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Original Research Article

In vitro Antioxidant, Antimicrobial and Antidiabetic activities of Insulin plant rhizome extracts - A comparative study

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EAEIP – Ethyl Acetate Extract of Insulin Plant
EEIP - Ethanolic Extract of Insulin Plant
MEIP - Methanolic Extract of Insulin Plant.

ABSTRACT

Background: *Costusigneus* retained the name as Insulin plant through traditional practice, further phytochemical and pharmacological evaluation of leaves of the plant has proven record on wider therapeutic potential such as antioxidant, antimicrobial and antidiabetic activity. Our interest of current research is to explore the *Costusigneus*rhizome by multiple extraction and to screen and find out the specific therapeutic potential of the specific extracts through relevant phytochemical and pharmacological *in vitro* evaluation.
Methods: *In vitro* DPPH, Disc Diffusion and DNS methods were chosen to evaluate antioxidant and antibacterial and antidiabetic activity on various *Costusigneus* rhizome extracts (ethyl acetate, methanol, ethanol and aqueous).
Results: The *in vitro* anti-oxidant effect is significantly high and almost same for all *Costusigneus* rhizome extracts. *Costusigneus* ethyl acetate, ethanol, methanol has moderate antibacterial activity against gram positive (*B. subtilies* and *S. aureus*) and gram negative organism (*E.coli* and *P. aeruginosa*) by disc diffusion method as compare to the standard drug (Ceftrioxone sodium). However, *Costusigneus* aqueous extract lacs antibacterial activity. The α-amylase inhibition activity by DNS method result infers, high antidiabetic activity for *Costusigneus* ethyl acetate and ethanolic rhizome extracts whereas mild activity for *Costusigneus* methanolic and aqueous rhizome extracts.
Conclusion: Thereby *Costusigneus*e thonolicrhizome extract might be a good choice for antioxidant, antibacterial and antidiabetic therapeutic segments among the *Costusigneus* rhizome extracts.

1. Introduction

In the present scenario the life threatening microbial infections is on the rise on worldwide, irrespective of available and newer synthetic antimicrobial agents and moreover the usage of these agents are limited by the prompt development of multiple drug resistant after a very short exposure of these agents. The multiple drug resistant might be addressed by developing the drug originated from plant. *Costus igneus* famously also known as Insulin plant as twice daily intake of 2-3 leaves was shown to be effective on the management of diabetic mellitus patent, traditionally practiced in the state of Kannada, India.[1–4] Pharmacognatical analysis leaf of the various extracts found to possess important phytochemicals such as such as alkaloids, flavonoids, terpenoids, glycosides, steroids and saponins and the antidiabetic activity was correlated due to the presence of flavonoids and saponins.[5] The present study was focussing the phytochemical and pharmacological screening on multiple extracts on rhizomes of *Costus igneus* on specific activities such as antioxidant, antibacterial, and antidiabetic activities to conclude the therapeutic potential of the *Costus igneus* rhizome.

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2. Materials and Methods

2.1. Plant material

The *Costus igneus* fresh rhizomes were collected from Kodaikanal hills, Tamilnadu, India. The plant material was authenticated by School of Agriculture & Animal Sciences Gandhigram Rural Institute. The rhizomes were shade dried, powered by pulveriser and stored in an air tight plastic container, until further use.

2.2. Preparation of multiple Rhizome extracts of costus igneus

The 500g of the dried coarse powder of *Costus igneus* rhizome was first treated with petroleum ether, the defatted powder was extracted with series of extraction by ethyl acetate, methanol, ethanol and distilled water by cold maceration, each extract was filtered separately by whatman No. 1 filter paper, filtrates were evaporated to remove the solvent at 40 to 45°C by electrical water bath, obtained filter cakes of each extracts were freeze dried and stored separately at −20°C until use [Figure 1).

2.3. Phytochemical analysis

Qualitative phytochemical analysis performed on various *Costus igneus* rhizome extracts in order to screen and confirm the phytochemical presence in extract and to correlate with the results with *in vitro* pharmacological activity.

