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Original Article

Phytochemical Analysis and hypoglycemic potential of *Filago hurdwarica* (Wall. ex DC.) Wagenitz in alloxan induced diabetic mice

Análise fitoquímica e potencial hipoglicemiante de *Filago hurdwarica* (Wall. ex DC.) Wagenitz em camundongos diabéticos induzidos por aloxana

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Abstract

Plants have profound therapeutic benefits, more economical treatments, fewer side effects, and a relatively cheap cost, making them a source of drugs for protective, preventative, curative, or conducive purposes and creating novel phytomedicines. Plant derived medicines are relatively safe compared to synthetic medicines. Many plants have proved to successfully aid in the treatment of diabetes including *Filago hurdwarica* (Wall. ex DC.) Wagenitz. The current investigations were therefore designed to assess the phytochemical, antioxidant, antidiabetic, and antihyperlipidemic activities of *F. hurdwarica*. The phytochemical investigations and antioxidant activities of different extracts were carried out using standard chemical tests, DPPH, and H_2O_2 scavenging assays. *F. hurdwarica* plant extract in Hydromethanolic solution were prepared by Soxhletation method and stored in refrigerator at 4°C for two days before use. Swiss Albino mice were made diabetic by a single dose of alloxan (150 mg/kg). Hydromethanolic plant extract and fractions of *F. hurdwarica* were screened for antidiabetic activity and given to the alloxan-induced diabetic mice at a concentration of 150-250 mg/kg of body weight in different groups of 6 diabetic mice each orally once a day for 15 days. Glibenclamide is also given to another group to as a standard drug to support the result at a dose of 10 mg/kg of body weight orally once a day for 15 days. Blood glucose levels and body weights of mice were measured on 0, 4, 7, 11 and 15th days. The study found that the extract was safe up to the dose level of 2000 mg/kg and the dose response effect of chloroform extract (150-250 mg/kg) of *F. hurdwarica* showed expressive antihyperglycemic effects and also improved other altered biochemical parameters associated with diabetes. The FTIR and XRD spectra demonstrated the occurrence of phenols, alcohols, alkenes, alkyl halides, ketones, and aromatic compounds and confirmed the amorphous nature of the extract. GC-MS spectral analysis showed the tentative presence of 31 phytochemical constituents in the chloroform extract of *F. hurdwarica* with different retention time. To conclude, the chloroform extract (250 mg/kg) of *F. hurdwarica* revealed considerable antioxidant, antihyperglycemic, and antihyperlipidemic potential and is safe for treating diabetes and related complications.

Keywords: alloxan, toxicity, antidiabetic, antihyperlipidemic, *Filago hurdwarica*.

Resumo

As plantas têm profundos benefícios terapêuticos, tratamentos mais econômicos, menos efeitos colaterais e um custo relativamente barato, tornando-as uma fonte de medicamentos para fins protetores, preventivos, curativos ou propícios e criando novos fitomedicamentos. Medicamentos derivados de plantas são relativamente seguros em comparação com medicamentos sintéticos. Muitas plantas provaram ajudar com sucesso no tratamento de diabetes, incluindo *Filago hurdwarica* (Wall. ex DC.) Wagenitz. As investigações atuais foram, portanto, projetadas para avaliar as atividades fitoquímicas, antioxidantes, antidiabéticas e anti-hiperlipidêmicas de *F. hurdwarica*. As investigações fitoquímicas e atividades antioxidantes de diferentes extratos foram realizadas usando testes químicos padrão, DPPH e ensaios de eliminação de H_2O_2. O extrato da planta *F. hurdwarica* em solução hidrometanólica foi preparado pelo método Soxhletation e armazenado em geladeira a 4 °C por dois dias antes do uso. Camundongos Swiss Albino foram tornados diabéticos por uma única dose de aloxana (150 mg/kg). Extrato de planta hidrometanólica e frações de *F. hurdwarica* foram rastreados quanto à atividade antidiabética e administrados aos camundongos diabéticos induzidos por aloxana em uma concentração de 150-250 mg/kg de peso corporal em diferentes grupos de 6 camundongos diabéticos cada, por via oral, uma vez ao dia por 15 dias. A glibenclamida também é administrada a outro grupo como medicamento padrão para apoiar o resultado na dose de 10 mg/kg de peso corporal por via oral

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uma vez ao dia por 15 dias. Os níveis de glicose no sangue e os pesos corporais dos camundongos foram medidos em 0, 4, 7, 11 e 15 dias. O estudo descobriu que o extrato era seguro até o nível de dose de 2.000 mg/kg e o efeito dose-resposta do extrato de clorofórmio (150-250 mg/kg) de *F. hurdwarica* mostrou efeitos anti-hiperglicêmicos expressivos e também melhorou outros parâmetros bioquímicos alterados associados com diabete. Os espectros de FTIR e DRX demonstraram a ocorrência de fenóis, álcoois, alcenos, haletos de alquila, cetonas e compostos aromáticos e confirmaram a natureza amorfa do extrato. A análise espectral por GC-MS mostrou a presença tentativa de 31 constituintes fitoquímicos no extrato clorofórmio de *F. hurdwarica* com diferentes tempos de retenção. Para concluir, o extrato de clorofórmio (250 mg/kg) de *F. hurdwarica* revelou considerável potencial antioxidante, anti-hiperglicêmico e anti-hiperlipidêmico e é seguro para o tratamento de diabetes e complicações relacionadas.

Palavras-chave: alloxan, toxicidade, antidiabético, anti-hiperlipidêmico, *Filago hurdwarica*.

1. Introduction

Diabetes mellitus (DM) is a chronic multi-etiological metabolic disorder characterized by inadequate glucose homeostasis with a deficiency of protein, carbohydrate and fat metabolism disorders resulting from insulin action, insulin secretion, or both the defects (Akhtar et al., 2016; Fatima et al., 2019). DM has been characterized by hyperglycemia, hyperlipidemia, hypoinsulinemia, hyperaminoacidemia and oxidative stress due to a drop in insulin (Jyothi et al., 2012). Diabetes mellitus is known to cause hyperlipidemia because of the interrelationship between carbohydrates and lipid metabolism and through various metabolic derangements. Among several metabolic derangements, insulin deficiency has been known to stimulate lipolysis in the adipose tissue and give rise to hyperlipidemia. It involves Long-term complications of skin, kidneys, eyes, nerves, and blood vessels (Elosta et al., 2012).

Diabetes has turned up as a major health problem worldwide with serious health-related and socioeconomic impacts on individuals and communities alike. In both developed and developing countries, diabetes mellitus has affected about 25 percent of the global population (Benalla et al., 2010; Emdin et al., 2015). The multiple factors related to the development of diabetes are called risk factors or predisposing factors. The factors which increase the risk of diabetes include a sedentary lifestyle, high family aggregation, age, nutritional status, obesity, and insulin resistance (Deepashree and Prakash, 2007).

In both developed and developing countries, herbal medicine has gained growing attention in the treatment and management of diabetes, due to its origin and fewer side effects (Hasani-Ranjbar et al., 2009; Rahimi et al., 2005). The discovery of antidiabetic drugs has shifted its attention to medicinal plants to offer new potential and competent drugs with fewer adversarial effects and lower costs (Marles and Farnsworth, 1995; Nabi et al., 2013). In the development of novel therapeutic agents, natural products from plants play a major role and have gained great attention as sources of bioactive substances, including hypoglycemic, hypolipidemic, and antioxidant agents (Akhtar et al., 2016). The data from ethnobotanical studies reports about 1200 rare plants that may possess antidiabetic ability worldwide (Arumugam et al., 2013).

