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In-vitro Evaluation of Antioxidant and Antidiabetic Potential of Traditional Herb Rosa Centifolia L.

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Abstract
Objective: The current work aimed to discover the antioxidant and antidiabetic potential of the *Rosa centifolia L.* fresh Petals extract, which belongs to the Family Rosaceae.
Material and Methods: The authenticated flowers of *Rosa centifolia* were subjected to the soxhlet extraction method. The phenolic content of ethanolic extract has attracted a great deal of attention. The crude ethanolic extract of *Rosa centifolia L.* flower was fractionated with different solvents, chromatographic separation was performed, and their TLC parameters were studied to know the presence of active phytochemicals. The *Rosa centifolia L.* petals antioxidant and antidiabetic potential have been studied as there were no previous research data available in the literature. The eluted fractions were screened for bioactivity through an *in-vitro* assay.
Results: The extract yielded various fractions through column chromatography. Sub-fraction F6.4.2 demonstrated a potent *in-vivo* antioxidant and antidiabetic potential compared to the other mother fraction and fraction. The bioactive effects of the sub-fractions F6.4.2 against antioxidant and antidiabetic in vitro show maximum.
Conclusion: The present research work would be beneficial to enhance the data availability concerning antioxidant and antidiabetic assessment to the investigation in the traditional system and in modern medicine for the medicinal use of herbal drugs.

Keywords: *Rosa centifolia L.*; TPC; TLC; *In-vitro* antioxidant; *In-vitro* antidiabetic

1. Introduction

The incidence of diabetes mellitus is increasing worldwide specifically in adults and public health is in trouble globally. A prediction related to diabetes is that some countries like India, China, and the US will have the highest number of individuals with this disease by 2030 [1], [2]. The type 2 diabetes is a much more common category [3], [4]. Diabetes mellitus is mainly because by an unhealthy lifestyle, urbanization, and ageing [5]. In the case of diabetes or hyperglycemia body creates reactive oxygen species (ROS) they are responsible for the development of secondary complications in diabetes mellitus because they cause lipid peroxidation, and cell membrane injury [6]. The β-cell dysfunction is the principal reason for causing type II diabetes resulting in resistance to insulin [7,8]**.** 90% of the population is affected by Type-II diabetes approximately 10% of the diabetic population [8]. The disease is considered hypercholesterolemia, hyperglycemia, and hypertriglyceridemia [9,10]. In the scientific community development of new drugs is

the great interest for the prevention and cure of secondary complications associated with various diseases [11]. Within the Indian subcontinent, in the area of ethnomedicine wide spectrum of scientific work has been accomplished to obtain the best possible medicinal plant use for the management of diabetes [12,13]. Another problem for many patients with diabetes is oxidative stress [14,15]. The hypoglycemic properties of the plants are reported due to their higher contents of flavonoids and different secondary metabolites. Plant *Rosa centifolia L.* (Rosaceae) is a perennial plant commonly known as a hundred-leaved rose or Shatapatri or Taruni and is available throughout India. It is a complex hybrid, bred from *Rosa gallica L.*, *Rosa moschata Herrm. Rosa canina L.* and *Rosa damascene Mill.* The genus Rosa (family Rosaceae) includes about 200 species spread in the temperate and subtropical zones of the Northern hemisphere [16,17]. Years ago the essential oils of roses were used in the field of cosmetology as perfumery due to their aromatic (strong) fragrance, and rose essential oils have repotted market worth. The current study was conducted to explore the antidiabetic potential of rose petal mother extract and its fraction, which is used as a raw material for the manufacturing of pharmaceutical products.

2. Material and methods

2.1 Chemical and instruments

All reagents and chemicals used are analytical grades purchased from some standard chemical suppliers such as Fischer Inorganic and Aromatic Limited, Chennai, India, Sigma Aldrich, Merck India Ltd, and Fine Chemical. Chemical used in this work were 1, 1-diphenyl-2-picryl-hydrazil (DPPH) free radicals, sodium carbonate, sodium phosphate, potassium ferricyanide, ascorbic acid, gallic acid, Follin-Ciocalteu, 2-deoxyribose, and 30%, v/v H2O2. TLC plate (pre-coated plate 0.2 mm thick) from E. Merck, Germany. The ultraviolet-visible spectrophotometer (UV mini-1240, Shimadzu Co., Kyoto, Japan) was used to record the absorbance spectra of the sample.

