Antidiabetic and antioxidant activities of *Roylea cinerea* extracts: a comparative study

Reena Sharma¹, Deepak Yadav², Mohd. Asif²*, MA Jayasri³, Vijai K Agnihotri⁴ & PC Ravikumar¹,**

¹School of Basic Sciences, Indian Institute of Technology-Mandi, Kamand Campus, Himachal Pradesh-175 005, India  
²Department of Pharmacology, Faculty of Medicine, Jamia Hamdard, New Delhi-110 062, India.  
³School of Biosciences and Technology, VIT University, Vellore-632 014, Tamil Nadu, India.  
⁴Natural Plant Products and Process Development Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur-176 061, Himachal Pradesh, India

Received 16 July 2015; revised 08 March 2016

*Roylea cinerea* (D.Don) Baill. (Fam. *Lamiaceae*), commonly known as Ashy Royleais, and locally, kattu is widespread in the Himalaya from Kashmir to Nepal, and known for its antidiabetic activity. In this study, we evaluated the crude ethanolic extract and its sub-extracts obtained from the aerial part of *R. Cinerea* for antioxidant and antidiabetic activities. The antioxidant activity was evaluated by different chemical-based assays, viz. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant potential (FRAP) and oxygen radical absorbance capacity (ORAC). The total phenolic content in *R. cinerea* extracts varied widely 3.87-95.3 mg GAEs/g of dry plant extract. Reduction in the blood glucose level was observed in albino male wistar rats after treatment for 15 days and the treatment with ethyl acetate extract showed highest reduction (41.13%) in blood glucose level. Serum lipid profile, hepatic glycogen content, SOD, GPx, GSH and TBARS of pancreatic tissue were also examined. The plant extract treatment increased the levels of SOD, GSH and GPx in the tissues and reduced the level of TBARS as compared to the control group. Histopathological studies showed that the treatment with plant extracts normalized the histology of pancreas and liver. These findings suggest that *Roylea cinerea* extracts has potent antidiabetic and antioxidant properties and thereby substantiate its use in traditional medicines.

**Keywords:** ABTS assay, Ashy Royleais, Diabetes, DPPH assay, Herbal, FRAP, ORAC, Phenolics

Diabetes is one of the most common endocrine disorders in human beings and has already emerged as one of the greatest global health challenges. About 415 million people are reported to be affected by diabetes and it is estimated to escalate to 642 million by 2040. In 2015 alone, this life-threatening disease has consumed 5 million adult lives across the globe and the International Diabetes Federation (IDF) has already declared Diabetes to be a global emergency¹. Generally, resistance of the target tissues to insulin or decrease in the number of β cells contributes to this disorder².³ Abnormality in insulin secretion leads not only hyper conditions but sometimes hypo conditions of blood sugar and lipid occurred⁴. Oxidative stress plays an important role in complications of diabetes⁵.

Involvement of oxidative stress in the development of diabetes mellitus is not only by oxygen free-radical generation but it can be suggested due to non-enzymatic protein glycosylation, impaired glutathione metabolism, auto-oxidation of glucose, formation of lipid peroxides and alteration in antioxidant enzymes⁶. Oxidative stress also leads to decrease in antioxidant status⁷. Antioxidants overcome the oxidative stress by obstructing the destruction of β cells and by inhibiting the peroxidation chain reaction, in this way they help in preventing the development of diabetes⁸.⁹ Antioxidants from natural sources have gained importance because of the health hazards from synthetic antioxidants viz. butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylinhydroquinone (TBHQ) and propyl gallate (PG)¹⁰,¹¹. These synthetic reagents are widely being used in industries but they are suspected to be responsible for chronic diseases like alzheimer’s disease, mutations through DNA damage, lipid peroxidation and carcinogenic effects¹²,¹³. Recently

---

*Correspondence:  
Phone: +91 674 2494321  
E-mail: pcr@niser.ac.in; asifdoctor2003@yahoo.co.in  
*Present add.: School of Chemical Sciences, National Institute of Science Education and Research (NISER) Bhubaneswar, Jatni, Odisha-752050, India
research on natural antioxidants has received considerable attention due to the side effects associated with synthetic drugs. A number of assays have been developed to estimate the antioxidant potential of plant extracts and pure compounds.\textsuperscript{14} Oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP), ABTS and DPPH are frequently used methods.