3. Gas Chromatography-Mass spectroscopy (GC-MS) Analysis

GC-MS is a hybrid analytical instrument that couples the separation capabilities of GC with the detection properties of the mass spectrometer (MS). The sample solution is injected into the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas. The sample flows through the column and the compounds comprising the mixture of interest are separated by their relative interaction with the coating of the column and the carrier gas. The latter part of the column passes through a heated transfer line and ends at the entrance to the ion source where compounds eluting from the column are converted to ions. The next component is a mass analyzer, which separates the positively charged ions according to various mass-related properties depending upon the analyzer used. After the ions are separated they enter into the detector and the output from which is amplified to boost the signal.

3.1. GC-MS operating conditions

The initial column temperature was 35ºC with a hold time of 3 minutes. The temperature was programmed to rise by 8ºC/ minute with a final temperature of 280ºC. In the process, 1μl of the sample was injected into the port and immediately vaporized and moved down the column with helium as the carrier gas with a flow rate of 1 ml/minute. The MS Spectrum was taken at 70 eV. After the separation in the column, the components were identified and further analyzed by FID. The identification of the compounds was done by comparing in NIST MS 2.0 Structural library to find out the names, molecular weight, and structure.

3.2. Antioxidant activity in vitro (DPPH-Method)

It is determined by DPPH (1, 1-diphenyl2-picrylhydrazyl) by the principle based on the free radical scavenging activity by using ascorbic acid as a standard by measuring the absorbance at 517 nm by UV-Visible spectrophotometry against the blank methanol.[6] Inhibition of free radical DPPH in percent was calculated as follows

$$\%\ Inhibition = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} = x\ 100$$

IC_{50} values were calculated as the concentration of each sample required to give 50% DPPH radical scavenging activity from the graph (linear regression curve).

3.3. Antibacterial activity - in vitro (Disc-diffusion method)

Antibacterial study was carried out on 2 gram positive (*B. subtilies*and *S. aureus*) and 2 gram negative (*E.coli*and *P.aeruginosa*) pathogenic organisms by disc diffusion method.[7] The sample crude extracts of ethyl acetate, methanol, ethanol and aqueous extracts of 50 μg ig/ml and standard (ceftrioxone sodium 30 μg/ml) of each sample sterile disc was inoculated with 4 bacteria in sterile nutrient agar medium containing petri dish with the help of laminar flow, then all the petri dishes are transferred aseptically into incubator and incubated at 37°C for 24 h, after the incubation period of 24 h the zone of inhibition is measured, (in mm) tabulated and the results were compared with standard.

3.4. In vitro diabetic activity: Inhibition assay for α-amylase activity (DNS)

Acabose (standard) and various *Costus igneus* rhizome extracts (ethyl acetate, methanol, ethanol and aqueous (tests) of five concentrations (40, 80, 120, 160 and 200μg/ml) were prepared by dissolving in double distilled water. A total of 1000μl of each plant extract and 500 μl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α-amylase solution (0.5mg/ml) were incubated for 10 minutes at 25°C.After pre-incubation, 500μl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was added to each tube at 5s intervals. This reaction mixture was then incubated for 10 minutes at 25°C.1ml of DNS colour

reagent was added to stop the reaction. These test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Finally, this reaction mixture was again diluted by adding 10ml distilled water following which absorbance was measured at 540nm.[8]

$$\%\ inhibition = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} = x\ 100$$

IC_{50} values were calculated as the concentration of each sample required to give 50% DNS α-amylase inhibitory activity from the graph (linear regression curve).