Filago hurdwarica is a wild herb belonging to family Asteraceae and is native to Pakistan, India, Afghanistan and Iran. The plant is utilized for the treatment of diabetes mellitus in herbal medicine of Pakistan (Zain-ul-Abidin et al., 2018) and in decoction form for curing skin allergies and itching (Zeb et al., 2020). There are no aforementioned cited research reports about the antidiabetic and antioxidant nature of *Filago hurdwarica* in this context. Therefore, the current research work was undertaken to investigate the antidiabetic, antihyperlipidemic, and antioxidant activities of *Filago hurdwarica* in alloxan- induced diabetic model.

2. Materials and methods

2.1. Ethics statement

All the experimental protocols involving animal experiments were developed in accordance with the approval of an ethical committee of the Department of Botany, Abdul Wali Khan University, Mardan. The animals were given feed ad libitum and 5% dextrose solution after 30 min of alloxan administration in order to stave off the early hypoglycemic phase. At the termination of the experiments, the animals were sacrificed by cervical dislocation under isoflurane anesthesia as per ethics committee guidelines, and all endeavors were made to reduce sufferings.

2.2. Collection of plant materials

The whole plant of *F. hurdwarica* was collected from District Buner, Khyber Pakhtunkhwa, Pakistan. The taxonomic identity of the desired plant was determined by the plant taxonomists at the Department of Botany, Abdul Wali Khan University Mardan, Pakistan. The sample specimen was deposited under a voucher no. Bot.20192 (AWK) at the herbarium of Botany Department (David et al., 2017).

2.3. Preparation of extracts

F. hurdwarica whole plant was collected, dried under shade, and then grossly powdered by a mechanical grinder. The powdered plant was weighed (1 Kg) and extracted with 80% methanol by direct maceration and allowed to stand for 3-4 days (David et al., 2017). The mixture was filtered after 72 h of maceration using a muslin cloth and then by Whatman filters paper No. 1. The filtrates were collected and the solvent was vaporized using a rotary evaporator (Heidolph Laborota 4000, Germany) at 40–50 °C. The dried extract was conveyed into a vial and preserved in a refrigerator until utilization (Mbiri et al., 2016). Solvent fractions were then prepared by liquid-liquid separation method. Thirty grams of the crude

methanolic extract was draped in 200 mL of distilled water in a separating funnel and it was then fractioned subsequently with 200 mL of various solvents of increasing polarity, commencing from n-hexane, followed by chloroform, and ethyl acetate, each in triplicate. The different solvent fractions were collected in flasks and then dried in an oven at 40°C (Tarekegne et al., 2016). The dried fractions were then transferred into vials and kept in refrigerator until used.

2.4. Phytochemical screening

The preliminary phytochemical screening tests were conducted for determining secondary metabolites such as alkaloids, glycosides, tannins, saponins, reducing sugars, terpenoids, phenols, anthraquinones, phlobatannins, steroids, coumarins, proteins, and quinones in the plant by using standard qualitative methods (Trease).

2.5. Quantitative determination of phytoconstitutents

The quantitative analysis of phytochemical constituents in the methanolic extract and fractions were carried out spectrophotometrically for the presence of phenol, flavonoid, tannin, sugar and protein contents by the "Folin Ciocalteu method" (Chun et al., 2003), Aluminum chloride assay (Chantiratikul et al., 2009), Folin Ciocalteu assay, Phenol Sulphuric acid method (Masuko et al., 2005) and Lowry's method (Ali and Sayeed, 1988) respectively.

2.6. Antioxidant study

The antioxidant potential of extract and various fractions of *F. hurdwarica*, was determined by using DPPH and Hydrogen peroxide assay methods (Ayoola et al., 2008). Ascorbic acid was used as a reference compound.

2.7. Experimental animals

The male Swiss albino mice of 3-5 weeks and weighing 25-30 g were used in the experiment. The animals were permitted to acclimatize in the animal house at the VIR (Veterinary Research Institute), Peshawar for a period of two weeks. The animals were kept at a narrowly sustained temperature of 25 ± 2 °C under optimum laboratory conditions. The experimental animals were used with the approval of an ethical committee of the Department of Pharmacy, Abdul Wali Khan University Mardan, (0012/2019).

2.8. Induction of diabetes

The diabetes was induced in Swiss albino mice by giving a single intraperitoneal (IP) injection of 150 mg/kg of alloxan monohydrate (Sigma Aldrich, Germany) (Ahmed et al., 2010). The animals were given feed ad libitum and 5% dextrose solution after 30 min of alloxan administration to overcome the early hypoglycemic crisis (Carvalho et al., 2003). The mice whose blood glucose level was more than 200 mg/dl were considered diabetic (Kesari et al., 2006). The treatment with the plant extracts was started after 3 days of alloxan injection in diabetic mice.

2.9. Acute toxicity test

The primary toxicological effect of the extract of *F. hurdwarica* was determined based on OECD guidelines 2008:425 (Sosa et al., 2020) to choose the suitable dose for antidiabetic assessments before commencement of the main study. The animals were observed for 96 hours to note the presence or absence of noxiousness and behavioral signs like tremor, restlessness, sluggishness, diarrhea, weight reduction and paralysis at regular intervals (Jörns et al., 1997).

2.10. Experimental design

The experimental animals were randomly divided into nine groups with six animals in each group for the evaluation of antidiabetic activity. Group 1, were normal saline-treated only with Tween 80, Group 2, were diabetic control received only alloxan 150 mg/kg, Group, 3 were diabetic mice treated with glibenclamide (10 mg/kg) (Ragavan and Krishnakumari, 2006), Group 4 and 5, had diabetic mice treated with crude methanolic extracts of 150 mg/kg and 250 mg/kg respectively, Group 6, 7, 8, and 9, included alloxan-induced diabetic mice provided with n-hexane, chloroform, ethyl acetate, and aqueous extracts respectively at a concentration of 250 mg/kg (Alema et al., 2020; Tafesse et al., 2017). They were marked separately and the treatment was continued for fifteen (15) consecutive days.

2.11. Determination of blood glucose

The blood glucose level was checked for each group on day 0, 4, 7, 11, and 15 during the study period by collecting blood from the mice tail vein of overnight fasted mice using a glucometer (Roche Diagnostics, Germany) and oxidase-peroxidase reactive strips (Morikawa et al., 2007). The tails were then rubbed with ethanol to prevent infection. The fluctuations in the weight of the animals were also recorded on the same days.

2.12. Determination of serum and lipid profile

The blood samples for serum profile were collected from overnight fasted mice under diethyl ether anesthesia by retro orbital plexus puncture method and were kept aside for 30 min for clotting. The serum was separated by centrifuging the sample at 3000 rounds per minute, for 10 min at 25°C (Morikawa et al., 2007). It was subsequently analyzed for alkaline phosphatase (ALP), serum creatinine, total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL) and low-density lipoproteins (LDL) respectively by the methods (Friedewald et al., 1972). The SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamate Pyruvate Transaminase) activities were determined according to the method of (Reitman and Frankel, 1957). These biochemical assays were measured by using commercial diagnostic kits.