2.2 Collection, Preparation of plant extract, fractions, and its phytochemical analysis

Fre petals of *Rosa centifolia L*. were collected from the botanical garden Koni Bilaspur C.G. and taxonomical authentication was made by a Botanist (Professor A. K. Dixit) at the Department of Botany, Guru Ghasidas University (C.G.) The collected fresh flower petals were converted into small pieces, and samples (200 gm) were extracted by distillation method using a soxhlet apparatus for 4 hours [17]. After the completion of the extraction cycle, the obtained sample extract was filtered and the excess solvent was evaporated using a rotary evaporator under pressure for 30 min resulting in a semisolid crude extract (5.17 g) [18]. The crude extract of *Rosa centifolia L.* was subjected to column chromatography to separate the extract into its component fractions [19]. The ethanolic crude extract (0.75 g) was fractionated by column chromatography using silica gel (60–120 mesh), the column used (85 cm × 5.7 cm) as shown in [20]. Elution was performed with different proportions of petroleum ether: chloroform of increasing polarity (100:0 to 0:100 with constant polarity increase of 10%) to give 10 fractions (F1 to F10), monitored TLC study. The extract fractions with similar Rf values with reference compounds (quercetin and gallic acid) in the TLC pattern were, further applied to in vitro assay. The antioxidant and antidiabetic activity was mainly detected in fractions F5 and F6. Further column purification of F6 (with greater antioxidant and antidiabetic activity than F5) eluted with chloroform: ethyl acetate (100:0 and 0:100) yielded fractions denoted F6.1 to F6.10. Fractions F6.4 were subjected to different dimension silica gel columns (27.5 × 2 cm) and fractionated with ethyl acetate: ethanol (100:0 and 0:100, 500 ml each fraction). Fraction F6.4 afforded ten sub-fractions (F6.4.1 to F6.4.10) of which F6.4.2 (0.14 g). The TLC pattern of this fraction represented similar Rf value, in vitro assays (test for antioxidant and antidiabetic biological activity).

2.3Total phenolic content determination (TPC)

Folin-Ciocalteu (FC) assay as described by Zovko et al was followed with minor modifications in it to the determination of the total phenolic content of *Rosa centifolia L*. mother extract and its fractions. Gallic acid was used as standard with different concentrations for the calibration curve [21].

2.4Analytical thin-layer chromatography (TLC)

The collected mother extract and its fractions were separately applied on a TLC plate along with a reference compound (gallic acid). Approximately 200 ml of petroleum Chloroform: Methanol: Ammonia (solvent

mixture) (7: 3: 0.3). Later the developed chromatograms were examined under the UV-visible light. The relative Retentive factor (Rf) value was calculated by the formula given below.

$$Rf = \frac{\text{Distance traveled by the streak from the starting point}}{\text{Distance traveled by the solvent from the starting point to the solvent front.}}$$

2.5In-vitro antioxidant activity

2.5.1 The 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging method

Antiradical activities of extracts were examined by comparing them to those of known antioxidants such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) [22].
The anti-radical activity was calculated based on the formula given below:
Antiradical activity (%) = (absorbance of control−absorbance of sample/absorbance of control) ×100
Where:
Aa = absorbance of the sample
Ab = absorbance of the blank (prepared by replacing the volume of DPPH solution with an equal volume of ethanol)
Ac = absorbance of the control (prepared by replacing the volume of a crude extract with an equal volume of ethanol).
After complete incubation (45 min), the absorbance for all samples was measured at 517 nm with a spectrophotometer (UV mini-1240, Shimadzu Co., Kyoto, Japan) [23].

2.5.2 Ferric reducing power assay

The reducing power of the crude extract was determined according to the method previously described [24]. The absorbance was measured at 700 nm with a spectrophotometer (UV mini-1240, Shimadzu Co., Kyoto, Japan).