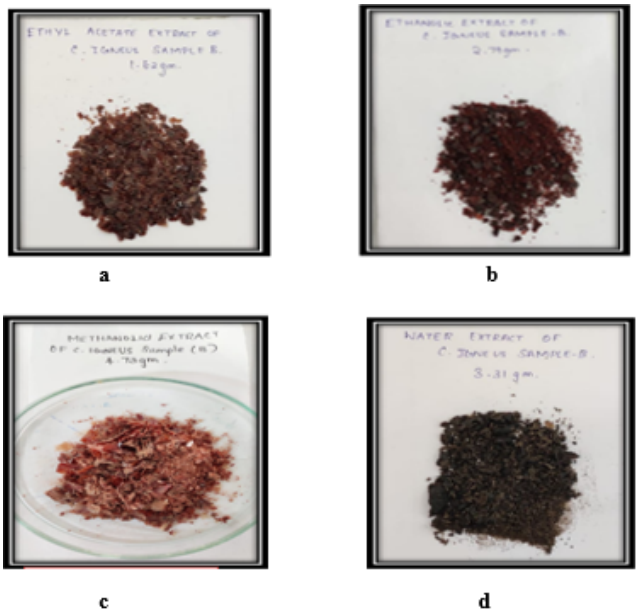

Fig. 1: a: Ethyl acetate Extract of *C. igneus* of *C. igneus* b: Ethanolic Extract of *C. igneus* c: Methanolic Extract of *C. igneus* d: Aqueous Extract of *C.igneus*

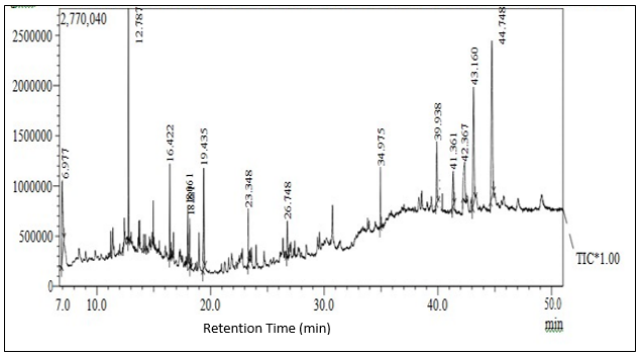

Fig. 2: GC- MS analysis chromatogram

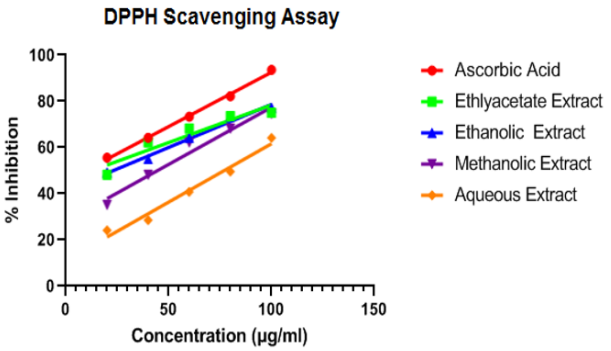

Fig. 3: Thein vitro antibacterial activity (disk diffusion method) of insulin plant extracts

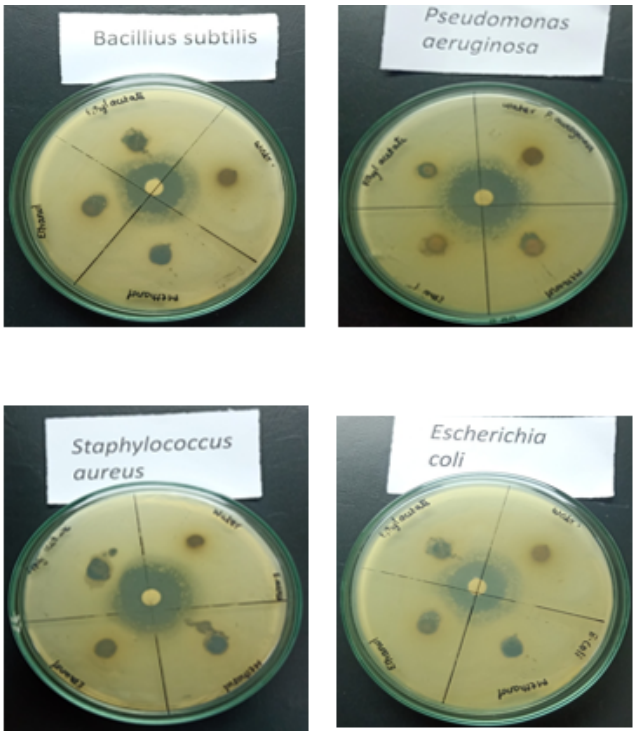

Fig. 4: Zone of Inhibition

4. Results

4.1. Preliminary phytochemical screening

The *Costus igneus* rhizome extracts contained alkaloids, glycosides, saponins, flavonoids, terpenoids, and steroids as presented on the Table 1.