2.13. Histopathological examination

The liver, pancreas and kidney of mice were cautiously dissected at the termination of the experiment and preserved in 10% formaldehyde. These were then cut into smaller pieces and fixed with 10% formalin solution and immediately processed for histopathological evaluation

by parrafin method. The tissue segments were primed with microtome and stained with a hematoxylin and eosin dyes so as to be observed under a light microscope (Dwivedi and Daspaul, 2013).

2.14. In vitro membrane stabilizing activity

The membrane-stabilizing effects of the extracts were determined on human erythrocytes to predict the *in vitro* antidiabetic activity (Omale and Okafor, 2008). The percentage of hemolysis or membrane stabilization was calculated using the following Equation 1:

$$\% \, haemolysis = OD1 - OD2 / OD1 \times 100 \quad (1)$$

where,

$OD1 =$ optical density of control

$OD2 =$ optical density of test sample

2.15. Measurement of antioxidant enzyme activity

Filago hurdwarica was investigated for its antioxidant potential in terms of antioxidant enzymatic capacities to support its traditional medicinal utilization. Enzymatic antioxidant activities were investigated by carrying out the Superoxide dismutase (SOD), peroxidase (POD, and Catalase (CAT) assays, spectrophotometrically at 25ºC by monitoring the absorbance at 420 nm, 560 nm, and 240 nm respectively (Onsa et al., 2004).

2.16. FTIR analysis

The FTIR (Fourier transform infrared) spectrum was used to detect the typical functional groups in the powdered extract of *F. hurdwarica* and was recorded from KBr pellet in FTIR Spectroscope (Shimadzu, IR Affinity1, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4-1 cm (Pramila et al., 2012).

2.17. XRD analysis

XRD measurement was performed to validate the phase analysis of chloroform extract of *F. hurdwarica* according to the methods of (Li et al., 2012; Rajakumar et al., 2017).

2.18. GC-MS exploration of extract

The GC-MS analysis for the tentative identification of the compounds in the chloroform extract of F. hurdwarica was carried out by using GC-MS spectral analysis (Thermo Scientific Co.) (Hema et al., 2010). Interpretation of mass spectrum obtained from GC-MS analysis was done using the database of National Institute of Standard and Technology (NIST) library.

2.19. Statistical Analysis

The data was quantified as the Mean ± Standard Deviation (SD). The statistical investigations were accomplished using ANOVA followed by Dunnet post comparison test with the aid of IBM Statistical Package for Social Scientist (SPSS 20) software for data analysis. The variations were considered significant at $P<0.05$.

3. Results

3.1. Extractive values

The percent yield of the crude methanolic extract and each solvent fraction, such as n-hexane, chloroform, ethyl acetate and aqueous extract obtained was 93.3%, 13.3%, 40%, 11.1%, and 28.8% respectively.

3.2. Qualitative phytochemical analysis

The phytochemical analysis of *Filago hurdw*arica revealed the presence of alkaloid, flavonoid, sugar, protein, glycosides, saponins, tannin, phytosterol, phenol, quinones, terpenes, fats, phlobatanin, and coumarin.

3.3. Quantitative determination of phytoconstitutents

The quantitative estimation of phytochemicals revealed that the plant extracts of *F. hurdawarica* contained phenols, flavonoids, tannins, total sugar, and protein as shown in Table 1.

3.4. In Vitro Antioxidant assay

The chloroform extract of *Filago hurdwarica* possesses the strongest ability to scavenge against DPPH radical (Table 2, Figure 1) and Hydrogen peroxide (Table 3, Figure 2) as compared to other extracts.

3.5. Acute toxicity

The extracts have been shown to be safe in mice up to the dose level of 2000 mg/kg body weight. There were no sensory and motor alterations, no changes in body weight, no toxicological effects, and no lethality detected in any mice.

Table 1. Quantitative determination of phytoconstitutents.

S.NO	Extract/Fractions	TF (mg/g)	TP1 (mg/g)	TT (mg/g)	TS (mg/g)	TP2 (mg/g)
1	**Methanolic**	0.9333 ± 0.21	1.7444 ± 0.18	4.1547* ± 0.26	2.638 ± 0.53	1.9821* ± 0.01
2	**n-Hexane**	0.6213 ± 0.52	1.9018 ± 0.07	3.6746 ± 0.07	1.986 ± 0.37	0.3423 ± 0.04
3	**Chloroform**	1.4663* ± 0.12	2.929* ± 0.04	4.2723* ± 0.15	2.669* ± 0.09	0.4163 ± 0.04
4	**Ethyl acetate**	0.2340 ± 0.09	1.005 ± 0.03	3.4876 ± 0.30	1.305 ± 0.27	0.9444 ± 0.07
5	**Aqueous**	1.3486 ± 0.18	1.672 ±0.27	3.4488 ± 0.13	1.729 ±0.11	0.0417 ± 0.03

TF=Total flavonoids, TP1=Total phenol, TT=Total tannin, TS=Total sugar, TP2=Total protein content. Data expressed as mean ± SD, n=3.

Table 2. DPPH scavenging activity of *Filago hurdwarica.*

S.NO	Extract/Fraction	Conc. mg/ml	DPPH	% Inhibition
1	**Methanolic**	0.1	0.0260 ± 0.003	70.5
2	**n-Hexane**	0.1	0.0324 ± 0.004	63.3
3	**Chloroform**	0.1	0.0125* ± 0.003	85.8
4	**Ethyl acetate**	0.1	0.0413 ± 0.003	53.2
5	**Aqueous**	0.1	0.0432 ± 0.004	51.1
6	**Ascorbic acid (control)**	0.1	0.0884 ± 0.003	93.2

Data presented as mean ± SEM of each triplicate test.

Table 3. Hydrogen peroxide scavenging activity.

S.NO	Extract/Fractions	Concentration mg ml^{-1}	Hydrogen peroxide Assay	% Inhibition
1	**Methanolic**	1.00	0.0401 ± 0.015	49.4
2	**n-Hexane**	1.00	0.0711 ± 0.089	10.3
3	**Chloroform**	1.00	0.0207* ± 0.005	73.8
4	**Ethyl acetate**	1.00	0.0688 ± 0.007	13.2
5	**Aqueous**	1.00	0.0513 ± 0.035	35.3
6	**Ascorbic acid**	1.00	0.0793 ± 0.017	86.2

Data presented as mean ± SEM of each triplicate test.

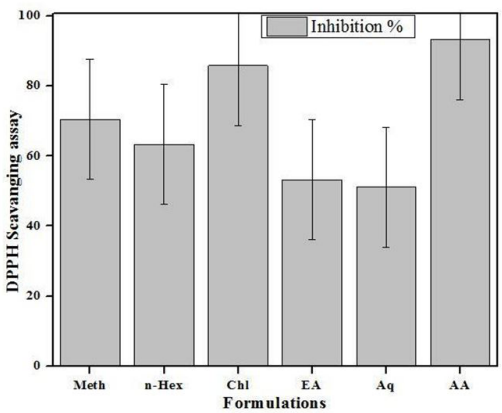

Figure 1. DPPH scavenging activities of *F. hurdwarica.*

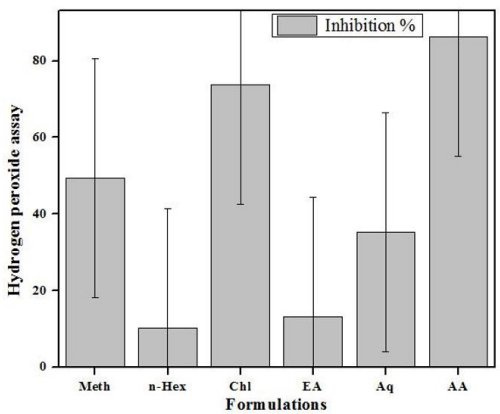

Figure 2. Hydrogen peroxide scavenging activity.