2.6 In-vitro antidiabetic activity

2.6.1 Inhibition assay for α-amylase activity

acarbose was used as Positive control, obtained by dissolving in phosphate buffer and DMSO (negative control vehicle used to dissolve the extracts). The assays were conducted by mixing 80 µL of plant extract, 20 µL of the α-amylase solution, and 1 mL of CNPG3. The incubation time for the mixture was 5 min at 37 °C. The absorbance of all the samples was measured at 405 nm by spectrophotometer (UV mini-1240, Shimadzu Co., Kyoto, Japan) [25].

Percentage inhibition (PI) was calculated by the expression:
PI = [(Absorbance Control-Absorbance Test)/Absorbance Control] X 100

2.6.2 Pancreatic lipase in vitro assay

The method used to measure the pancreatic lipase inhibitory activity of all the extracts. Orlistat, 100 mM used as a positive control, and DMSO (negative control vehicle used to dissolve the extracts) were pipetted into respective wells of a 96-well plate. Freshly prepared porcine pancreatic lipase was added at four times the volume of the test samples, positive and negative controls (40 mL). The absorbance for all the samples was recorded at 405 nm [26].
The expression calculated percentage lipase inhibitory activity:
% Lipase inhibition = [1- (A/B)] X 100.

2.7 Statistical analyses

The experimental triplicate data were analyzed using Student's t-test by GraphPad Prism (8.01) (Microsoft Corporation, USA) and reported as mean SD. The confidence limits used in this study were based on 95% (P<0.05).

3 Result

3.1 Estimation of the total phenolic content of extract and its fractions

In our present study, it was found that the F6.4.2 fraction of ethanolic extract shows a higher phenolic content. The content of total phenolic in ethanolic extract and its fractions was determined by using the Gallic acid equivalent (Table 1). Linear regression for the final fraction (F6.4.2) was found to be r^2= 0.9989 as illustrated in figure 1.

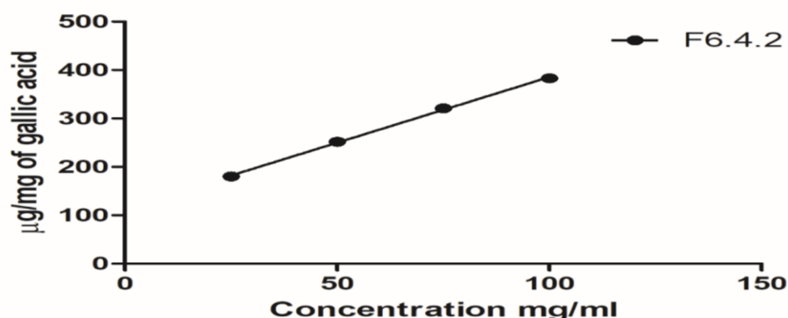

Fig.1. Linear regression of fraction F6.4.2 for TPC.

Table 1. Total phenolic content of various fractions of *Rosa centifolia* fresh petal.

Concentration (mg/ml)	Total phenolic content (µg/mg of gallic acid)
ME	210.7 ± 0.152
F6	235.3 ± 0.333
F6.4	320.3 ± 0.666
F6.4.2	387.0 ± 0.577

3.2 TLC study for extract and its fractions

TLC study of Rosa centifolia extract and fraction has resulted leading towards the presence of polyphenolic compounds. A total of four sub-fractions of *Rosa centifolia L.* petal extract were examined. A similar separation (Rf) was recorded in all samples of extract as shown in Figure 2 and, the results of TLC studies are concise in Table 2.

Table 2. Characteristics of fraction from the extract of *Rosa centifolia* petals, TLC Screening of Tannins-Gallic acid and Flavonoids – Quercitin

Fraction		ME	F6	F6.4	F6.4.2
Quantity (mg)		1.14	0.19	0.59	0.28
Spot	Gallic acid (Rf)	0.75	0.75	0.76	0.75
	Quricitine (Rf)	0.82	0.83	0.84	0.82
UV-Visible light	Gallic acid	Grayish yellow	Grayish yellow	Grayish yellow	Grayish yellow
	Quaricitine	Greenish brown	Greenish brown	Greenish brown	Greenish brown

Eluent: Petroleum ether - ethyl acetate - ethanol (3:2:1)

Fig.2. Comparative TLC observations of Phenolic compound from different fractions of Rosa centifolia.