\textit{Roylea cinerea} belongs to the family Lamiaceae. The plant is a medium sized shrub reaching a height of 0.9-1.7 m widespread in the Himalaya from Kashmir to Nepal, in Indian subcontinent at 1200-3700 m altitude. Leaves of this plant are widely used in traditional medicine as decoction for febrifuge, and as a tonic in contusions.\textsuperscript{15} Leaves are also used against skin diseases and crushed pieces of branches are given to infants to treat jaundice. Shoots are crushed and eaten with salt to strengthen the liver. Young shoots are used as insect repellent for cattle during rainy season.\textsuperscript{16} Flowers are used in snuffing during cold season.\textsuperscript{16} Ethanolic extracts of the leaves of \textit{R. cinerea} were reported to have central nervous system depressant and skeletal muscle relaxant activity.\textsuperscript{17} This plant has also been reported to be used as antimalarial agent by people of Garhwal hills.\textsuperscript{18} \textit{R. cinerea} is traditionally used for the treatment of diabetes.\textsuperscript{19} The diterpenes isolated from aerial part of this plant have been reported as antitumor agents against P-388 lymphocytic leukaemia.\textsuperscript{20}

This study is the first extensive analysis of the antidiabetic and antioxidant activities of different extracts in the increasing order of solvent polarity from aerial part of \textit{R. cinerea}.

**Materials and Methods**

**Chemicals**

DPPH (1,1-Diphenyl-2-picrylhydrazyl), ABTS (2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diaminonium salt), 2,2´-azobis-2-methyl-propanimidamide dihydrochloride (AAPH), gallic acid, Folin-Ciocalteu reagent, ethylene diamine tetra acetic acid (EDTA), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), ascobic acid, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ferrozone and alloxane monohydrate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Biochemical kits and all other chemicals utilized were of analytical grade and purchased from HIMEDIA (India) and Qualigens (India/Germany). Other chemicals were of highest quality commercially purchased from local suppliers.

**Plant Material**

Aerial parts of \textit{R. cinerea} were collected from Yol region (Dharamshala district of Himachal Pradesh in Western Himalayas of India) in March 2013. The taxonomical identification of plant material was confirmed by Dr. Brij Lal senior scientist, Biodiversity department, CSIR-IHBT and voucher specimen (16532) deposited in the Herbarium of CSIR-Institute of Himalayan Bioresource Technology, Palampur, India.

**Preparation of plant extracts**

Aerial part of the plant was shade dried and ground to a fine powder in a mechanical grinder. The powdered sample (1000 g) of the plant was initially extracted with ethanol at room temperature (27 ± 1ºC) for 24 h in a percolator. This process was repeated three times. After filtering through filter paper, the solution was collected and supernatant was recovered. The solvent was evaporated through vacuum rotary evaporator to have the crude extract. This dried crude ethanolic extract was dissolved in water and fractionated with four different solvents in the increasing order of polarity viz. chloroform, ethyl acetate, butanol and water to obtain the respective subextracts. This method was used for the fractionation of soluble compounds from aerial part of \textit{R. cinerea}. After evaporating to dryness each extract was lyophilized to obtain the dry extract which was stored at 4ºC until analysis.

**Preliminary phytochemical screening**

Crude plant extract was subjected to preliminary phytochemical screening for phenolics, saponins, flavonoids, alkaloids, steroids and terpenoids.\textsuperscript{21}

**Animal housing**

Experiment was done using 6 weeks old Albino Male Wistar rats of 150 ± 20 g body wt., and housed in polypropylene cages in the Central Animal House of Hamdard University, New Delhi, India. The rats were acclimatized for one week to new environmental conditions before the beginning of experiment. Animals were divided in groups of five rats per cage and kept maintained at 25 ± 2ºC with a 12 h light and dark cycle. They were given free access to standard laboratory feed (Amrut Laboratory, Navmaharashtra Chakan Oil Mills Ltd, Pune, India) and water \textit{ad libitum}. The experiments were done according to the guidelines of the Institute’s Animals Ethics Committee (registration number 173/CPCSEA).
Determination of total phenolic content

The total phenolic content of the extracts of *R. cinerea* was measured by Folin-Ciocalteu method as described previously by Gao et al.\(^2\)\(^6\). 100 µL of plant extract was mixed with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of water, and left for incubation at room temperature for 3 minutes following the addition of 1 mL of 20% sodium carbonate to the mixture. The absorbance relative to that of blank was measured at 765 nm after 1 h of incubation at room temperature. Gallic acid was used as a standard for quantification. The results were expressed as gallic acid equivalents (GAE), milligrams per gram of extract. All determinations were performed in triplicate.

Reducing power

The reducing power of different solvent extracts of aerial part of *R. cinerea* was determined according to the method of Oyaizu\(^2\)\(^3\). About 2 mg of the extract in 1 mL of distilled water was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K\(_3\)Fe(CN)\(_6\)]. The mixture was incubated at 50ºC for 20 min, 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm (thermofisher multifuage X3R) for 10 min. After centrifugation 2.5 mL of this solution was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. With increase in concentration the absorbance of reaction mixture also increased. This increase in absorbance indicated increased reducing power. All measurements were done in triplicate.

