-inflammatory
30	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	37.330	2713	1.12	$C_{21}H_{38}O_{4}$	Antimicrobial
31	α.-Tocopheryl acetate	40.688	3308	0.32	$C_{31}H_{52}O_{3}$	Anti-inflammatory

32	Ferruginol	40.906	2225	4.11	$C_{20}H_{30}O$	Antibacterial, antitumour, antimalarial and cardioprotective effects
33	δ-Tocopherol	48.940	2923	0.18	$C_{27}H_{46}O_2$	Anticarcinogenic
34	alpha.-Tocopherol-.beta.-D-mannose	52.928	3149	0.17	$C_{29}H_{50}O_2$	Antioxidant, vitamin E supplement
35	Stigmasterol	54.866	2739	0.32	$C_{29}H_{48}O$	Antidiabetic, antioxidant, anticancer, anti-inflammatory
36	Campesterol	55.854	2632	1.00	$C_{28}H_{48}O$	Anticarcinogenic and cholesterol-lowering activity
37	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-	60.699	3140	0.69	$C_{31}H_{50}O_3$	Antimycobacterial

revealed that O-H groups were the most frequently found group. A detailed description of the analysis is depicted in Table 2.

3.3.3 *Toxicity Test*

The toxicity of plant extracts was not noted up to a dose of 2000 mg/kg, and there were no indications detected, deviations from their usual behaviour, fatalities, or other adverse effects. Therefore, for the current investigation,the plant was chosen.

3.3.4 *Effects of a Plant Extract on Body Weight and Blood Sugar Level*

Tables 3 and Table 4 show that group 3 and group 4 had significantly decreased blood glucose levels ($p \leq 0.001$) and gain in body weight than those of group 2. It is assumed, based on the results, that the ethanolic extract of *R. rugosa* exhibited antihyperglycemic action.

3.3.5 *Effect on Lipid Profile*

As shown in Figure 3, after 14 days of treatment, total cholesterol and LDL level decreased significantly ($p \leq 0.001$), triglycerides, and a considerable rise in HDL levels.

3.3.6 *The Repercussion on Liver Functions*

The efficacy of the extract of *R. rugosa* on liver functions is demonstrated in Fig 4. SGOT, SGPT, and ALP levels substantially rose in subject matter rats. However, the level of liver parameters substantially decreased ($p \leq 0.001$) in rats medicament with ethanolic extracts of *R. rugosa* and standard drug.

3.3.7 *Impact on Renal Functions*

In the subject matter, rats cured with ethanolic extracts of *R. rugosa* and standard drug glibenclamide (group 3, group 4) they exhibited significant restoration ($P < 0.01$) of renal marker enzymes to the normal range after the 14th day of treatment. A detailed description of the analyzed data is shown in Figure 5.