3.6. Glucose tolerance test

Alloxan treated diabetic mice showed increased levels of blood glucose compared to normal mice (Table 4, Figure 3). After treatment with chloroform extract (250 mg/kg) of *F. hurdwarica* induced a significant hypoglycemic effect ($p< 0.01$) by restoring glucose levels to normal and its effect was comparable to that caused by standard hypoglycemic agent, glibenclamide used in the study.

3.7. Changes in body weight

In diabetic mice, the body weight was significantly decreased compared to normal mice. The body weight decline was significantly increased ($p< 0.01$) by the crude methanolic extract and chloroform fraction (250 mg/kg) at the end of 15 days of treatment. Other extracts were not effective (Table 5, Figure 4).

3.8. Antihyperlipidemic activity of plant

There was a significant elevation in the levels of serum cholesterol, triglycerides, LDL and reduced HDL in diabetic mice. The oral administration of chloroform extract (250 mg/kg) significantly reduced ($p < 0.01$) the levels of TC, TG, and LDL and significantly elevated the levels of HDL (Table 6, Figure 5).

3.9. Serum profile

A significant decrease ($p< 0.01$) in serum creatinine and substantial increase ($p<0.01$) in SGPT, SGOT, and ALP

Table 4. Effect of extracts of *F. hurdwarica* on blood glucose.

Category	Treatments	Dose (mg/kg)	Blood glucose (mg/dl)				
			DAY 0	DAY 4	DAY 7	DAY 10	DAY 15
1	Normal Saline	0.03 ml	98.4 ± 3.3	96 ± 2.8	97 ± 3.4	95 ± 4.02	93 ± 2.5
2	Diabetic Control	0.03 ml	411.4 ± 3.2	473 ± 3.04	483 ± 5.1	491 ± 2.8	506 ± 2.3
3	Glibenclamide	0.03 ml	412.6 ± 6.4	387.4* ± 10.3	352.2** ± 13.2	318.6*** ± 10.8	286.4*** ± 11.7
4	Methanolic Extract (1)	150	415 ± 5.3	402.4 ± 6.5	391 ± 4.4	381 ± 4.4	370* ± 6.05
5	Methanolic Extract (2)	250	409.4 ± 3.2	388* ± 2.5	374* ± 3.3	368* ± 3.03	355* ± 4.4
6	n-Hexane	250	419.4 ± 3.6	412 ± 3.1	403.6 ± 4.6	396.6 ± 6.5	376 ± 8.9
7	Chloroform	250	410 ± 6.9	390.4* ± 9.6	366** ± 11.5	343*** ± 5.8	323*** ± 10.4
8	Ethyl acetate	250	405 ± 6.4	408 ± 3.6	414.4 ± 4.9	422 ± 4.02	434.6 ± 4.3
9	Aqua	250	409.2 ± 3.4	422 ± 5.2	435 ± 3.2	446.4 ± 4.03	455 ± 4.4

Values are expressed as mean ±SEM n=6 in each group. *P < 0.05; **P < 0.01, significant results compared with diabetic control; ***P<0.001.

Table 5. Effect of extracts of *F. hurdwarica* on body weight

Category	Treatments	Dose (mg/kg)	Body weight (g)				
			DAY 0	DAY 4	DAY 7	DAY 10	DAY 15
1	Normal Saline	0.03 ml	32 ± 0.73	32.3 ± 0.33	33 ± 0.23	33 ± 0.29	33.7 ± 0.20
2	Diabetic Control	0.03 ml	32 ± 0.8	29.4 ± 0.31	29.1 ± 0.28	27.3 ± 0.23	25.4 ± 0.30
3	Glibenclamide	0.03 ml	26.3 ± 0.34	28.4* ± 0.28	29.7* ± 0.23	32.7** ± 0.31	33.5** ± 0.31
4	Methanolic Extract (1)	150	25.7 ± 0.4	26 ± 0.57	27.6 ± 0.50	28.8 ± 0.31	30 ± 0.38
5	Methanolic Extract (2)	250	24.6 ± 0.35	25.3 ± 0.39	28.88 ± 0.36	29.5 ± 0.36	30.1* ± 0.33
6	n-Hexane	250	25.4 ± 0.4	24.8 ± 0.25	26.4 ± 0.65	27.8 ± 0.36	28.5 ± 0.24
7	Chloroform	250	27 ± 0.54	26.5* ± 0.27	28.4* ± 0.30	31.3* ± 0.27	32.4**± 0.27
8	Ethyl acetate	250	25.3 ± 0.33	24 ± 0.47	24.5 ± 0.62	25.4 ± 0.22	24 ± 0.79
9	Aqua	250	28.6 ± 0.38	27.6 ± 0.35	26.2 ± 0.46	25.3 ± 0.34	25 ± 0.46

Values are expressed as mean ±SEM n=6 in each group. *P < 0.05; **P < 0.01, significant results compared with diabetic control.

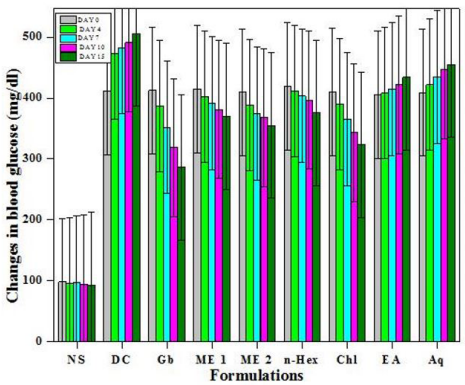

Figure 3. Changes in blood glucose level of mice.

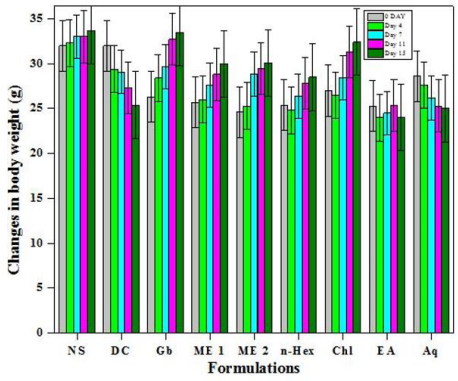

Figure 4. Changes in body weight of mice.

were observed in diabetic mice which showed renal and liver dysfunction caused by diabetogenic agent alloxan. The chloroform extract (250 mg/kg) of *F. hurdwarica* significantly improved the above parameters to about normal (Table 7, Figure 6).

3.10. Antioxidant enzymes

Diabetes mellitus significantly reduced antioxidant enzymes like catalases (CAT), peroxidases

(POD), and superoxide dismutase (SOD) levels and elevated the action of reactive oxygen species.

Table 6. Effect of extracts of *F. hurdwarica* on lipid profile.