DPPH radical scavenging activity

Radical scavenging activity of the extracts against stable DPPH radical was determined using Shimadzu UV-2450 Spectrophotometer at 517 nm. The radical scavenging activity of extracts was measured by the method of Williams et al.\(^2\)\(^4\) with slight modification. DPPH (100 µM) was prepared in ethanol. Varying doses of the extracts were prepared with respective solvents. About 1.9 mL of 100 µM DPPH was added to each test tube and incubated in dark at room temperature. The solution was kept at 30 min incubation. Absorbance was read at 517 nm. Percentage DPPH radical scavenging activity (% DRSA) was calculated using the following formula.

\[
\% \text{ DRSA} = \left(\frac{A_c - A_s}{A_c}\right) \times 100
\]

where \(A_c\) is the absorbance of the control and \(A_s\) is the absorbance of the extract. All measurements were done in triplicate.

Free radical scavenging by ABTS radical

The free radical scavenging capacity of plant extracts was also studied using ABTS radical cation decolorization assay Re et al.\(^2\)\(^5\). ABTS was dissolved in deionized water to a 7 mM concentration. ABTS radical cation was produced by reacting ABTS solution with 2.5 mM potassium persulphate and allowing the reaction mixture to stand in dark at 25ºC for 12-16 h before use. ABTS radical solution was diluted in deionized water to an absorbance of 0.7 (±0.05) at 734 nm. The absorbance reading was taken after 10 min of the addition of 100 µL of different extracts solutions to 3 mL of ABTS radical cation solution. All solutions were used on the same day of preparation and all determinations were carried out in triplicate.

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the procedure of Benzie and Strain\(^2\)\(^6\) with slight modification. This method is based on the principle that there is reduction of ferric 2,4,6-Tris(2-pyridyl)-s-triazine (Fe\(^{3+}\) TPTZ) to its ferrous, blue coloured form (Fe\(^{3+}\) TPTZ) in the presence of antioxidants. The FRAP reagent was prepared from 0.3 M acetate buffer (pH 3.6), 10 mmol of TPTZ solution in 40 mmol of HCl and 20 mmol of iron (III) chloride solution in ratio of 10:1:1 (v/v), respectively. The FRAP reagent was freshly prepared daily and warmed at 37ºC before use. 1.5 mL of FRAP reagent was added to 50 µL of extract after 4 min the absorbance of reaction mixture was recorded at 593 nm using blank. FRAP values were calculated using Fe\(_2\)O\(_4\).7H\(_2\)O as standard. All measurements were done in triplicate.

Free radical scavenging by oxygen radical absorbance capacity (ORAC) assay

The ORAC assay is based on the principle of scavenging of peroxyl radicals generated by thermal decomposition of 2,2’-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) which prevent the quenching of the fluorescent probe. The ORAC assay was applied according to the method of Ou et al.\(^2\)\(^7\) with little modification. The reaction was carried out in 75 mM phosphate buffer (pH 7.4), 300 µL of plant extract solutions and 1.8 mL of fluorescein (70 mM final concentrations) were mixed in a cuvette and for 5 min pre-incubated at 37ºC. AAPH solution (900 µL, 12 mM, final concentration) was added to
the incubated mixture, and then fluorescence was recorded for 60 min at the interval of every 5 min on Agilent Technologies Cary eclipse fluorescence spectrometer. A blank sample using 300 µL of phosphate buffer instead of antioxidant solution and four calibration solutions of Trolox (10, 30, 50, 70 µM concentration) were also tested to establish a standard curve. The measurements were taken in triplicate. The area under the curve (AUC) was calculated by applying the following formula.

\[
AUC = (1 + f_1/f_0 + f_2/f_0 + f_3/f_0 + \ldots \ldots + f_n/f_0)
\]

where \( f_0 \) is the initial fluorescence and \( f_n \) is the fluorescence at time ‘n’.

The net AUC of the sample was calculated by subtracting the AUC of the blank. The regression equation between net AUC and Trolox concentration was determined and ORAC values were expressed as µM Trolox equivalents by using the standard curve of trolox.

**Diabetes induction**

Animals (Albino Male Wistar rats) were used for the experiment. Animals were kept fasting overnight. After fasting alloxan monohydrate (150 mg kg\(^{-1}\) body wt.) was administered intraperitoneal to the experimental rats and diabetes was induced, while 5 animals were given 0.1M sodium citrate buffer only.