3.3.8 *Histopathological Study of Pancreas, Liver, and Kidney*

The histopathological analysis of rat's pancreas,

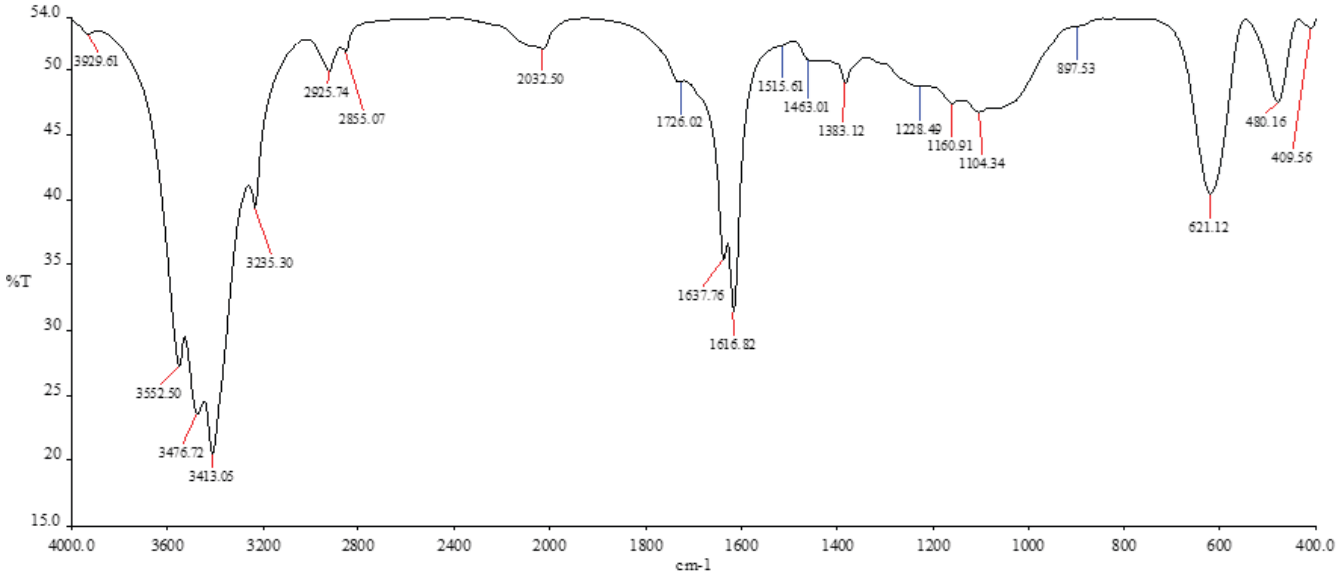

Figure 2. FTIR spectrum scrutiny of bioactive compounds.

Table 2. Interpretation of a functional group of compounds of *R. rugosa* extract

Peak value cm-1	Bond	The intensity of a functional group	Class of functional group
3929.61	O - H stretch	Medium, sharp	Alcohol
3552.50	O - H stretch	Medium, sharp	Alcohol
3476.72	O - H stretch	Strong, broad	Alcohol
3413.05	O - H stretch	Strong, broad	Alcohol
3235.30	O - H stretch	Strong, broad	Alcohols
2925.74	O - H stretch, N - H stretch, C - H stretch	Strong, broad, weak, broad, strong, broad, medium	Carboxylic acid, alcohol, amine salt, alkane
2855.07	O - H stretch, N - H stretch, C - H stretch	Strong, broad, strong, broad, medium	Carboxylic acid, alcohol, amine salt, Alkane
2032.50	N = C = S stretch	Strong, broad	Isothiocyanate
1726.02	C = O stretch	Strong	α, β - unsaturated esters
1637.76	C = C stretch, N - H bending	Medium	Cyclic alkene, conjugated alkene, amine, alkane
1616.82	C = C stretch	Strong, medium	α, β - unsaturated ketones, conjugated alkene
1515.61	N - O stretch	Strong	Nitro compounds
1463.01	C - H bending	Medium	Alkanes
1383.12	C - H bending, S = O stretch, O - H bending	Medium, strong, medium,	Aldehyde, alkane, sulfate, sulphonyl chloride, alcohol, Phenols
1228.49	C - O stretch, C - N stretch	Strong, medium	Alkyl aryl ether, amine
1160.91	C - O stretch	Strong	30 alcohols
1104.34	C - O stretch	Strong	20 alcohols
897.53	C - H bending	Strong	1,2,3-trisubstituted
621.12	C - Br stretch	Strong	Halo compounds

Table 3. Impact of ethanolic extract of aerial parts of *R. rugosa* on % change in body weight in alloxan-induced diabetic rats.

Treatment groups	Blood weight(g)			% Change in body weight
	Initial day	7th day	14th day	
Normal control	234 ± 2.00	240 ± 3.71	265 ± 3.54	-22.23%
Diabetic control (150 mg/kg alloxan)	276 ± 2.10^{x}	248 ± 2.13	226 ± 2.53^{x}	13.38%
Alloxan + SD glibenclamide (5mg/kg)	273 ± 4.94^{x}	289 ± 3^{x,a}	304±3.83^{x,a}	11.15%
Alloxan + extract of R. rugosa (50 mg/kg)	242 ± 3.80^{a}	252 ± 4.5	260 ± 5.54^{a}	7.65%

Data is implied as mean ± SEM, n = 6 (six rats per group), x = p ≤ 0.001 (when equated with group 1), a = p ≤0.001 (when equated with group 2)

Table 4. Impact of ethanolic extracts of *R. rugosa* on the blood sugar levels in diabetic rats (n=6).