4.2. GC-MS phytochemical analysis

Among the *Costus igneus* rhizome extracts, it is evident that *Costus igneus* ethanolic rhizome extract possess all 3 *in vitro* pharmacological activity at moderate level, thereby we selected the same extract for GC-MS analysis in order to identify and correlate the specific phytochemicals

Table 1: Qualitative phytochemical analysis

S. No	Tests	EAEIP	EEIP	MEIP	AEIP
1	Test for Anthroquinones	-	-	-	-
2	Test for Tannins	-	-	-	-
3	Test for alkaloids	+	+	+	+
4	Test for saponins	+	+	+	+
5	Test for flavonoids	+++	+++	++	+
6	Test for Glycosides	+	+	+	-
7	Test for Terpenoids	++	++	++	+
8	Test for Steroids	++	++	++	-

Table 2: GC-MS analysis chromatogram with pharmacological activity of EEIP

S. No	Compounds	Retention Time	Base m/z	Pharmacological Activity
1	1,1,3-triethoxybutane	6.977	73.05	
2	2,4-ditert-butylphenol	12.787	191.05	Antioxidant
3	Isopropyl Myristate	16.422	59.95	Treatment for head lice.
4	Methylpalmitate	18.061	74.00	Anti-inflammatory
5	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	18.221	57.05	Antioxidant, Antibacterial.
6	Ethyl Palmitate	18.221	88.05	Hair and skin conditioning agent
7	Ethyl (9E,12E)-octadeca-9,12-dienoate	23.348	67.05	
8	2-Ethylhexyl 4-methoxycinnamate	26.748	78.00	Minimize DNA photodamage
9	Squalene	34.975	69.05	Antioxidant, Anticancer
10	Cholesterol	39.938	55.05	Vitamin D
11	9,19-Cyclocholestan-3-ol, 14-methyl-, (3.beta.,5.alpha.)	41.361	55.00	Antibacterial
12	Campesterol	42.367	55.05	Anticancer
13	Stigmasterol	43.160	55.05	Diuretic
14	Gamma-Sitosterol	44.748	57.05	Antidiabetic

Table 3: In vitro Antioxidant activity by DPPH scavenging assay

S. No	Conc. (μg/ml)	Percentage Inhibition					IC_{50} Value
		Standard	EAEIP	EEIP	MEIP	AEIP	
1	20	55.6	48.2	49.2	35.2	24.2	9.36
2	40	64.2	62.2	55	48.2	28.6	12.49
3	60	73.4	68.2	64.2	62.2	40.8	22.90
4	80	82.2	73.6	73.4	68.2	49.6	44.45
5	100	93.6	75	77.2	74.6	64.2	76.87

AEIP – Aquous Extract of Insulin Plant, EAEIP – Ethyl Acetate Extract of Insulin Plant, EEIP - Ethanolic Extract of Insulin Plant, MEIP - Methanolic Extract of Insulin Plant.

Table 4: Antibacterial activity by disc diffusion method

S. No	Drug/Extract	Concentration (μg/ml)	Zone of Inhibition (mm)			
			E.coli	S.aureus	P.aeruginosa	B.subtilis
1	Standard	30	20	23	26	22
2	EAEIP	50	10	12	10	11
3	EEIP	50	11	8	9	7
4	MEIP	50	7	10	8	9
5	AEIP	50	0	0	0	0

AEIP – Aquous Extract of Insulin Plant, EAEIP – Ethyl Acetate Extract of Insulin Plant, EEIP - Ethanolic Extract of Insulin Plant, MEIP - Methanolic Extract of Insulin Plant.

Table 5: In Vitro Antidiabetic activity by α-amylase enzyme inhibitory activity

S. No	Conc. (μg/ml)	Standard	EAEIP	EEIP	MEIP	AEIP	IC_{50} Value
1	40	56.56	51.09	52.55	35.03	24.08	10.04
2	80	68.24	55.10	62.40	44.52	32.48	38.18
3	120	75.18	63.86	68.97	55.83	40.87	20.11
4	160	87.22	68.61	74.45	62.04	49.63	105.05
5	200	95.25	75.91	83.21	68.24	62.77	153.99

AEIP – Aquous Extract of Insulin Plant, EAEIP – Ethyl Acetate Extract of Insulin Plant, EEIP - Ethanolic Extract of Insulin Plant, MEIP - Methanolic Extract of Insulin Plant.

