https://doi.org/ 10.33472/AFJBS.6.5.2024. 1646-1658

African Journal of Biological Sciences

An antidiabetic and antioxidant activity of an unexplored plant of *Diapensia himalaica* in Alloxan-induced diabetic rats

Arnab Bagchi[1] , Bapi Ray Sarkar[2]*, Prosanta Pal[3]

[1] Himalayan Pharmacy Institute, Majhitar, Rangpo, East Sikkim. Pin- 737136, India.

[2] Associate Professor and Head Department of Pharmaceutical Technology at University of North Bengal(NBU), Raja Rammohunpur, Darjeeling, West Bengal . Pin-734013, India.
[3] Department of Pharmaceutical Technology at University of North Bengal(NBU), Raja Rammohunpur, Darjeeling, West Bengal . Pin-734013, India.

Corresponding Author
Associate Professor and Head Department of Pharmaceutical Technology,
University of North Bengal (NBU),
Raja Rammohunpur, Darjeeling,
West Bengal . Pin-734013, India.
E-mail: brspublication@gmail.com
Mobile no.: 9707927297

Article History
Volume 6, Issue 5, Apr 2024
Received: 01 May 2024
Accepted: 09 May 2024
doi: *10.33472/AFJBS.6.5.2024. 1646-1658*

ABSTRACT: Aim: Medicinal plants can effectively lower the oxidative stress that causes diabetes mellitus. This study investigated the potential benefits of Diapensia himalaica Methanolic Extract (DHME) for the treatment of diabetes. The study evaluated the antihyperglycemic activity of DHME in both normal off-feed animals and glucose-loaded animals with diabetes, supporting its anti-diabetic effects. Additionally, the chronic multiple dosage therapy lasting twenty-eight days examined DHME's confirmed antidiabetic effectiveness and its role in ameliorating toxicity. **Material andMethods**: The study utilized a single dose of alloxan (120 mg/kg) to induce hyperglycemia, leading to elevated levels of total lipids, triglycerides, and cholesterol, along with reduced insulin levels. The investigation aimed to determine the effectiveness of DHME in alleviating these diabetic symptoms. Results: The results demonstrated that DHME exhibited significant antihyperglycemic effects, as evidenced by reduced blood glucose levels in both normal off-feed animals and glucose-loaded diabetic animals. Furthermore, DHME treatment led to a decrease in total lipids, triglycerides, and cholesterol levels, while insulin levels showed improvement. These findings suggested that DHME could potentially serve as an anti-diabetic agent.Conclusion: The findings from this study support the use of Diapensia himalaica Methanolic Extract (DHME) as an effective treatment for diabetes

INTRODUCTION: When the pancreas is unable to make enough insulin or when the body is unable to use the insulin that is produced properly, diabetes mellitus (DM), a metabolic disorder, occurs.(Vaghela, 2011). Hyperglycemia can result in either impairments in cellular glucose uptake or pancreatic dysfunction, which is typified by an inability to produce enough insulin for the body to break down sugar.The prevalence of diabetes has rapidly increased recently all across the world. In fact, more than 642 million patients are expected by the year 2040, up from the forecasted 422 million in 2014. In 2012, 1.5 million individuals worldwide died from diabetes; by 2040, that number will increase to 3.7 million(Korsmeyer,1983). Injections of alloxan or streptozotocin result in diabetes in animals and also produce Reactive oxygen species (ROS) that kill pancreatic beta-cells. Numerous long-term effects of diabetes are mostly caused by chronic hyperglycemia. Protein glycation, a key source of free radicals, is mostly brought on by hyperglycemia. A rising body of research indicates that free radicals play a crucial role in the onset and effects of diabetes. These effects include vascular tissue, neurological, renal, and metabolic alterations(Alladi,2012). Herbal medicine has gained popularity for the treatment and management of diabetes in both developed and developing countries due to its origin and fewer adverse effects. Since the creation of antidiabetic drugs, researchers have shifted their attention to medicinal plants in an effort to create fresh, powerful drugs with fewer undesirable side effects and cheaper costs (Chakraborty, 2016). In order to prevent oxidative damage, antioxidants operate along four fundamental mechanisms. Reduced ROS levels, halting chain reactions, scavenging free radicals, and chelating transition metals that catalyse free radical synthesis are a few of these (Shakir, 2016).Additionally, oxidative stress-induced diabetes mellitus may be avoided by eating a nutritious, high-antioxidant diet. Actually, artificial hypoglycemic medications can be used to treat diabetes mellitus. The bulk of these traditional diabetes treatments, however, had numerous serious side effects when taken regularly over an extended period of time.