Treatment groups	Blood glucose level mg/dl			% Change in blood glucose level
	Initial day	7th day	14th day	
Normal control	115.2 ± 2.03	113.7 ± 2.64	144.7 ± 1.40	25.60
Diabetic control (150 mg/kg alloxan)	596.3 ± 2.40^{x}	591.83 ± 3.43^{x}	595.3 ± 3.12^{x}	-0.16
Alloxan+SD glibenclamide (5 mg/kg)	587 ± 3.92^{x}	273.3± 3.04^{x,a}	132 ± 3.11^{a}	-77.51
Alloxan + Ethanolic extract of *R. rugosa* (50 mg/kg)	594±2.23^{x}	360±3.74^{x,a}	90± 4.9^{y,a}	-84.84

Data is implied as mean ± SEM, n = 6 (six rats per group), x = p ≤ 0.001 (when equated with group 1), a = p ≤0.001 (when equated with group 2)

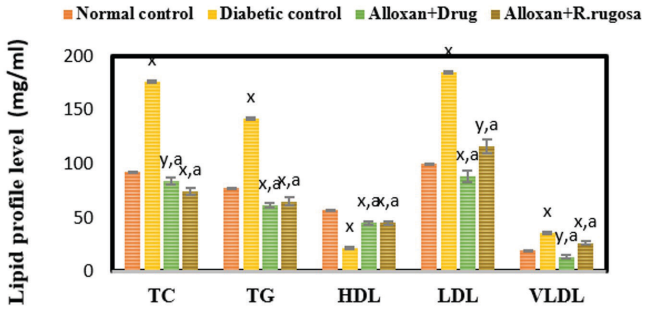

Figure 3. Impact of *R. rugosa* aerial parts ethanolic extract on lipid profile mg/dL in diabetic rats.

Every value is stated as mean ± SEM, n=6 (number of rats in each group); x = $p \leq 0.001$, y = $p \leq 0.005$ (in comparison to group 1), a = $p \leq 0.001$ (in contrast to group 2)

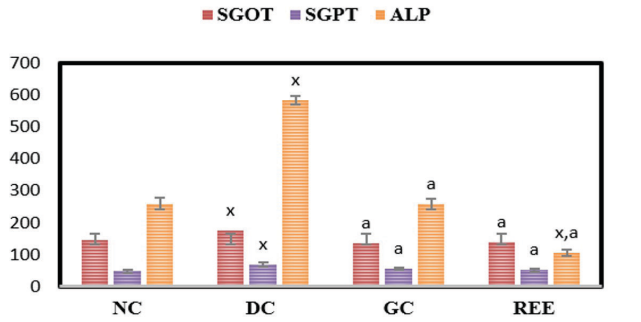

Figure 4. Impact of ethanolic extract of aerial parts of *R. rugosa* on liver marker enzymes in diabetic rats.

Every value is stated as mean ± SEM, n =6 (number of animals in all groups); x = $p \leq 0.001$ (in comparison to group 1); a= $p \leq 0.001$ (in comparison to group 2)

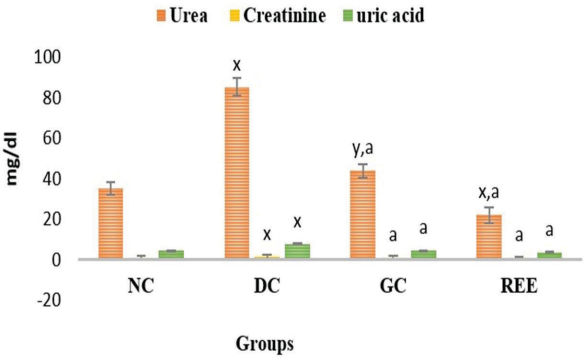

Figure 5. Impact of ethanolic extract of aerial parts of *R. rugosa* on renal parameters in diabetic rats.