Group	Treatments	Dose (mg/kg)	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
1	Normal Saline	0.03 ml	126 ± 2.08	131.6 ± 3.2	88.3 ± 2.5	38.2 ± 0.64
2	Diabetic Control	0.03 ml	195.6 ± 1.45	179.6 ± 1.76	168.4 ± 2.0	27.4 ± 0.52
3	Glibenclamide	0.03 ml	142.8* ± 1.18	131.3** ± 0.56	107.7** ± 1.31	39.5** ± 0.85
4	Methanolic Extract (1)	150	157.9 ± 4.5	149.1 ± 2.8	131.3 ± 1.65	31.6 ± 1.12
5	Methanolic Extract (2)	250	154.3* ± 2.3	144.6 ± 2.8	114.7* ± 0.72	35.1* ± 0.75
6	n-Hexane	250	155.3 ± 5.51	147 ± 1.24	127.5 ± 3.9	32.4 ± 1.27
7	Chloroform	250	148.5** ± 0.57	134.9** ± 1.10	110.6** ± 4.8	35.4* ± 0.57
8	Ethyl acetate	250	182.7 ± 3.3	167.3 ± 2.3	155.8 ± 0.61	30.8 ± 1.25
9	Aqua	250	188.9 ± 0.78	171.6 ± 2.08	161± 2.02	29.8 ± 0.84

Values are expressed as mean ±SEM. TC, total cholesterol; TG, triglycerides; LDL, low density lipids and HDL, high density lipids. * P <0.05; **P <0.01, significant results compared with diabetic control.

Table 7. Effect of extracts on serum profile.

Group	Treatments	Dose (mg/kg)	SGPT (U/l)	SGOT (U/l)	ALP (U/l)	CREATININE (mg/dl)
1	Normal Saline	0.03 ml	22.3 ± 1.12	26.33 ± 0.89	195.3 ± 3.35	1.23 ± 0.42
2	Diabetic Control	0.03 ml	43.8 ± 0.79	41.46 ± 0.81	308.7 ± 2.02	3.42 ± 0.22
3	Glibenclamide	0.03 ml	24.6** ± 2.99	27.36** ± 0.72	212** ± 3.05	1.33** ± 0.29
4	Methanolic Extract (1)	150	29.7 ± 1.38	33.76 ± 2.31	275.7 ± 1.21	1.92 ± 0.22
5	Methanolic Extract (2)	250	31.8 ± 2.15	32.53** ± 0.82	272.1* ± 3.36	2.34 ± 0.13
6	n-Hexane	250	28.8 ± 1.62	34.1 ± 1.28	274.9 ± 4.21	2.37 ± 0.22
7	Chloroform	250	27.5* ± 1.36	29.6** ± 1.04	268** ± 2.07	1.24* ± 0.30
8	Ethyl acetate	250	35.4 ± 1.47	36.83 ± 1.10	284.8 ± 3.77	2.53 ± 0.29
9	Aqua	250	36.9 ± 1.71	37.63 ± 1.34	283.7 ± 3.02	2.44 ± 0.20

Values are expressed as mean ±SEM. SGPT, Serum Glutamate Pyruvate Transaminase; SGOT, Serum Glutamic Oxaloacetic Transaminase; ALP, alkaline phosphatase. *P < 0.05. **P < 0.01, significant results compared with diabetic control.

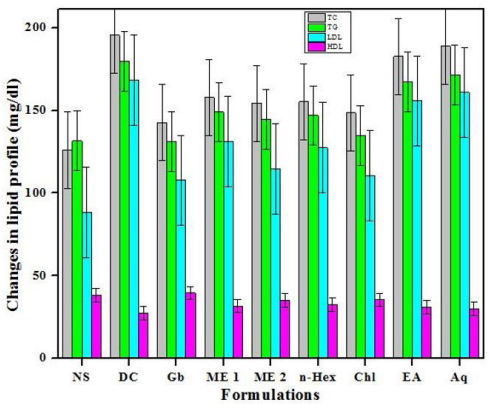

Figure 5. Antihyperlipidemic activity of plant extracts.

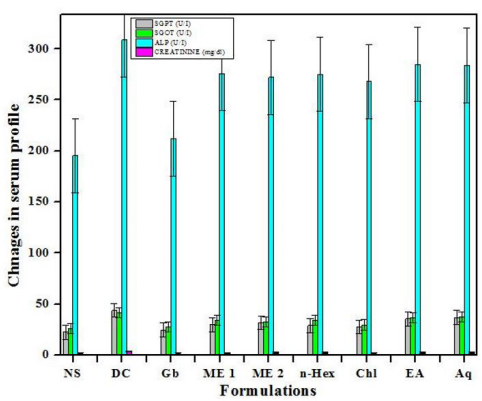

Figure 6. Serum profile of plant extracts.

The chloroform extract at the dose of 250 mg/kg increased significantly increased the activities of antioxidant enzymes. However other extracts treated group showed no significant difference in comparison to the glibenclamide treated group (Table 8, Figure 7).

3.11. In vitro membrane stabilizing activity

In hypotonic solution induced condition, chloroform extract showed the highest protection of erythrocyte membrane (83.71%) which was comparable to haemolysis inhibited by the standard acetyl salicylic acid (Table 9).

Table 8. Effect of *F. hurdwarica* extracts on antioxidant enzymes.

Group	Treatments	Dose (mg/kg)	SOD (U/mg)	POD (U/mg)	CATALASE (U/mg)
1	Normal Saline	0.03 ml	2.24 ± 0.12	5.27 ± 0.25	3.91 ± 0.27
2	Diabetic Control	0.03 ml	1.92 ± 0.90	1.84 ± 0.09	2.37 ± 0.05
3	Glibenclamide	0.03 ml	3.58** ± 0.03	7.25** ± 0.04	4.32** ± 0.03
4	Methanolic extract (1)	150	1.68 ± 0.27	3.24 ± 0.04	2.99 ± 0.25
5	Methanolic Extract (2)	250	2.02* ± 0.04	4.29** ± 0.08	2.03 ± 0.05
6	n-Hexane	250	1.09 ± 0.10	3.73 ± 0.03	2.77 ± 0.08
7	Chloroform	250	2.14** ± 0.09	4.85** ± 0.22	3.65* ± 0.03
8	Ethyl acetate	250	1.36 ± 0.13	2.31 ± 0.10	2.07 ± 0.04
9	Aqua	250	1.4 ± 0.16	2.16 ± 0.17	2.81 ± 0.11

Values are expressed as mean ±SEM. SOD, superoxide mutase, POD, peroxidase. *P < 0.05. **P < 0.01, significant results compared with diabetic control.

Table 9. Effect of plant extracts on RBCs membrane stabilization.

S.NO	Extract/Fractions	Concentration mg ml^{-1}	Thrombolytic activity	% Inhibition
1	Methanolic	1.00	0.101 ± 1.02	41.08
2	n-Hexane	1.00	0.129 ± 0.52	28.98
3	Chloroform	1.00	0.038 ± 2.03	83.71
4	Ethyl acetate	1.00	0.124 ± 1.23	32.06
5	Aqueous	1.00	0.136 ± 0.34	25.24
6	Acetyl salicylic acid (Control)	0.10	0.182 ± 0.81	88.2

Data are expressed as mean ± SD (n=3).

3.12. FTIR analysis

The FTIR spectrum of *Filago hurdwarica* chloroform extract of showed distinguishing spectral configurations in the fingerprint region (400–4000 cm^{-1}). The spectral peaks showed that the extract possesses phenols, aliphatic compounds, amines, ketones, and halogen compounds (Table 10, Figure 8).