Therefore, finding innovative, safe, and effective natural antidiabetic drugs to treat diabetes is one of the most important fields of research and one that continues to pique the interest of many researchers (Devaki,2016). The non-edible botanical compounds known as phytochemicals serve a number of purposes. They have a significant role in determining the colour and flavour of raw or cooked fruits and vegetables. Recent research has revealed that many of the substances plants produce to defend themselves can also shield people from sickness(Miliauskasa,2004). Diabetes has been linked to a number of chronic conditions that can lead to death, including neuropathy, nephropathy, and a range of vascular conditions that affect the heart, kidneys, brain, peripheral blood vessels and eyes (Abdullah, 2018).

MATERIALS AND METHODS:

Chemicals, Reagents: All chemicals used were of analytical reagent grade The following reagents are used in entire research work those are- 1,1-Diphenyl-2-Picrylhydrazyl Radical (DPPH), 3,5 Dinitro Salicylic Acid (DNSA), α-amylase Aspergillus oryzae, Dimethylsulfoxide (DMSO), Alloxan, sitagliptin.

Plant Material: In the month of June 2021, leaf samples of Diapensia himalaica were obtained in the North Sikkim region of India. The species was verified by Dr. Manoranjan Chowdhury of the Department of Botany at the University of Northbengal in West Bengal, India. For future use, the accession number for the plant is 11778.

Experimental animals: For this experiment, adult male Wistar albino rats weighing 180–200g were employed. They were kept in a spotless polypropylene cage and given a regular pellet diet along with unlimited access to water. Prior to the trial, the animals spent 7 days becoming used to the lab environment. The university's animal ethics committee (HPI/2021/60/IAEC/PP-0186) evaluated and approved each of the mentioned procedures (Sujata, 2016).

Preparation of plant extracts: Before being turned into powder, Diapensia himalaica leaves were dried in the shade. The powder was repeatedly extracted with ethyl acetate and

methanol at 60–800C in a soxhlet extraction apparatus. The solvent was completely drained out under reduced pressure to produce a dry bulk. The extracts were stored in vacuum desiccators for further use (Umamageswari, 2017).

DPPH radical scavenging activity: The DPPH test was used to measure the free radical scavenging capacity of Diapnia himalacia leaf extract. To prepare samples, plant extract was diluted in methanol at different concentrations ranging from 50 to 1000 g/ml. A newly produced methanolic solution of DPPH (0.1 M) was stored in the dark at 4 °C until use. One millilitre of the extract was mixed with one millilitre of methanolic DPPH solution. The control reaction was carried out by substituting 100% of the methanol solution for the extract. The mixture was allowed to sit at room temperature in the dark for 30 minutes, and then a spectrophotometer with a UV-visible wavelength of 517 nm was used to measure the absorbance.

The antioxidant activity was calculated using the following formula (Alladi, 2012).

$$\% \ \alpha \text{ amylase inhibition} = \frac{100 \times Abs_{100\% \text{ control}} - Abs_{Sample}}{Abs_{100\% \text{ Control}}}$$

In vitro α-amylase inhibitory studies: The 3,5-dinitrosalicylic acid (DNSA) technique was used to conduct the α -amylase inhibition experiment.[12] To generate concentrations ranging from 10 to 1000 g/ml, the leaf extract of *Diapnsia himalacia* was diluted in buffer solution and NaCl with a minimum of 10% DMSO. After mixing the extract with 200 µl of α-amylase solution (2 units/ml), it was incubated at 30 °C for 10 minutes. Subsequently, 200µl of the starch solution was added to each tube, and it was incubated for three minutes. After stopping the reaction using 200µl of the DNSA reagent, the mixture was heated in a water bath for 10 minutes at 85–90 °C. After dilution with 5 ml distilled water and cooling to room temperature, the liquid was measured for absorbance at 540 nm using a UV-Visible spectrophotometer. To construct a blank with 100% enzyme activity, 200 µl of buffer was substituted for the plant extract. Using the plant extract at each concentration, a blank reaction was created in the same way without the enzyme solution. A positive control sample was prepared using acarbose (100 g/ml–2 g/ml), and the reaction was conducted similarly to that with plant extract. The -amylase inhibitory activity % inhibition was calculated using the following equation: The graph's IC50 values were produced by plotting the extract concentration against the percentage of -amylase inhibition (Sheikh-Ali, 2011).