The experimental animals were divided into eight groups of 5 rats each as follows: Group I, Normal control (NC): Normal rats treated with vehicle alone; Group II, Alloxan-induced diabetic control (DC): Diabetic rats treated with vehicle alone; Group III, Standard drug treatment (SD): Diabetic rats treated with glibenclamide; Groups IV-VIII, diabetic rats treated with (IV) parent extract; (V) water subextract; (VI) ethylacetate subextract; (VII) chloroform subextract of \( R. \ cinerea \); and (VIII) butanol subextract of \( R. \ cinerea \).

Group IV-VIII and VIII were extract treated (ET) groups parent extract, water subextract, ethylacetate subextract, chloroform subextract and butanol subextract, respectively. Following the diabetes induction, the experimental rats were administered 50 % glucose solution to provoke hypoglycemic shock. After 1 wk of administration of alloxan, fasting blood glucose (FBG) concentration was measured with the help of ultra glucometer (Johnson & Johnson Company, USA) with strips. Animals showing FBG higher than 140 mg/dL, (Groups II-VIII) were considered as diabetic\(^{28}\). Animals were treated with drug glibenclamide (Gr. III) (50 mg kg\(^{-1}\) body wt.) and different extracts (Gr. IV-VIII) (50 mg kg\(^{-1}\) body wt.), orally per day and diabetic control (Gr. II) was left without treatment. The control group was administered 0.1M sodium citrate buffer.

**Biochemical assays**

Biochemical parameters were estimated for serum. After 15 days of treatment, animals were kept fasted overnight and blood was collected through retro orbital plexus on day 16\(^{th}\) and serum was separated. Different cholesterol levels as total cholesterol (TC), HDL-cholesterol, VLDL, LDL cholesterol and triglyceride (TG), were measured in mg/dL with the help of diagnostic kits (SPAN Biotech Pvt. Ltd. India).

After 15 days of treatment the animals were sacrificed under diethyl ether anesthesia. Pancreases were removed from the body and washed with chilled saline solution (0.9%). Tissues were left for drying and thereafter the weight of the pancreas was measured. One gram pancreatic tissue of each experimental animal was homogenized in 10 mL of 0.2 M tris-HCl using homogenizer (Proscientific, US). The homogenate was filtered, cooled and centrifuged at 10000 rpm for 20 min at 4°C. Obtained supernatant was estimated for the hepatic glycogen content\(^{29}\), superoxide dismutase (SOD)\(^{30}\), glutathione peroxidase (GPx)\(^{31}\), reduced glutathione (GSH)\(^{32}\) and thiobarbituric acid reactive substances (TBARS)\(^{33}\).

**Histopathological observation**

Liver and pancreas of all the experimental groups were further studied for their histopathological changes followed by Yadav et al.\(^{34}\). The extract treated groups were compared with normal control, diabetic control and glibenclamide treated groups for any changes. Parts of selected organs of experimental rats were fixed in 10% formalin. The organs were then processed in graded series of alcohol ororbital plexus on day 16\(^{th}\) and serum was separated. Following the diabetes induction, the experimental rats were administered 50 % glucose solution to provoke hypoglycemic shock. After 1 wk of administration of alloxan, fasting blood glucose (FBG) concentration was measured with the help of ultra glucometer (Johnson & Johnson Company, USA) with strips. Animals showing FBG higher than 140 mg/dL, (Groups II-VIII) were considered as diabetic\(^{28}\). Animals were treated with drug glibenclamide (Gr. III) (50 mg kg\(^{-1}\) body wt.) and different extracts (Gr. IV-VIII) (50 mg kg\(^{-1}\) body wt.), orally per day and diabetic control (Gr. II) was left without treatment. The control group was administered 0.1M sodium citrate buffer.

**Statistical analysis**

All the assays were done in triplicates. Results were expressed as mean ± standard deviation of three measurements. Statistical analysis were executed following the student’s ‘t’-test with the help of software Sigma Plot (version 8.0) and one-way (ANOVA) by PRISM software \( P <0.05 \) were considered significant.
Results
The results of phytochemical screening showed the presence of phenolics, saponins, flavonoids, alkaloids, steroids and terpenoids.

Total phenolic content
The total phenolic contents in different extracts were determined from calibration curve of gallic acid using linear regression equation and were expressed in gallic acid equivalents per gram of dry plant extract. Table 1 gives the total phenolic content of all the extracts. Our report demonstrates the high total phenolic contents from different extracts of aerial part of *R. cinerea*.