Every value is stated as mean ± SEM, n = 6 (number of rats in all groups); x = $p \leq 0.001$, y=$p \leq 0.005$ (by post hoc analysis, values are compared with a group1); a = $p \leq 0.001$ (in comparison to group 2)

liver and kidneys stained with hematoxylin-eosin stain is demonstrated in Fig. 6 with a detailed description. A – Acini; IL – Islet of Langerhans, CV – Central Vein; H – Hepatocytes, G – Glomerulus; BS – Bowman's Space **A1**–In an untreated normal control group, the regular architecture of islets of Langerhans and acinar cells was seen **A2**–The diabetic control group, in which inflammation area and rupture of IL were observed. **A3**–Diabetic rats treated with the drug showed improved IL, and the inflammation region was also regenerated. **A4**–Diabetic rats treated with extract showed remarkable improvement in histological architecture as a normal control group. **B1**–Untreated normal control group showing normal hepatocytes and central vein. **B2**–The diabetic control group showed destructive architecture of hepatocytes and central veins. **B3–**Diabetic rats treated with the reference drug showed improvement in the destructive structure of hepatocytes and central veins. **B4**–Diabetic rats treated with extract showed immense improvement in the structure of hepatocytes and central veins. Extract-treated groups show a better result than the drug-treated groups. **C1**–Normal control group showing normal glomeruli enclose in bowman's space**. C2–**Diabetic control group showing the destructive glomeruli and vasculature. **C3–**Diabetic rats treated with the drug showed improvement in glomeruli structure. **C4**–Rats with diabetes given plant extract showed normal glomeruli and finely tuned tubular structure.

4. DISCUSSION

The current investigation reported that the selected plant species has incredible anti-diabetic activity. In a previous report, we disclosed the *in vitro* antioxidant and antidiabetic activity of the ethanolic extract of R.rugosa. Therefore, the present work checked the antidiabetic potency of the same plant extract on alloxan-induced diabetic rats.

Plants are rich sources of phytoconstituents, and as their outstanding health benefits, medicinal plants are in demand nowadays. Former studies reported that the oil of *R. rugosa* exhibited major constituents identified as sesquiterpene hydrocarbons. Our GCMS results showed differences in compound compositions, and some additional compounds were reported, which could be due to the method of extraction, climate change, and altitude variation. FTIR analysis uncovered the functional group of bioactive compounds. The occurrence of hydroxyl functional groups indicates the presence of the majority of phenolic chemicals, including flavonoids and tannins. N-H stretching suggests alkaloids and terpenes due to the appearance of C-H stretch. The Hydroxyl group mostly occurred in our findings. The occurrence of the OH group in various bioactive compounds contributes a major role in antidiabetic, antioxidant, and antibacterial activities.

Body weight, blood glucose level, liver parameters, lipid profile, renal parameters, and histological features all improved in rats with alloxan-induced diabetes following treatment with plant extract. The weight of the diabetic rats decreased as their condition deteriorated. The previous findings showed that phytochemicals might be the primary factor in controlling abnormally high blood sugar levels. The earlier research confirmed the usefulness of plant extract in bringing blood sugar and weight back to normal. Diabetes is linked to an increased risk of cardiovascular illnesses by lipid abnormalities such as raised TC, TG,