$$\% \ \alpha \text{ amylase inhibition} = \frac{100 \times Abs_{100\% \text{ control}} - Abs_{Sample}}{Abs_{100\% \text{ Control}}}$$

Acute Toxicity Study: Dose selection was made using OECD TG-423. The method enabled a judgment for dividing the test substance into a series of toxicity classes defined by fixed LD50 cut-off values (Akhila JS, 2007).

Alloxan-induced diabetic model: To test the hypothesis, a single i.p. dose of 120 mg/kg alloxan monohydrate in sterile saline. After five days of alloxan administration, the rats with blood glucose levels greater than 250 mg/dl were classified as hyperglycaemic and segregated.The experimental animals were treated with the methanol and ethyl acetate extracts of *Diapnsia himalacia* (DHME, DHEAE) for 28 days. The blood sugar levels were investigated on 0^{th}, 1^{st}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day intervals. The animals were divided into 7 groups containing 6 animals in each group.

Group I: Normal control group (Normal saline treated group).

Group II: Disease Control group (treated with alloxan monohydrate 120mg/kg, single dose treatment).

Group III: Standard control group, Sitagliptin (10mg/kg).

Group IV: Low dose of DHME (100mg/kg).

Group V: High dose of DHME (200mg/kg).

Group VI: Lowdose of DHEAE (100mg/kg).

Group VII: High dose of DHEAE (200mg/kg) (Zaid, 2022).

RESULTS AND DISCUSSION:

DPPH Radical Scavenging Evaluation: With reference to DPPH, several types of solvents and plant phytochemicals were mostly in charge of the free radical scavenging action. The evaluation of antioxidant activity using spectrophotometry is a highly used technique. Diapnsia himalacia Methanolic extract had the lowest IC50 value reported as 39.14 g/ml, followed by ethyl acetate 65.20 g/ml, petroleum ether 220.94 g/ml, chloroform 238.03 g/ml, and water 261.43 g/m. The results are shown graphically in Figure 1.

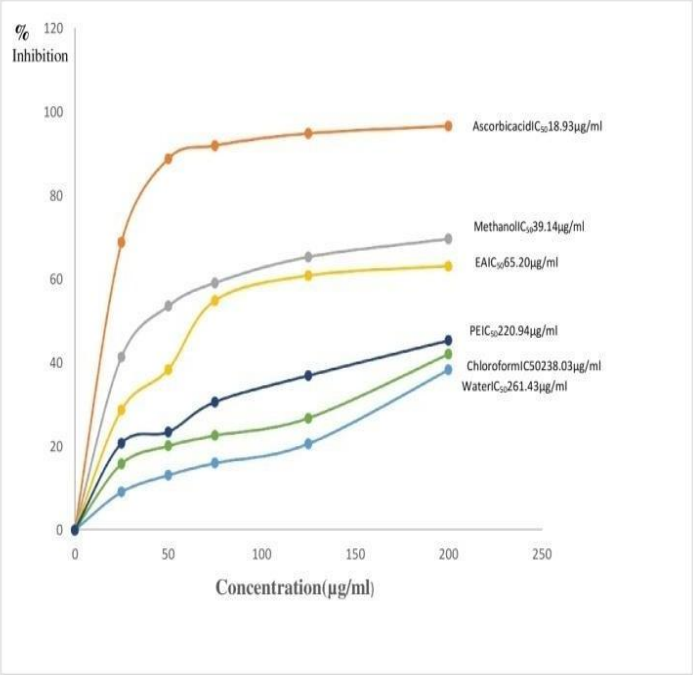

Fig.1. DPPH radical scavenging activity of *Diapnsia himalacia* plant extract and ascorbic acid

In vitro α-amylase inhibitory Evaluation: In this study acarbose used as a standard. The IC_{50} value was reported in *Diapnsia himalacia* Methanolic extract 76.3 µg/ml followed by Ethyl acetate extract 79.4 µg/ml and the standard positive control Acarbose showed an IC50 of 35.84 µg/ml. The results are shown graphically in Figure 2.