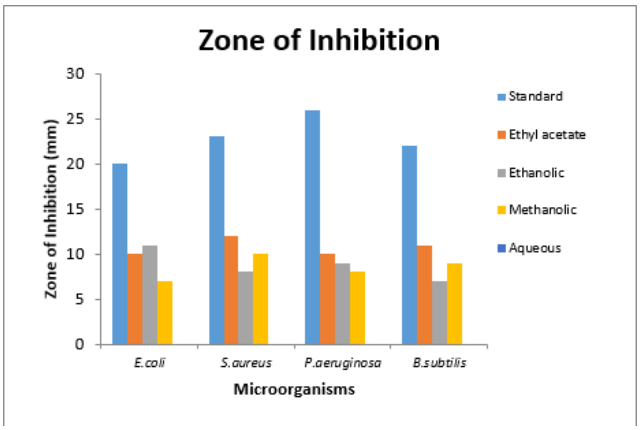

Fig. 5: Thein vitro antimicrobial activity on insulin plant extracts

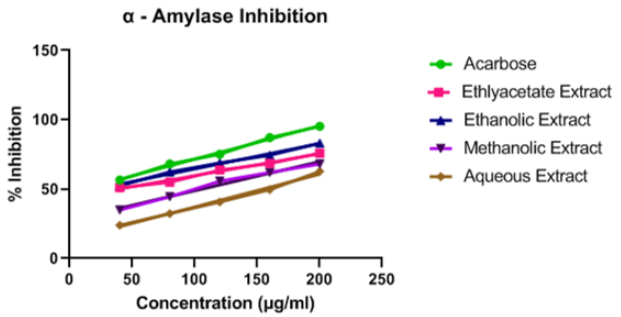

Fig. 6: Thein vitro antidiabetic activity (dns activity) of insulin plant extracts.

possibly play a role in respective pharmacological actions. The results showed about 14 specific phytochemicals and its specific pharmacological actions as represented in the Figure 2 and Table 2.

4.3. Antioxidant activity - in vitro (DPPH-method)

Ascorbic acid showed the maximum antioxidant effect with the percentage inhibition of 93.6 % at 100 μg/ml concentration by the DPPH method, nearly close antioxidant effect was observed with *Costus igneus* rhizome extracts of ethyl acetate, ethanolic, methanolic and aqueous at the same concentration with the percentage inhibition of as 75%, 77.2%, 74.6 % and 64.2% respectively [Table 3 and Figure 3] and the IC_{50} value of ascorbic acid, ethyl acetate extract, ethanolic extract, methanolic extract and aqueous extract of *Costus igneus* rhizome was found to be 9.36 μg/ml, 12.49 μg/ml, 22.90 μg/ml, 44.45 μg/ml and 76.87 μg/ml respectively.

4.4. Antibacterial activity - in vitro (disc-diffusion method)

The antibacterial activity various rhizome extracts (50 μg/ml) of *Costus igneus* was compared with the standard drug (Ceftrioxone sodium 30 μg/ml) determined by disc diffusion showed highest zone of inhibition (22, 23, 20 and 26 mm) for the standard drug for *B. subtilies, S. aureus, E.coli* and *P. aeruginosa* respectively, the antibacterial activity of *Costus igneus* ethyl acetate, ethanol, methanol has moderate antibacterial activity as compare to the Ceftrioxone sodium (standard). Ethyl acetate has slightly higher antibacterial activity among the other extracts, however *Costus igneus* aqueous extract has no antibacterial against any of the bacteria [Table 4 and Figure 4].

4.5. α-Amylase inhibition activity by DNS method (in vitro)

The percentage inhibition for anti-diabetic activity was assessed at concentration ranges from 40 μg/ml to 200 μg/ml for standard and test and the maximal inhibition effect at 200μg/ml concentration was found to be 95.25%, 75.91%, 83.21, 68.24 and 62.77% for acarbose, ethyl acetate extract, ethanolic extract, methanolic extract and aqueous extract of *Costus igneus* rhizome extract respectively [Table 5 and Figure 5] and the IC_{50} value of acarbose, acetate extract, ethanolic extract, methanolic extract, and aqueous extract was found to be 10.04 μg/ml, 38.18 μg/ml, 20.11 μg/ml, 105.05 μg/ml and 153.99 μg/ml respectively. It implies *Costus igneus* rhizome ethyl acetate and ethanolic extracts has more potent, whereas methanol and aqueous extract of has mild *in vitro* anti-diabetic effect by inhibition of α-amylase activity.