3.3 Anti-oxidant assay

3.3.1 DPPH radical scavenging activity

The free radical scavenging activity of *Rosa centifolia L*. petals extract was evaluated by applying the DPPH method and the outcomes were given in Figure 3. On increasing the concentration of All the above four fractions of *Rosa centifolia L.* petal (25 mg/L to 100 mg/L), the percent inhibition obtained for ME, F6, F6.4, and F6.4.2 were 34.7±0.180 to 56.6±0.114, 41.6±0.152 to 69.9±0.008, 46.7±0.152 to 77.9±0.011, 52.1±0.854 to 80.56±0.020, respectively, the test samples showed excellent reducing power at 100 mg/mL, Amongst these, for the F6.4.2 fraction recorded the maximum and mother extract (ME) observed with the lowest reducing. Samples are comparable to the standard ascorbic acid with 59.8±0.589 to 81.77±0.062 at 25 mg/mL to 100 mg/mL radical scavenging activity is (Table 3).

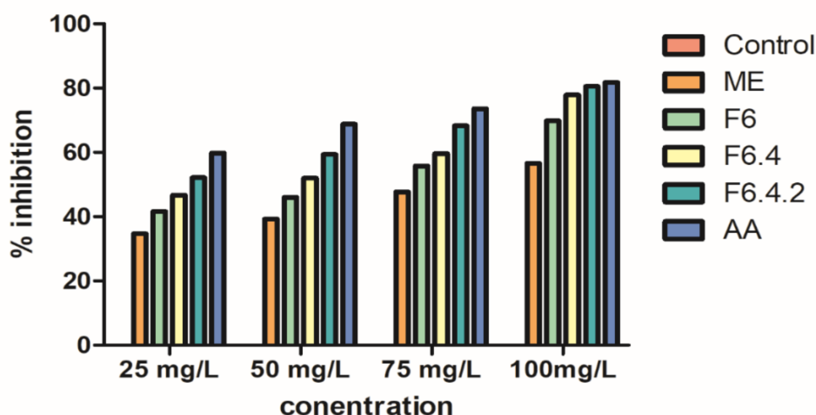

Fig.3. Effects of mother extracts, fraction, sub-fraction of *Rosa centifolia* at varying concentrations on DPPH radical scavenging method. The results are expressed as mean ± SD of three replicates. AA=ascorbic acid, ME= Mother Extract, F6=F6 fraction, F6.4= F6.4 fraction, F6.4.2=F6.4.2 fraction. The figures are in percentage with the control showing 0% inhibition.

3.3.2 Reducing power

The reducing power activity of crude extract outcomes is given in Figure 4. Shows the reducing power of all the above four fractions of *Rosa centifolia L.* petal of their concentration. On increasing the concentration of crude extract fraction (25 mg/L to 100 mg/L), the reducing power obtained for ME, F6, F6.4, and F6.4.2 were 0.745±2.038 to 1.81±0.098, 1.30±0.028 to 2.25±0.156, 1.36±0.044 to 3.38±0.205, 2.29±0.026 to 3.65±0.052, respectively; the reducing power obtained for the test samples was excellent at 100 mg/mL, Amongst these, the F6.4.2 showed the highest and ME the lowest reducing power against the tested concentrations. The reducing power for ascorbic acid was 3.48±0.027 to 4.09±0.111 at 25 mg/mL to 100 mg/mL, respectively (Table 3).