Radical scavenging activity using DPPH and ABTS assays
The DPPH and ABTS free radical scavenging assays are widely used for antioxidant activity determination of various natural products. Antioxidant activity was expressed in terms of percentage inhibition for different extracts and a graph was plotted between percentage inhibition and concentration of different extracts (Fig. 1). Antioxidant activity showed an increase with the increase in concentration. The IC$_{50}$ value for butanol subextract was lowest as compared to other extracts. IC$_{50}$ of extracts were ranged from 84.83 µg/mL (butanol subextract) 198.55 µg/mL (ethyl acetate subextract), 426.9 µg/mL (parent extract), 610.09 µg/mL (chloroform subextract) to 661.8 µg/mL (water subextract). The IC$_{50}$ value for standard (ascorbic acid) was 6.3 µg/mL. The results showed that the butanol subextract possess the highest DPPH scavenging activity among all the extracts. ABTS assay with different extracts showed similar order of antioxidant potential as DPPH assay. In ABTS assay butanol subextract showed highest antioxidant activity as was shown by DPPH assay (Fig. 2). The IC$_{50}$ of extracts were ranged from 60.3 µg/mL (butanol subextract), 95.84 µg/mL (ethyl acetate subextract), 152.11 µg/mL (parent extract), 259.68 µg/mL (chloroform subextract) to 544.3 µg/mL (water subextract) and for standard (ascorbic acid) IC$_{50}$ value was 8.07 µg/mL. Our results have proved that butanol and ethyl acetate subextracts of *R. cinerea* have significant free radical scavenging activity and therefore exhibited higher antioxidant activities.

FRAP
In our results, the butanol subextract showed higher reducing power in comparison to all other extracts from aerial part of the plant. FRAP results were in linear correlation with DPPH and ABTS results. Table 2 showed the value of Fe$^{3+}$ reduction by different extracts in terms of mill moles of Fe$^{2+}$ equivalents per gram of dry weight of extracts. Fe$^{2+}$ equivalents were calculated from the calibration curve of standard FeSO$_4$.7H$_2$O by using linear regression equation.

---

Table 1 — Total phenolic contents of different extracts of *R. cinerea*.

<table>
<thead>
<tr>
<th>Different extracts</th>
<th>Phenolic content (mg GAEs/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylacetate subextract</td>
<td>65.53±3.25</td>
</tr>
<tr>
<td>Chloroform subextract</td>
<td>33.8±2.71</td>
</tr>
<tr>
<td>Butanol subextract</td>
<td>95.3±2.72</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>45.03±3.00</td>
</tr>
<tr>
<td>Water subextract</td>
<td>3.87±0.45</td>
</tr>
</tbody>
</table>

* Gallic acid equivalents

Data expressed as mean ± SD from three individual experiments

---

Figs. 1 — Percentage Scavenging by different solvent extracts of *Roylea cinerea* in DPPH assay

Figs. 2 — Percentage Scavenging by different solvent extracts of *Roylea cinerea* in ABTS assay.
ORAC

ORAC is used as one of the consistently good and standard assays to investigate the antioxidant activity of food products. ORAC value gives an indication of the antioxidant activity of the substance. The values for butanol, chloroform, ethyl acetate, crude and water extracts were found to be 49.73 ± 6.12, 20.31 ± 4.50, 36 ± 2.94, 28.16 ± 4.33 and 8.55 ± 3.49, respectively (Table 2). ORAC assay results were also showing the similar order of antioxidant potential as was shown by DPPH, ABTS, FRAP and reducing power assay.

Total reducing power

Reducing power assay is based on the principle of electron donation which also indicates antioxidant potential of the extract. Reducing power of all the extracts was in linear correlation with their concentration. The increase in absorbance indicated increase in reducing power. The total reducing power of ethanolic extract and its subextracts of R. cinerea along with standard was shown in Fig. 3, among different extracts butanol subextract showed the highest reducing power.

Effect of treatment on body weight and blood sugar level

Experimental animals showed reduced body wt. to groups that administered alloxan (Table 3) and also showed increased blood glucose in alloxan administered rats. All extracts reduced the glucose level after 15 days treatment while ethyl acetate extract showed highest reduction (41.13%) as comparison with standard and other extracts (Table 4).

Effect of treatment on hepatic glycogen content

A significant decrease of (56.33 ± 8.5 to 33.66 ± 9.07) was observed in the hepatic glycogen content of DC as compared to NC. But after the treatment with extracts a significant increase of hepatic glycogen content was observed compared to DC. Similarly, treatment of SD (glibenclamide) increased the hepatic glycogen content while ethyl acetate and butanol subextracts showed highly significant results as compared to SD also (Table 5).

Effect of treatment on serum lipid profile

Alloxan introduction increased the level of serum total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG), and very low density lipoprotein (VLDL), in diabetic control (group 2), whereas the level of high density lipoprotein (HDL) considerably reduced in DC. However extract treatment reduced the level of serum total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and increased the level of high density lipoprotein (HDL). Treatment with butanol and ethyl acetate subextract of ethanolic extract of R. cinerea reduced the level of total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) as compared to SD (Fig. 4).

Table 2 — FRAP and ORAC values for different extracts of R. cinerea.