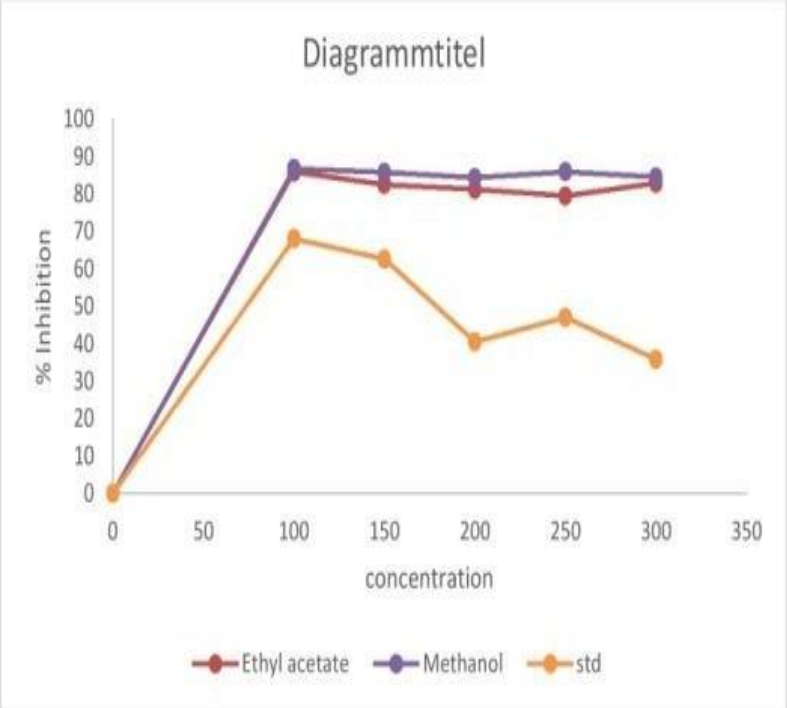

Figure 2 : α-amylase Inhibitory effect of *Diapnsia himalacia* plant extract

Acute toxicity: Mice were taken to carry out an acute toxicity study. DHME and DHEA extracts were administered orally using oral gavage. No mortality or fatal was found during acute toxicity.

Effect of *Diapnsia himalacia* on fasting blood glucose levels: All the animals survived the treatment period. Alloxan monohydrate treated diabetic control (DC) animal's serum glucose level was raised. On the other hand, the standard group experienced a significant decrease in glucose levels. The extract reduced elevated glucose levels at the doses from the 1st day to the 28th day than diabetic control. The high dose group shows the effective result till 28th day than the diabetic control and low dose group. The results are shown graphically in Figure 3.