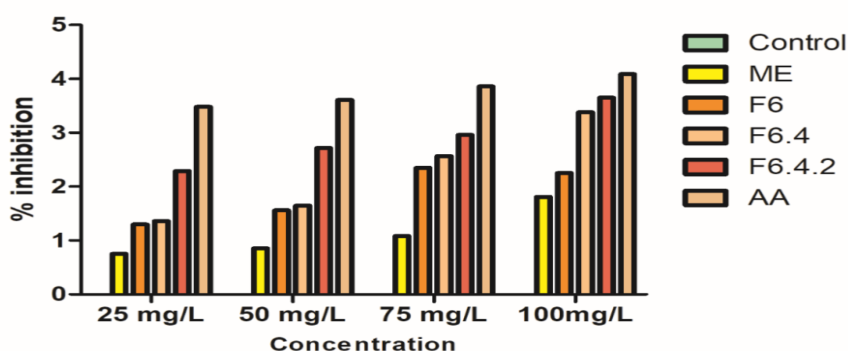

Fig.4. Effects of mother extracts, fraction, sub-fraction of *Rosa centifolia* at varying concentrations on reducing power. The results are expressed as mean ± SD of three replicates. AA=ascorbic acid, ME= Mother Extract, F6=F6 fraction, F6.4= F6.4 fraction, F6.4.2=F6.4.2 fraction. The figures are in percentage with the control showing 0% inhibition.

Table 3. Antioxidant activities of the different fractions of *Rosa centifolia.*

Fraction	Concentration	DDPH	Reducing power
		% Inhibition	% Inhibition
ME	25	34.7± 0.180	0.754±2.038
	50	39.3± 0.0709	0.857±0.049
	75	47.7± 0.135	1.08±0.020
	100	56.6 ± 0.114	1.81±0.098
F6	25	41.6 ± 0.152	1.30±0.0281
	50	46.3 ± 0.0504	1.56±0.046
	75	55.8 ± 0.0120	2.89±0.0338
	100	69.9 ± 0.008	2.25±0.153
F6.4	25	46.7 ± 0.152	1.36±0.0448
	50	52.5 ± 0.0869	1.65±0.0344
	75	59.6 ± 0.052	2.77±0.132
	100	77.9 ± 0.0115	3.38±0.205
F6.4.2	25	52.1 ± 0.854	2.29±0.026
	50	59.4 ± 0.0448	2.72±0.086
	75	68.3 ±0.0115	2.75±0.116
	100	80.56 ± 0.020	3.65±0.052
Standard			
Ascorbic acid	25	59.8±0.598	3.48±0.027
	50	68.8±0.026	3.61±0.039
	75	73.6±0.043	3.86±0.025
	100	81.8±0.062	4.09±0.111

3.4In-vitro antidiabetic study

3.4.1 Inhibition assay for α-amylase activity

The results of the study are summarized in Figure 5. All the above four fractions Rosa centifolia Patel showed varying effects on glucose utilization. On increasing all concentrations of crude extract fraction (25 mg/L to 100 mg/L) the α-amylase activity obtained for ME, F6, F6.4, and F6.4.2 were 35.6±0.124% to 49.2±0.070%, 54.6±0.052% to 56.7±0.017%, 60.5±0.003% to 71.7±0.041%, 73.1±0.003% to 84.1±0.032% respectively; *Rosa centifolia L.* fraction F6.4.2 showed maximum inhibition of the enzyme with the highest value seen at 100mg/L and ME the lowest reducing power against the tested concentrations. Although, the percentage inhibition of the positive control (acarbose) was 76.8±0.041% to 88.4±0.037% at 25 mg/L to 100 mg/L mg/mL (Table 4).

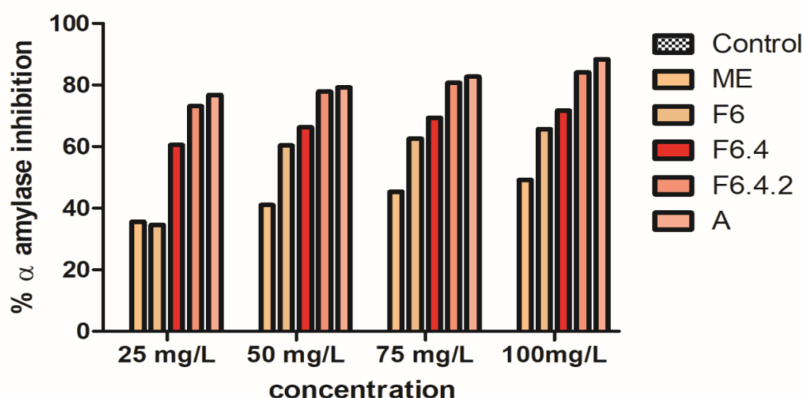

Fig.5. Effects of mother extracts, fraction, sub-fraction of *Rosa centifolia* at varying concentrations on α-amylase activity extract. The results are expressed as mean ± SD of three replicates. A= acarbose, MF= Mother Extract, F6=F6 fraction, F6.4= F6.4 fraction, F6.4.2=F6.4.2 fraction. The figures are in percentage with the control showing 0% inhibition.