3.13. XRD analysis

No distinctive diffraction peaks were observed in the whole spectrum, indicating the amorphous nature of chloroform extract of *F. hurdwarica* (Figure 9).

3.14. GC-MS analysis

The GC-MS analytical spectrum tentatively identified 31 bioactive compounds in the chloroform extract of *F. hurdwarica* with different peak area and retention time (Table 11, Figure 10). The efficient compounds stigmasterol and ß-sitosterol as depicted in Figure 11, identified in the GC-MS spectrum possess potential hypoglycemic and antioxidant properties.

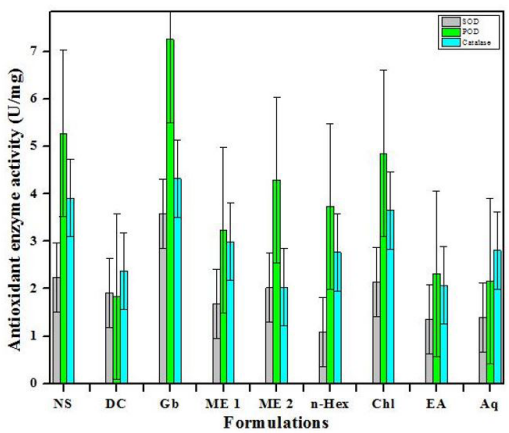

Figure 7. Antioxidant enzymes activity of plant extracts.

4. Discussion

The current investigations were designed to evaluate the antidiabetic, antihyperlipidemic, and antioxidant potential of *Filago hurdwarica* in alloxan-induced diabetic mice with the view to validate its traditional utilization in the management of diabetes mellitus (DM) in humans. Alloxan generates diabetes in mice by specific toxicity effects, destroying pancreatic β cells and exhibiting the production of free radicals both *in vivo* and *in vitro* (Carvalho et al., 2003; Kamalakkannan and Prince, 2003; Lenzen, 2008). The fasting blood glucose levels above 200 mg/dl on the fourth day of administration of alloxan using glucose strips confirmed the induction of diabetes.

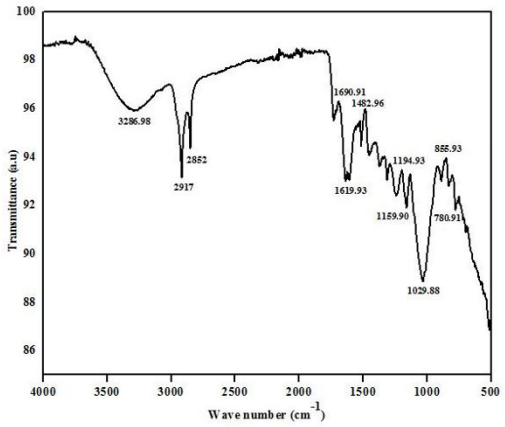

Figure 8. FTIR spectrum of methanolic extract of *F. hurdwarica*.

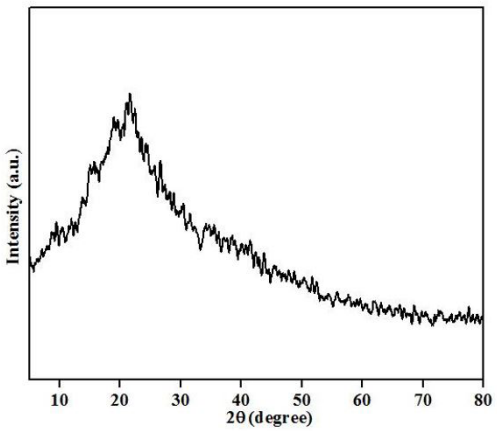

Figure 9. XRD pattern of *F. hurdwarica*.

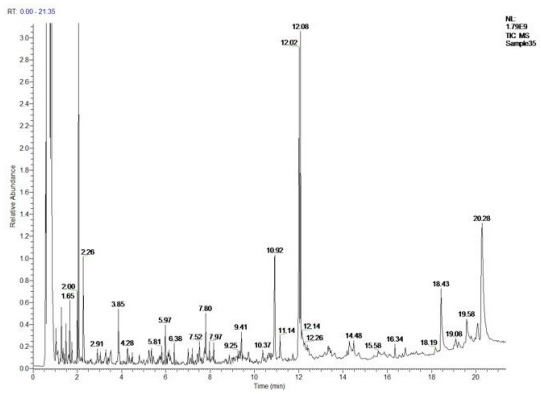

Figure 10. GC-MS chromatogram of *F. hurdwarica* extract.

The plasma glucose-lowering activity of the extracts was compared with glibenclamide, a standard hypoglycemic agent, used to cure diabetes mellitus for many years, by invigorating insulin secretion from pancreatic beta cells (Tian et al., 1998).

Table 10. Structural features of the *F. hurdwarica* chloroform extract by FTIR spectrum.

Wave numbers (cm-1) Stretching	Assignments
3286.98 O-H	Alcohols, Phenols
2917 C-H	Alkanes
2820 C=C	Alkenes
1690.91 C-H	Nitro compounds
1619.93 C=O	Ketones
1482.96 N-H	Amides
1194.93 C-O	Ethers
1029.88 N-H	Aliphatic amines
855.93 C-X	Alkyl halides
780.91 C-H	Aromatic compounds

The oral administration of crude methanolic extract and the chloroform fraction of *F. hurdwarica* demonstrated a significant lowering of blood glucose level in the alloxan-induced diabetogenic animal model. The antihyperglycemic effect of *F. hurdwarica* may be due to the presence of active hypoglycemic agents or their ability to prevent free radical formation induced by alloxan. The reduction in blood glucose by the extract may also be attributed to the enhancement of peripheral glucose utilization in abdominal muscle and augmenting the pancreatic secretion of insulin from β-cells of islets of Langerhans (Jadhav et al., 2009; Singh, 2011).

In alloxan-induced diabetic mice, the reduction of body weight may be due to the breaking down of tissue protein and muscle wasting via unattainability of carbohydrates and catabolism of fats (Gougeon et al., 2008). However, treatments with orally administered methanolic, and chloroform extracts of 250 mg/kg significantly improved the body weight compared with diabetic control which signifies its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis, and subduing the free radicals produced due to hyperglycemia (Ma et al., 2017; Mestry et al., 2016).

In diabetes mellitus, hyperlipidemia occurs as a result of the excess mobilization of fats from the adipose tissue due to the underutilization of glucose (Akpan et al., 2012; Okon et al., 2007). Besides, hyperglycemia the study also demonstrated irregularities in the lipid metabolism, significant elevation in serum TC, TG, LDL, while reduction in serum HDL levels was observed in the diabetic mice before treatment.The glibenclamide (Group III) and chloroform fraction of *F. hurdwarica* (Group VII) treated mice showed a significant reduction in TC, TG, and LDL compared to diabetic control mice and a significant increase in HDL level. This could be due to increase utilization of glucose that led to lipid peroxidation inhibition and controlling of lypolytic hormones (Adeneye et al., 2011). A number of plants have been reported to possess anti-hyperlipidemic effects in this manner due to the presence of various phytochemicals in the plants extracts (Chaudhry et al., 2016; Yusufoglu et al., 2015).