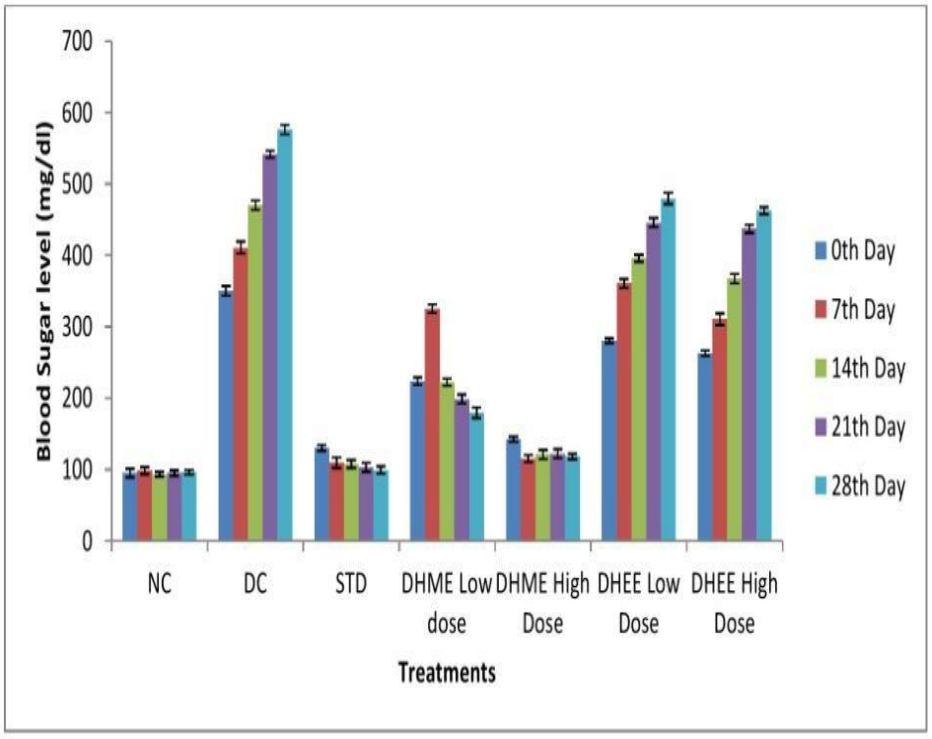

Fig3: Effect of *Diapnsia himalacia* on blood sugar level against Alloxan induced diabetes

Effect of *Diapnsia himalacia* on Body weight: Diabetic control showed extreme significant (P< 0.001) increase in blood sugar level on 0^{th} , 7^{th} , 14^{th} , 21^{st} and 28^{th} days compare to normal control group standard, high and low dose of methanol extract , high dose of ethyl acetated showed significant (P< 0.001) decrease in blood sugar level on the day 0, 7, 14, 21 and 28 days compare to diabetic control group where as low dose of ethyl acetated showed significant (P< 0.05) decrease in weekly intervals blood sugar level compared diabetic control group. Table 1 Effect of *Diapnsia himalacia* extract on the relative body weight in alloxan-induced diabetic rats

Groups	Day 0 (after induction without treatment)	Day 7	Day 14	Day 21	Day 28 (final reading day after last treatment)
Normal control group	95.26 ± 5.79	98.39± 4.82	93.64 ± 3.29	95.39±3.78	96.28±3.49
Diabetic control	350.83 ± 6.82***	410.78 ± 8.62***	470.14 ± 6.76***	541.57 ± 4.94***	576.28±6.45***
Standard	130.49 ± 4.83*###	109.62 ± 7.35###	107.92 ± 5.36###	103.34 ± 6.29###	99.25 ± 5.28###
Methanol extract (low dose)	223.84±5.28***###	325.27±5.87***###	222.38±4.92***###	198.62±6.29***###	179.72±7.39***###
Methanol extract (high dose)	142.47±3.56*###	115.29±4.97###	121.32±6.27###	122.21±6.43###	118.29±3.72 ###
Ethyl acetate extract (low dose)	280.56±3.45***#	360.62±6.34***#	395.91±4.78***#	445.67±6.23***#	479.73±7.72***#
Ethyl acetate extract (high dose)	262.93±3.78***###	310.59±8.31***###	367.45±6.39***###	437.29±5.68***###	462.54±4.78***###

The highest antioxidant activity of methanolic extract are reported. It was also reported the other potential antioxidant activities such as superoxide, nitric oxide, hydroxyl radical scavenging activities. Oxidative stress- induced free radicals are scavenged by dietary phenol and flavonoids. Several antioxidant enzyme defense systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) were initiated by polyphenolic compounds to restraint reactive oxygen species followed by preventing the cell injuries It excluded n-hexane extract from our study as it showed poor antioxidant activity. So, the methanolic extract has more potential in terms of total phenolic contents, total flavonoid contents and free radical scavenging activities with DPPH than other extracts (Marinova).

Low IC_{50} contain Methanolic extract shows more antidiabetic activity than others extract. From the graphical diagram it can be observed the order of antidiabetic activity of *Diapnsia himalacia* Methanolic extract > Ethyl acetate extract . Methanolic extract of *Diapnsia himalacia* shows more alpha amylase activity than Ethyl acetate extract (Sb Viana, 2004).

Prior to the main study, an acute toxicity investigation on mice was conducted. There was no evidence of death at any of the doses tested. In our research, alloxan was chosen to induce diabetes. Alloxan is responsible for pancreatic β-cell damage, resulting in diminished insulin secretion. Alloxan raises the fasting blood glucose levels significantly in laboratory animals. It is due to pancreatic β-cell toxicity and subsequent insulin insufficiency (Chatatikun, 2013).