<table>
<thead>
<tr>
<th>Different extracts</th>
<th>FRAP (mmol Fe²⁺/g)</th>
<th>ORAC (μM TEAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylacetate subextract</td>
<td>0.59 ± 0.02</td>
<td>36 ± 2.94</td>
</tr>
<tr>
<td>Chloroform subextract</td>
<td>0.33 ± 0.01</td>
<td>20.31 ± 4.50</td>
</tr>
<tr>
<td>Butanol subextract</td>
<td>1.09 ± 0.08</td>
<td>49.73 ± 6.12</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>0.40 ± 0.01</td>
<td>28.16 ± 4.33</td>
</tr>
<tr>
<td>Water subextract</td>
<td>0.14 ± 0.03</td>
<td>8.55 ± 3.49</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.D from three individual experiments. Trolox equivalents

Table 3 — Effect of the 15 days treatment of different solvent extracts of R. cinerea on body weight.

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>NC (Control)</th>
<th>Diabetic</th>
<th>SD (Standard)</th>
<th>ET (Parent)</th>
<th>ET (Water)</th>
<th>ET (Ethylacetate)</th>
<th>ET (Chloroform)</th>
<th>ET (Butanol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>152.6±2.1</td>
<td>160±0.5</td>
<td>155.2±1.8</td>
<td>154.4±2.58</td>
<td>170.5±1.8</td>
<td>164.4±1.58</td>
<td>156.3±1.8</td>
<td>161.4±1.8</td>
</tr>
<tr>
<td>Final</td>
<td>174.2±1.9</td>
<td>115±1.15</td>
<td>180.5±3.4</td>
<td>150.4±1.64</td>
<td>176.4±3.4</td>
<td>183.4±1.14</td>
<td>172±2.4</td>
<td>169.7±2.16</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Level of significance: *significant (P <0.05) difference and **insignificant difference (P>0.05) compared to basal values.
Effect of treatment on SOD, GSH, GPx and TBARS

SOD activity was reduced after alloxan administration in pancreas. The 15 days treatment with extracts notably improved SOD activity, while ethyl acetate subextract showed high activity as compared to other extracts as well as glibenclamide treated animals, which confirmed to be more significant to normalize the SOD activity as compared to standard drug also (Table 6).

Results from the experiments showed that alloxan also reduced the GSH and GPx activity in experimental rats possibly due to their increased usage by the liver cells or which may be the result of their reduced synthesis and increased degeneration by oxidative stress. Treatment with different extracts showed increased GSH and GPx activity in the pancreatic tissue. Moreover, induction of alloxan significantly \((P<0.05)\) increased the level of TBARS (Table 4). The increased concentration of TBARS has been observed in diabetic condition which is the part of lipid peroxidation. TBARS concentrations were

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glycogen content (mg g(^{-1}) tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>56.3±3.85</td>
</tr>
<tr>
<td>DC</td>
<td>33.5±6.05</td>
</tr>
<tr>
<td>SD</td>
<td>81.0±8.85</td>
</tr>
<tr>
<td>ET Parent</td>
<td>75.0±7.05</td>
</tr>
<tr>
<td>ET Water</td>
<td>64.0±3.65</td>
</tr>
<tr>
<td>ET Ethylacetate</td>
<td>100.3±6.05</td>
</tr>
<tr>
<td>ET Chloroform</td>
<td>82.3±4.15</td>
</tr>
<tr>
<td>ET Butanol</td>
<td>87.3±3.55</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. One-way ANOVA is significant at \(P<0.05\). *significant \((P<0.05)\) difference, compared to NC; **significant \((P<0.05)\) difference, compared to DC; ***significant \((P<0.05)\) difference compared to SD.

Table 4 — Effect of the 15 days treatment of different solvent extracts of *R. cinerea* on blood sugar after diabetes induction.