5. Discussion

Insulin plant medicinal value was established traditionally for the treatment of diabetes and other ailments and

substantiated by recent work on *Costus igneus* various leaf extract. The phytochemical analysis of *Costus igneus* rhizome extracts confirm the presence glycosides, saponins, flavonoids, terpenoids and steroids. Based upon rich pharmacological activities observed on ethanolic extract we have selected and performed GC-MS anlaysis for specific phytochemicals, results showed 14 important specific compounds as mentioned.

In vitro anti-oxidant effect was significantly high and almost same for all *Costus igneus* rhizome extracts by DPPH assay. The results also showed *Costus igneus* rhizome extract of ethyl acetate, ethanol and methanol possess the moderate antibacterial activity against gram positive (*B. subtilies and S. aureus*) and gram negative pathological organism (*E.coli* and *P. aeruginosa*) by disc diffusion method as compare to the standard drug (ceftriaxone sodium), but no antibacterial activity was found against aqueous extract. α-amylase inhibition activity by DNS method results infer, high anti-diabetic activity for *Costus igneus* ethyl acetate and ethanolic rhizome extracts whereas mild activity for methanolic and aqueous rhizome extracts.

6. Conclusion

Among the *Costus igneus* rhizome extracts, ethonolic extract has maximal antioxidant, antibacterial and antidiabetic activities also found to possess nearly similar phytochemicals as that of the leaf, especially the flavonoids were found to be more as compare to the remaining extracts. Thereby we would like to conclude that *Costus igneus* ethonolic extract might possess therapeutic potential on the antioxidant, antibacterial segment, and antidiabetic segments. Based on GC-MS Analysis in cognition with literature review we would like to conclude that antidiabetic activity might be mediated by gamma-sitosterol, antioxidant activity might be mediated by 7,9-Di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione, 2,4-ditert-butylphenol and squalene. The antibacterial activity might be mediated by 7,9-Di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione and 9,19 - Cyclocholestan-3-ol, 14-methyl-(3.beta.,5.alpha). However further *in vivo* and *in vitro* screening of *Costus igneus* ethanolic extract on specific phytochemicals are much needed to confirm the same.

7. Source of Funding

None.

8. Conflict of Interest

None.

References

1. Shetty AJ. Effect Of The Insulin Plant (Costus igneus) Leaves on Blood Glucose Levels In Diabetic Patients: A Cross Sectional Study. *J Clin Diagn Res*. 2010;4:2617–21.
2. Krishnan K, lakshmi RV. Beneficial Effects Of Costus Igneus And Dose responsestudies In Streptozotocin induced Diabetic Rats. *Int J Curr Pharm Res*. 2011;3(3):42–6.
3. Shetty C, Kuruvilla K. Effect of leaves on dexamethason induced hyperglycemia Shetty. *Int J Ayurveda Res*. 2010;1(2):100–2.
4. Devi VD, Urooj A. Hypoglycemic potential of Morus indica. L and Costus igneus. Nak.—A preliminary study. *Indian J Experimen Biol*. 2008;46:614616.
5. Kalailingam P. The Efficacy of Costus igneus Rhizome on Carbohydrate Metabolic, Hepatoproductive and Antioxidative Enzymes in Streptozotocin-induced Diabetic Rats. *J Health Sci*. 2011;57(1):37–46.
6. Williams WB, Me C, Berset C. Use of free radical method to evaluate antioxidant activity. *Lebens-Wiss Technol*. 1995;28:25–30.
7. Bauer AW, Kirby W, Sherris JC, Turck M, et al. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol*. 1966;45:493–6.
8. Bhatt UP, Sati SC, Chandra S, Kumar S, Anthwal S, Singh R, et al. Evaluation of in vivo and in vitro antidiabetic activity of Roylea Cinerea. *Int J Pharm Sci Rev Res*. 2015;32:210–3.

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