3.4.2 Pancreatic lipase in vitro assay

The results of the study are summarized in Figure 6. All the above four fractions of *Rosa centifolia* Patel showed a varying effect. At all concentrations of extract as a fraction (25 mg/L to 100 mg/L) the Pancreatic lipase activity obtained for ME, F6, F6.4, and F6.4.2 were 34.50±0.009% to 58.40±0.020%, 46.4±0.026% to 69.6±0.029%, 46.40±0.003% to 76.90±0.082%, 54.50±0.011% to 83.8±0.023% respectively; *Rosa centifolia* fraction F6.4.2 showed maximum inhibition of the enzyme with the highest value seen at 100mg/L and ME the lowest reducing power against the tested concentrations. The percentage inhibition of the positive control (orlistat) was 62.5±0.052 to 84.4±0.015 at 25 mg/L to 100 mg/L mg/mL (Table 4).

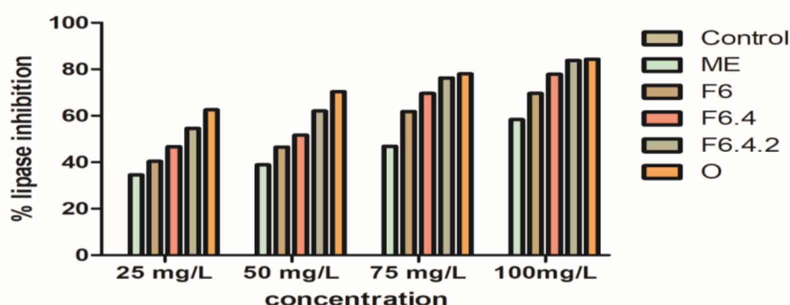

Fig.6. Effects of mother extracts, fraction, sub-fraction of *Rosa centifolia* at varying concentrations on lipase activity extract. The results are expressed as mean ± SD of three replicates. O=orlistate, MF= Mother Extract, F6=F6 fraction, F6.4= F6.4 fraction, F6.4.2=F6.4.2 fraction. The figures are in percentage with the control showing 0% inhibition.

Table 4. Antidiabetic activities of the different fractions of Rosa centifolia.

Fraction	**Concentrrarion**	**α-amylase**	**Pancreatic lipase**
		% Inihibition	**% Inihibition**
ME	25	35.6±0.124	34.5±0.009
	50	41.0±0.131	38.9±0.021
	75	45.4±0.066	46.9±0.018
	100	49.2±0.070	58.4±0.020
F6	25	54.6±0.052	40.4±0.026
	50	60.39±0.014	46.5±0.0145
	75	62.6±0.073	61.5±0.322
	100	65.7±0.017	69.6±0.029
F6.4	25	60.5±0.030	46.4±0.003
	50	66.3±0.063	51.7±0.032
	75	69.4±0.024	69.6±0.025
	100	71.7±0.041	76.9±0.082
F6.4.2	25	73.1±0.032	54.5±0.011
	50	77.9±0.0328	62.7±0.005
	75	80.9±0.406	76.3±0.014
	100	84.1±0.032	83.8±0.023
Standard			
Acarbose	25	76.8±0.041	NA
	50	79.3±0.123	NA
	75	82.7±0.055	NA
	100	88.4±0.037	NA
Olisterate	25	NA	62.5±0.053
	50	NA	70.4±0.007
	75	NA	78.5±0.069
	100	NA	84.4±0.015