<table>
<thead>
<tr>
<th>Blood sugar (mg/dL)</th>
<th>NC (Control)</th>
<th>DC (Diabetic)</th>
<th>SD (Standard)</th>
<th>ET (Parent)</th>
<th>ET (Ethylacetate)</th>
<th>ET (Chloroform)</th>
<th>ET (Butanol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>109.2±1.94</td>
<td>215.4±4.13&quot;</td>
<td>228.6±4.34&quot;</td>
<td>234.4±3.14&quot;</td>
<td>224.7±4.13&quot;</td>
<td>236.8±4.51&quot;</td>
<td>219.72±4.19&quot;</td>
</tr>
<tr>
<td>Day 4</td>
<td>111.3±4.21</td>
<td>226.2±5.4&quot;</td>
<td>206.2±3.5&quot;</td>
<td>216.36±2.2&quot;</td>
<td>208.8±3.54&quot;</td>
<td>218.56±4.23&quot;</td>
<td>206.53±3.23&quot;</td>
</tr>
<tr>
<td>Day 7</td>
<td>120.5±3.22</td>
<td>241.1±4.5&quot;</td>
<td>191.7±2.96&quot;</td>
<td>201.72±3.84&quot;</td>
<td>192.6±2.42&quot;</td>
<td>203.43±5.24&quot;</td>
<td>193.48±1.98&quot;</td>
</tr>
<tr>
<td>Day 10</td>
<td>157.6±5.24&quot;</td>
<td>168.18±5.1&quot;</td>
<td>186.8±2.9&quot;</td>
<td>181.3±2.83&quot;</td>
<td>188.87±4.42&quot;</td>
<td>181.18±3.53&quot;</td>
<td>175.28±2.89&quot;</td>
</tr>
<tr>
<td>Day 13</td>
<td>116.4±3.51</td>
<td>255.81±7.21&quot;</td>
<td>157.61±3.4&quot;</td>
<td>172.5±5.21&quot;</td>
<td>175.2±2.64&quot;</td>
<td>167.62±2.45&quot;</td>
<td>176.3±5.11&quot;</td>
</tr>
<tr>
<td>Day 16</td>
<td>121.1±2.21</td>
<td>257.5±4.6&quot;</td>
<td>145.68±3.16&quot;</td>
<td>154.6±2.37&quot;</td>
<td>164.1±4.41&quot;</td>
<td>139.4±3.21&quot;</td>
<td>167.26±4.27&quot;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. One-way ANOVA is significant at \(P<0.05\). *significant \((P<0.05)\) difference compared to NC, **significant \((P<0.05)\) difference compared to DC, ***significant \((P<0.05)\) difference compared to SD.

Fig. 4 — Effect of different solvent extracts of Roylea cinerea on serum lipid profile. TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein. Values are given as mean ±S.E.M.. *P < 0.05 significant difference compared to NC, **P < 0.05 significant difference compared to DC, ***P < 0.01 significant difference compared to DC.
significantly decreased after the treatment with extracts and ethylacetate subextract was found to be convincingly more active in reducing TBARS concentration in diabetic rats than the glimebclamide treated group (Table 6).

**Histopathological study**

Alloxan administration to the rats of group DC leads to many abrasions in the liver such as dilation in the sinusoids, infiltration in portal triad, granular cytoplasm degeneration and neutrophilic infiltration as compared to the normal control group (Fig. 5). However, the treatment with extracts resulted in normalizing almost all the parameters as compared to standard.

Pancreatic tissue of group NC shows normal islets of langerhans. But after the alloxan administration, damaged islets of langerhans were observed (Fig. 6). However, extract treatment resulted in normalising the deformities caused by alloxan. An interesting striking feature observed in the glimebclamide treated group was presence of granulomatous reactions in the lymph nodes adjacent to pancreas.

**Discussion**

In this study, alloxan was used to induce diabetes in the rats. Alloxan promotes hyperglycaemia by the destruction of β cells of islets of Langerhans and leads to excessive production of (ROS) which reduces the release of insulin. Oxidative stress as a consequence of hyperglycaemia leads to diabetes and related complications. Alloxan induces free radical damage due to the production of ROS. These ROS generates lipid peroxidation i.e. the oxidative degradation of the lipids by the free radicals or the ROS. This demoliition of the lipid membrane and the by products of lipid peroxidation are hazardous to the cells and the tissues.

In the present study it has observed that treatment with *R. cinerea* extracts brings back the level of blood glucose to normal and the ethyl acetate showed the highest percentage of decrease in blood glucose level in the fifteen days treatment in diabetes induced rats. Induction of diabetes also contributes to decreased body weight of animals while extract treatment reduced the weight loss in different animal groups.

Alloxan introduction decreased the level of hepatic glycogen content and increased the level of serum lipid profile. Decreased hepatic glycogen content may be due to unavailability of insulin that leads to glycogenolysis. Introduction of plant extract increased the level of hepatic glycogen content in diabetic rats, ethyl acetate extract increased the hepatic glycogen content better than the standard drug glimebclamide. Increased level of serum total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG), and very low density lipoprotein (VLDL), in diabetic control group, whereas the level of high density lipoprotein (HDL) considerably reduced in DC was observed. There was a convincing control in the levels of serum lipids in extract treated diabetic rats. Alloxan administration decreased the level of SOD in pancreas and also reduced the GSH and GPx activity. The decreased activity of SOD, GSH and GPx enzymes may be due to the formation of reactive oxygen species. SOD is an important antioxidant enzyme it damages the peroxides and prevents an organism from peroxides and superoxide radicals giving an antioxidant defense. GSH and GPx also play a dominant role in preventing the oxidative damage. Extract treatment increased the level of SOD, GSH and GPx and ethyl acetate subextract showed comparable results as comparable to standard drug glimebclamide.