CONCLUSION: The findings from this study support the use of Diapensia himalaica Methanolic Extract (DHME) as an effective treatment for diabetes. The demonstrated antihyperglycemic activity, as well as the ability to ameliorate toxic effects, highlight the potential of DHME as an anti-diabetic component. Further research is warranted to explore its mechanisms of action and evaluate its clinical applications.

ACKNOWLEDGEMENT: The authors acknowledge the University of North Bengal, West Bengal and Himalayan Pharmacy Institute, Sikkim, for continuous support for research.

REFERENCES:

1. Vaghela JS, Sisodia SS. (2011). *In vitro* antioxidant activity of *Terminalia chebula* fruit extracts. Res J Pharm Technol., 4, 1835-43.
2. Korsmeyer RW, Gurny R, Doelkar E, Buri P, Peppas NA. (1983). Mechanism of Solute Release from Porous Hydrophilic Polymers. Int J Pharm.,15(1), 25-35.
3. Alladi S, Sarath Prakash D, Malothu R. (2012) . Anti-hyperglycemic activity of the leaves of *Terminalia tomentosa* against normal and alloxan induced diabetic rats. Res J Pharm Technol., 5,1577-84.
4. Chakraborty R, Roy S, Mandal V.(2016). Assessment of traditional knowledge of the antidiabetic plants of Darjeeling and Sikkim Himalayas in the context of recent phytochemical and pharmacological advances. J Integr Med., 14(5),336-58.
5. Shakir M, Prashant K, Keerti J, Rakesh KT, Jain NK.(2016). Mucoadhesion a promising approach in drug delivery system. Reactive and Functional Polymers., 100,151-72.
6. Devaki K, Priyanga S, Hemmalakshmi S.(2016). Antidiabetic activity of aqueous extract of *Erythrina variegata* L. bark in streptozotocin induced diabetic rats. J Chem Pharm Res., 8(4), 306−12.
7. Miliauskasa G, Venskutonisa PR, Beekb TA.(2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem., 85(2), 231−7.
8. Abdullah KM, Alam MM, Iqbal Z, Naseem I.(2018). Therapeutic effect of Vitamin B_3 on hyperglycemia, oxidative stress and DNA damage in alloxan induced diabetic rat model. Biomed Pharmacother., 105, 1223-31.
9. Sujata K, Meenu N, Geeta A, Puneet UK, Jain PS.(2016). Microparticles Drug Delivery System. A Review World. Journal of Pharmacy and Pharmaceutical Sciences., 5(3), 543-66.

10. Umamageswari MS, Karthikeyan TM, Maniyar YA.(2017). Antidiabetic activity of aqueous extract of *Solanum nigrum* linn berries in alloxan induced diabetic Wistar albino rats. J Clin Diagn Res.,11(7), 16-9.
11. Alladi S, Sarath Prakash D, Malothu R. (2012). Anti-hyperglycemic activity of the leaves of *Terminalia tomentosa* against normal and alloxan induced diabetic rats. Res J Pharm Technol., 5(15), 77-84.
12. Vertuani S, Angusti A, Manfredini S.(2004). The antioxidants and pro-antioxidants network: an overview. Current pharmaceutical design., 10(14), 1677-94.
13. Sheikh-Ali M, Chehade JM, Mooradian AD.(2011). The antioxidant paradox in diabetes mellitus. American journal of therapeutics., 18(3), 266-78.
14. Akhila JS, Shyamjith D, Alwar MC.(2007). Acute toxicity studies and determination of median lethal dose. Current science., 10, 917-20.
15. Zaid AN, Abu Zaaror Y, Kaddumi A, Ghanem M, Jaradat N, Abu Salah T, Siaj S, Omari L.(2022). Stability of extemporaneously prepared sitagliptin phosphate solution. Plos one., 17(3), 0262-68.
16. Marinova G, Batchvarov V. (2011). Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulgarian Journal of Agricultural Science., 17(1), 11-24.
17. Sb Viana G, Carolina A, Medeiros C. (2004). Poglycemic and anti-lipemic effects of the aqueous extract from Cissus sicyoides Cissus sicyoides, alloxan-induced diabeteshypoglycemialipid profilehepatic enzymes. J Clin Diagn Res., 7, 1-7.
18. Chatatikun M, Chiabchalard A.(2013). Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (*Daucus carota* Linn.) root crude extracts. J Chem Pharm Res,, 5(4), 97−102.