It is acknowledged that the diabetes is linked to irreversible hepatocellular damage by destroying the liver

Table 11. GC-MS exploration of *F. hurdwarica.*

S.NO	Compound Name	Area %	RT	Molecular formula	MW
1	Pentane, 3-methyl-	93.49	0.65	C_6H_{14}	86
2	9-Octadecenoic acid	0.01	1.51	$C_{57}H_{104}O_6$	884
3	p-Xylene	1.22	2.06	C_8H_{10}	106
4	o-Xylene	0.21	2.20	C_8H_{10}	106
5	Oleic acid	0.01	5.95	$C_{38}H_{74}O_2$	562
6	Fenbuconazol	0.06	8.68	$C_{19}H_{17}ClN_4$	336
7	Eicosanebioic acid	0.06	8.68	$C_{22}H_{42}O_4$	370
8	Milbemycin b	0.07	11.39	$C_{33}H_{46}ClNO_7$	603
9	Lycopene	0.07	11.39	$C_{40}H_{56}$	536
10	9,12,15-Octadecatrienoic acid	10.47	17.83	$C_{27}H_{52}O_4Si_2$	496
11	2,3-Epoxycarane	0.04	4.81	$C_{10}H_{16}O$	152
12	Pregnane-3,20-dion	10.24	18.21	$C_{32}H_{62}N_2O_5Si_3$	638
13	1-chloro- Octadecane	0.12	5.28	$C_{18}H_{37}Cl$	288
14	Nonanoic acid	0.07	19.92	$C_{19}H_{30}O_2$	290
15	Dasycarpidan-1-methanol, acetate	0.03	21.23	$C_{20}H_{26}N_2O_2$	326
16	Heneicosane	0.44	9.41	$C_{21}H_{44}$	296
17	Hexadecanoic acid	0.01	28.70	$C_{17}H_{34}O_2$	270
18	Curan, 16,17-didehydro-, (20.xi.)-	0.03	21.23	$C_{19}H_{24}N_2$	280
19	Strychane	0.01	22.06	$C_{21}H_{26}N_2O_2$	338
20	1-Monolinoleoylglycerol trimethylsilyl ether	0.01	22.06	$C_{27}H_{54}O_4Si_2$	498
21	Hexasiloxane	0.01	24.59	$C_{12}H_{38}O_5Si_6$	430
22	Octasiloxane	0.01	24.59	$C_{16}H_{50}O_7Si_8$	578
23	Haloxazolam	0.13	29.07	$C_{17}H_{14}BrFN_2O_2$	376
24	1-Heptatriacotanol	0.06	14.28	$C_{37}H_{76}O$	537
25	17-Pentatriacontene	0.06	16.81	$C_{35}H_{70}$	490
26	Vitamin E	0.46	18.45	$C_{29}H_{50}O_2$	430
27	à-Tocopherol-á-D-mannoside	0.46	18.45	$C_{35}H_{60}O_7$	592
28	Ethyl iso-allocholate	0.08	19.08	$C_{26}H_{44}O_5$	436
29	Stigmasterol	0.31	19.60	$C_{29}H_{50}O$	414
30	Amiodarone	1.38	20.30	$C_{25}H_{29}I_2NO_3$	645
31	ß-Sitosterol	1.38	20.30	$C_{24}H_{26}ClFN_4O$	440

RT=Retention time, MW-Molecular weight.

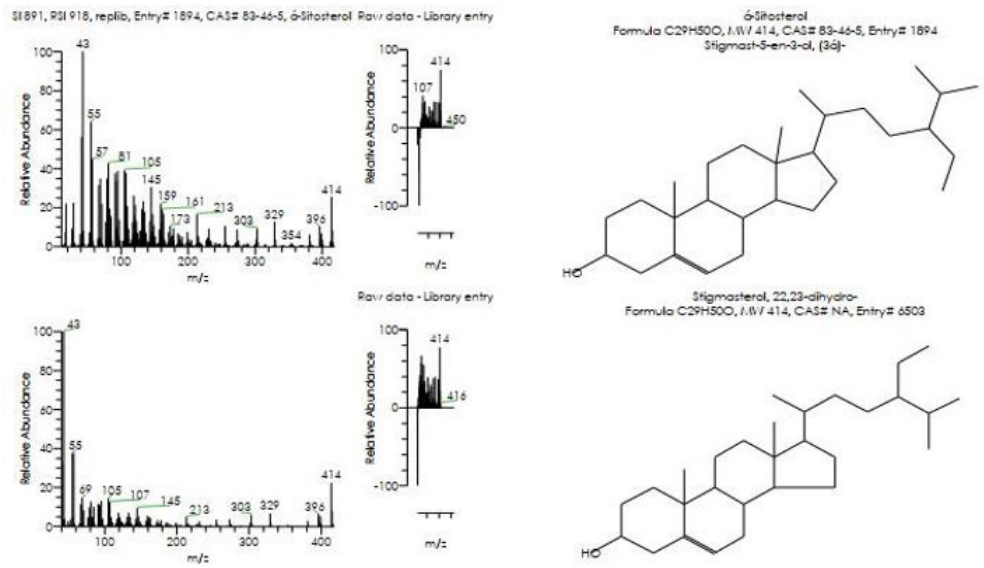

Figure 11. Structure of ß-Sitosterol and Stigmasterol.

microsomal cell, leading to the proliferation of different enzymes including SGOT, SGPT, and ALP into the blood (Pari and Latha, 2002). The crude methanolic extract and chloroform fraction (250 mg/kg) significantly lowered the levels of SGOT, SGPT, and ALP in the plasma compared to the diabetic control group. The diabetic mice showed a significant increase in serum creatinine level, which is considered as a marker of impaired renal dysfunction (Preethi and Kuttan, 2009). *F. hurdwarica* chloroform extract significantly reduced the serum creatinine thus treating and preventing the progression of renal damage in diabetic mice. Reports have shown that the reversal of such changes in above parameters is allied with the presence of secondary metabolites in the extract (Gupta and Gupta, 2009; Lal et al., 2009; Sharma et al., 2014, 2019).

In diabetes mellitus, high glucose can inactivate antioxidant enzymes SOD, POD and, CAT by glycating these proteins thus producing induced oxidative stress, which in turn, causes lipid peroxidation, which leads to both rise in ROS (reactive oxygen species) and to the demonetization of free radical scavenging compounds (Baynes and Thorpe, 1999; Kakkar et al., 1998; Karasu, 1999). The extensive array of antioxidant enzymes, SOD, POD, and CAT shield the body from the hostile effects of free radicals that are produced *in vivo* under normal physiological circumstances (Halliwell, 1999). SOD scavenges superoxide anion to form hydrogen peroxide reducing the toxic effects derived from a secondary reaction. POD and CAT is involved in the reduction of hydrogen peroxide thus protecting the erythrocytes from reactive oxygen species (Karasu, 1999). Treatment with methanolic and chloroform extract has reversed the activities of these enzymatic antioxidants which could be a result of decreased lipid peroxidation and decreased utilization (Yin et al., 2014).

Several studies revealed that injury to membranes of RBCs due to hyperglycemia makes cells more susceptible to secondary damage through lipid peroxidation, haemolysis, and oxidation of haemoglobin (Halliwell, 1999). The chloroform extract of *F. hurdwarica* showed significant inhibition of hypotonic solution-induced hemolysis of erythrocytes compared to control. The inhibition of erythrocyte lysis property of *F. hurdwarica* could be the possible mechanism for its anti-diabetic activity due to the presence of various biochemical compounds in the extract which exert a profound stabilizing effect on lysosomal membrane and their cations binding ability (Miliauskas et al., 2004; Oyedapo et al., 2004).