4. Discussion

Some of the secondary metabolites exhibited antioxidant and antidiabetic properties through different way of mechanisms. Phenolic compounds are considered to be primary antioxidants or free radical scavengers [27]. The amounts of total phenolics (387±0.577 µg/mg) contents were highest in the F6.4.2 plan extract as shown in table 1. TLC study of crude extract and final fraction of *Rosa centifolia L.* petal extract show similar Rf value to polyphenolic compound quercetin (Rf= 0.75 and 0.82). Polyphenolic compounds have an aromatic benzene ring with substituted hydroxyl groups, including their functional derivatives [28]. The presence of a high content of phenolic in plant extract as bioactive compounds are mainly responsible for antioxidant and antidiabetic activity. So further investigation is under progress by isolating the F6.4.2 and rescreening their *in-vitro* antioxidant and antidiabetic potency on different models. In this study, the antioxidant and antidiabetic activity of *Rosa centifolia L.* was measured using two different assay method. Evaluating the antioxidant properties through one method would not give the correct result. Therefore, it is important to carry out more than one type of antioxidant capacity measurement to cover the various mechanisms of antioxidant action [29]. A comparison of the DPPH radical scavenging activity of different fractions of plant extract against standard drugs has been depicted in figure 3. Ascorbic acid used as a positive control shows 59.8±0.598 to 81.80±0.062 scavenging activity, illustrating the DPPH radical scavenging activity of different fractions of crude extract at various concentrations. These results showed that the F6.4.2 fraction of *Rosa centifolia L.* contained a high amount of radical scavenging compounds with proton-donating ability. F6.4.2 fraction exhibited increased DPPH radical scavenging activity from 52.1±0.854 to 80.56± 0.020 mg/L. Moreover, other fractions showed less activity at all concentrations. A comparison of the FRAP activity of different fractions of plant extract against standard drug ascorbic acid shows 3.48±0.027 to 4.09±0.111 scavenging activity, used as positive control has been depicted in figure 4. This result shows that the F6.4.2 fraction exhibited higher reducing activity from 2.29±0.026 to 3.65±0.052 mg/L. The results obtained are consistent with the studies carried out on *Rosa centifolia L.* F6.4.2 fraction containing a higher amount of reducing activity. Moreover, other fractions showed that they have less activity at all concentrations.

In the current research, the experimental findings revealed that fraction F6.4.2 of ethanolic extract has significantly high DPPH free radical scavenging activity and FRAP. The results were calculated in Table 3 for DPPH and FRAP [30]. Natural polyphenols are reported to have inhibitory activity against enzymes like α-amylase, and α-glucosidase (carbohydrate hydrolyzing) [31]. In this study, the *in-vitro* α–amylase inhibitory activities of *Rosa centifolia L* petals ethanolic extract and its fraction were investigated. F6.4.2 showed potent inhibitory activity against α-amylase from 73.10±0.003 to 84.1±0.032% in a dose-dependent manner. The results were calculated in Table 4. Acarbose is a standard drug for α-amylase inhibitory activity 76.8±0.041% to 88.4±0.037% used as the positive control. An α-amylase inhibitory activity comparison between the reference compound (standard drug) and plant extracts and its fraction has been depicted in figure 5. Our results of various fraction and mother extracts of *Rosa centifolia L.* petals for the first time reported the potent inhibitory effect against key enzymes related to diabetes. This investigation showed The F6.4.2 showed potent α-amylase inhibitory activity from 54.50±0.011 to 83.80±0.032% in a dose-dependent manner. The inhibitory effect of *Rosa centifolia L.* extracts against pancreatic lipase was compared to that of orlistat, a lipase inhibitor showing inhibitory activity 62.5±0.052 to 84.4±0.015 used as a positive control [32].

5. Conclusion

The results were calculated in Table 4. An α-lipase inhibitory activity comparison between the reference compound (standard drug) and ethanolic plant extracts and its fraction has been depicted in figure 6. The *in-vitro* study provided partial regarding the pharmacological applications of *Rosa centifolia L.* in the treatment of diabetes. However, further research including *in-vivo* studies and isolation of the active constituents will be interesting to explore and to confirm the efficacy of *Rosa*

centifolia L. Petal extract before its extensive use in the management of diabetes and its secondary complications.

6. Acknowledgments

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