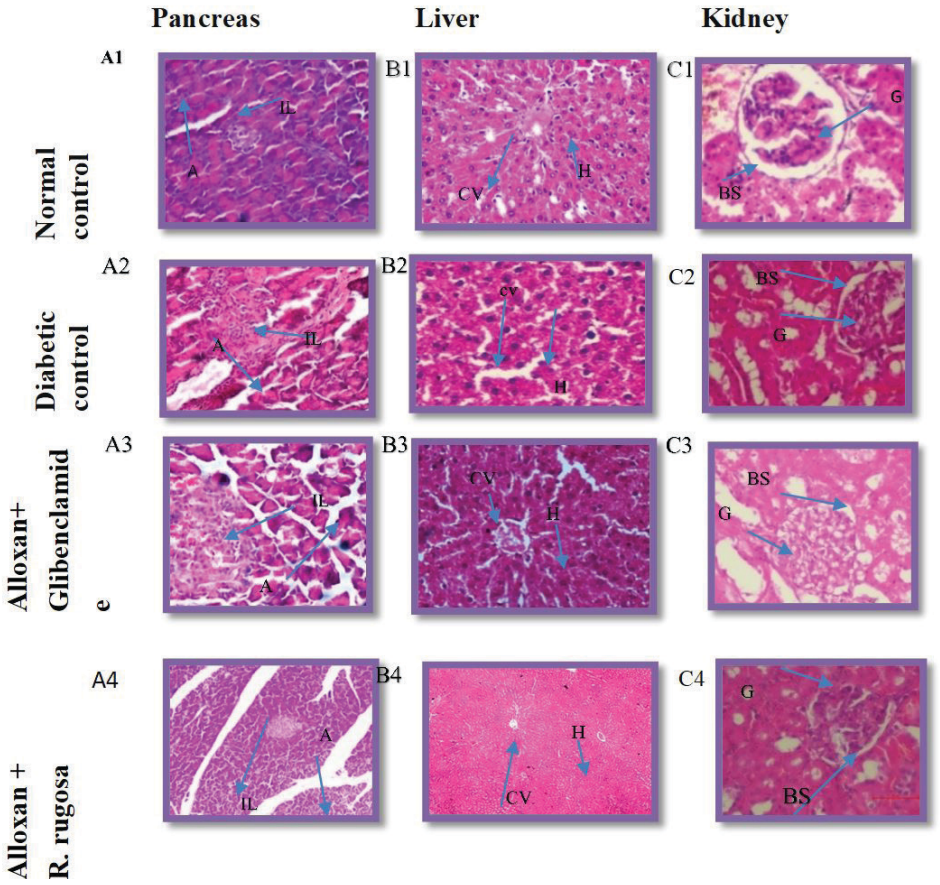

Figure 6. Histopathological study of the liver, pancreas, and kidneys.

LDL, and VLDL levels and falling HDL levels. It has been described as the combination of DM leading to increased lipid and free fatty acid breakdown from outline deposits in insulin deficiency. Nevertheless, when *R. rugosa* ethanolic extracts (50 mg/kg) and the medication glibenclamide (5 mg/kg) were administered, the levels of TC, TG, LDL, and VLDL significantly decreased over the course of two weeks, while HDL levels increased. The fact that *R. rugosa* extract has a healing impact on lipid parameters suggests that the high level of insulin in test rats may be the cause of the extract's effectiveness. ALP, SGOT, and SGPT levels increasing are signs of liver impairment. On diabetic rats, the extract has a notable impact on the histological structure that is correlated with biological parameters. This might be because some beta cells may still be alive and available for the extract from *R. rugosa* to act upon and release insulin.

5. CONCLUSION

The recent experimental work on alloxan-induced diabetic rats suggests that the ethanolic extract of *R. rugosa* has a significant hypoglycemic impact. The extract showed considerable improvements in blood sugar regulation, weight gain, and several biological specifications like lipid profile, liver parameters, urea, uric acid, and creatinine levels. Histological restoration of organs involved in glucose regulation, such as the pancreas, liver, and kidneys, demonstrated the plant's antidiabetic efficacy. The extract contained a wide variety of phytoconstituents, each of which had its own unique biological effects. An in vivo study has confirmed the effectiveness of our antidiabetic treatment. Isolating antidiabetic components is necessary for pinpointing the precise mechanism of action. If this were to materialize, it would lend credence to using *R. rugosa* as an antidiabetic medication in treatment. Our research shows promise as a natural source for the developing antidiabetic medication.

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