### Table 6 — Effect of different solvent extracts of *R. cinerea* on SOD, GPx, GSH and TBARS activity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/g)</th>
<th>GPx (U/g)</th>
<th>GSH (U/g)</th>
<th>TBARS (nmoles TBARS/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>76.13±3.4</td>
<td>48.16±3.5</td>
<td>79.16±2.4</td>
<td>38.2±2.6</td>
</tr>
<tr>
<td>DC</td>
<td>32.36±4.7</td>
<td>16.13±2.7</td>
<td>37.12±1.9</td>
<td>354.4±6.7</td>
</tr>
<tr>
<td>SD</td>
<td>69.50±2.7</td>
<td>45.3±4.1</td>
<td>74.5±2.7</td>
<td>134.2±4.1</td>
</tr>
<tr>
<td>ET Parent</td>
<td>62.21±2.6</td>
<td>37.6±4.5</td>
<td>66.25±2.0</td>
<td>231.4±5.6</td>
</tr>
<tr>
<td>ET Water</td>
<td>56.5±4.4</td>
<td>31.2±1.8</td>
<td>59.2±1.6</td>
<td>245.2±8.6</td>
</tr>
<tr>
<td>ET Ethylacetate</td>
<td>52.7±3.7, c</td>
<td>46.3±2.3, c</td>
<td>78.5±4.6, c</td>
<td>112.3±7.3, c</td>
</tr>
<tr>
<td>ET Chloroform</td>
<td>58.8±3.2, b</td>
<td>29.4±3.1, b</td>
<td>58.3±4.1, b</td>
<td>206.0±5.4, b</td>
</tr>
<tr>
<td>ET Butanol</td>
<td>68.2±2.5</td>
<td>42.2±5.2</td>
<td>75.2±3.1</td>
<td>123.7±4.5, b</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. One-way ANOVA is significant at *P* < 0.05. *a* significant (*P* < 0.05) difference, compared to NC; *b* significant (*P* < 0.05) difference, compared to DC; *c* significant (*P* < 0.05) difference, compared to SD.
TBARS were observed in diabetic control group as compared to normal control group\textsuperscript{44}. Treatment with the extracts decreased the level of TBARS in diabetic rats. Alloxan induced diabetes also changes the histopathology of liver and pancreas while administration of extracts prohibit the histopathological changes of the liver and the pancreas. Due to the differences between various free-radical scavenging assay systems, it is better to employ different methods for the evaluation of antioxidant activities. In this study different methods viz. DPPH, ABTS, FRAP, ORAC, reducing power assay were employed for the determination of antioxidant potential of different extracts. Butanol subextract from the crude ethanolic extract of \textit{R. cinerea} showed highest antioxidant activity in DPPH as compared to ethanol (parent)
extract, chloroform, ethyl acetate and water subextracts. In other assays also, butanol subextract showed highest antioxidant activity as compared to other extracts. A linear relationship was found between the total phenolic content and antioxidant activity. The positive correlation between phenolic content and antioxidant potential of different plant extracts have been thoroughly demonstrated in previous reports. The present study indicates that the ethyl acetate subextract of *R. cinerea* exhibit antidiabetic activities comparable with the standard drug, glibenclamide. The significant antioxidant and antidiabetic activity of *R. cinerea* extracts could be due to the presence of different secondary metabolites detected in the phytochemical screening.

**Conclusion**

*Roylea cinerea* is a known potent herbal medicinal plant and it contains diterpenoids and glycosides constituents. In the present study, antidiabetic and antioxidant activities of different solvent extracts of *R. cinerea* were investigated. To the best of our knowledge, this is the first report on the total phenolics, antioxidant and antidiabetic activities of this plant. Our results showed high amount of phenolics present and high antioxidant activities of butanol and ethyl acetate subextracts. This study was directed to investigate the comparison among various solvent extracts of *R. cinerea* in the treatment of alloxan induced diabetes in rats. We conclude that the different extracts of aerial part of *R. cinerea* can slow down the progression of diabetes in rats and also having high antioxidant activities. Currently, we are in the process of further isolation and characterization of the active molecules responsible for the observed antidiabetic and antioxidant properties of *R. cinerea*.

**Acknowledgement**

The authors are thankful for financial assistance in the form of SEED Grant by IIT Mandi and Department of Science and Technology, New Delhi and IIT Mandi for financial assistance (Sr/FT/CS-92/2011), AMRC Facilities at IIT Mandi and support from CSIR-IHBT Palampur and Jamia Hamdard, New Delhi is gratefully acknowledged.

**References**


dant effects and their relationship to ion prepared from –