Various secondary metabolites isolated from diverse plant species have been shown to possess potent anti-hyperglycemic and glucose suppressive effects. These secondary metabolites include alkaloids, flavonoids, terpenoids, and phenols (Ivorra et al., 1989; Sharma et al., 2010). These might stimulate glycogenesis in the liver, insulin release from pancreatic ß-cells, or inhibiting glucose absorption in the gut, or increasing glucose utilization by the body (Sezik et al., 2005). As a result, these secondary metabolites which were found in *F. hurdwarica* crude extracts and chloroform fraction may, therefore, be accountable for the observed glucose suppressive and anti-hyperglycemic activity (Kumar et al., 2011).The antioxidant effect of the extract may involve the scavenging of free radicals as seen with DPPH and H_2O_2, thereby protecting the cells from the damaging effect of the free radicals and successive complications. Antioxidants have been recognized to exhibit protective functions against oxidative stress and are associated with reduced risk of chronic complications (Gordon, 1990). The experimental finding revealed that the chloroform extract has significantly high DPPH and hydrogen peroxide radical scavenging activity which is an agreement with the previous studies (Ali and Sayeed, 1988; Asamenew et al., 2011).

The characteristic peaks of different functional groups from the FTIR spectroscopic characterization show the presence of relative active compounds in the sample that might be used as antidiabetic and antihyperlipidemic agents as reported in previous studies (Sidhu and Sharma, 2014; Wei et al., 2017). XRD analysis of *F. hurdwarica* showed no distinctive diffraction peaks in the whole spectrum suggesting the amorphous nature of the extract. However, the possible presence of amorphous phases could be attributed to the phenols, alkaloids, flavonoids, and tannins, which are evident from the qualitative and quantitative screening tests. Furthermore, the result of XRD confirmed the conclusion of FTIR analysis. These results are in accordance with previous studies suggesting the amorphous nature to the presence of phytoconstitutents (Bala et al., 2015; Rajaram et al., 2015; Varadharaj et al., 2019).The GC-MS spectral data tentatively identified that the chloroform extract contained several compounds that were found active in terms of anti-diabetic and antioxidant potentials as evident from their reported literature. In diabetic context stigmasterol and ß-sitosterol exhibit antidiabetic and membrane-stabilizing effects (Nualkaew et al., 2015; Ramu et al., 2016; Zeb et al., 2017). Similarly, amiodarone, hexadecanoic acid, and 2,3-dihydroxypropyl ester are also used as hypoglycemic agents either alone or in combination (Lai et al., 2020). Other antidiabetic compounds identified included, 1-Monolinoleoylglycerol trimethylsilyl ether (Senthil et al., 2016), vitamin E (Bharti et al., 2012), and Dasycarpidan-1-methanol, acetate (Al-Gara et al., 2019).

5. Conclusion

The current research study revealed for the first time the antidiabetic and antioxidant potential of *F. hurdwarica* in alloxan-induced diabetic model. The study demonstrated that the chloroform extract of *F. hurdwarica* possess substantial antihyperglycemic, antihyperlipidemic, and antioxidant potential in alloxan-induced diabetic mice models thus extenuating its traditional utilization. The preliminary information obtained from the FTIR, XRD, and GC-MS procedures recommended the therapeutic significance of the plant. Consequently, further study is indispensable for hypoglycemic mechanism and isolation of bioactive constituents for auxiliary validation of the plant. Future research needs to focus on bioassay guided isolation and confirmed identification of the active principles using

standards or spectral workup. This could inform better extraction and formulation procedures to lower the dose required, as well as lead to a validated phytochemical analysis of a standardized product.

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References

ADENEYE, A.A., AGBAJE, E.O. and OLAGUNJU, J.A., 2011. Metformin: an effective attenuator of risperidone-induced insulin resistance hyperglycemia and dyslipidemia in rats. *Indian Journal of Experimental Biology*, vol. 49, no. 5, pp. 332-338. PMid:21615056.

AHMED, M., AKTER, M.S., LEE, J. and EUN, J., 2010. Encapsulation by spray drying of bioactive components, physicochemical and morphological properties from purple sweet potato. *Lebensmittel-Wissenschaft + Technologie*, vol. 43, no. 9, pp. 1307-1312. http://dx.doi.org/10.1016/j.lwt.2010.05.014.

AKHTAR, M.F., ASHRAF, M., ANJUM, A.A., JAVEED, A., SHARIF, A., SALEEM, A. and AKHTAR, B., 2016. Textile industrial effluent induces mutagenicity and oxidative DNA damage and exploits oxidative stress biomarkers in rats. *Environmental Toxicology and Pharmacology*, vol. 41, pp. 180-186. http://dx.doi.org/10.1016/j.etap.2015.11.022. PMid:26710178.

AKPAN, E.J., OKOKON, J.E. and OFFONG, E., 2012. Antidiabetic and hypolipidemic activities of ethanolic leaf extract and fractions of Melanthera scandens. *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 7, pp. 523-527. http://dx.doi.org/10.1016/S2221-1691(12)60089-6. PMid:23569963.

ALEMA, N.M., PERIASAMY, G., SIBHAT, G.G., TEKULU, G.H. and HIBEN, M.G., 2020. Antidiabetic activity of extracts of terminalia brownii fresen. Stem bark in mice. *Journal of Experimental Pharmacology*, vol. 12, pp. 61-71. http://dx.doi.org/10.2147/JEP.S240266. PMid:32110120.

AL-GARA, N.I., ABU-SERAG, N.A., SHAHEED, K.A.A. and AL BAHADLY, Z.K., 2019. Analysis of bioactive phytochemical compound of (Cyperus alternifolius L.) By using gas chromatography–mass spectrometry. *IOP Conference Series. Materials Science and Engineering*, vol. 571, pp. 012047. http://dx.doi.org/10.1088/1757-899X/571/1/012047.

ALI, R. and SAYEED, S. (1988). A Novel dye for staining electrophoretically resolved protein. In: *Proceedings of the International Symposium on Protein Structure-Function Relationship*, Karachi. Pakistan. Amsterdam: Elsevier, vol. 15

ARUMUGAM, G., MANJULA, P. and PAARI, N., 2013. A review: anti diabetic medicinal plants used for diabetes mellitus. *Journal of Acute Disease*, vol. 2, no. 3, pp. 196-200. http://dx.doi.org/10.1016/S2221-6189(13)60126-2.

ASAMENEW, G., BISRAT, D., MAZUMDER, A. and ASRES, K., 2011. In vitro antimicrobial and antioxidant activities of anthrone and chromone from the latex of Aloe harlana Reynolds. *Phytotherapy Research*, vol. 25, no. 12, pp. 1756-1760. http://dx.doi.org/10.1002/ptr.3482. PMid:21452374.

AYOOLA, G., COKER, H., ADESEGUN, S., ADEPOJU-BELLO, A., OBAWEYA, K., EZENNIA, E. and ATANGBAYILA, T., 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, vol. 7, pp. 1019-1024.

BALA, N., SAHA, S., CHAKRABORTY, M., MAITI, M., DAS, S., BASU, R. and NANDY, P., 2015. Green synthesis of zinc oxide nanoparticles using Hibiscus subdariffa leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. *RSC Advances*, vol. 5, no. 7, pp. 4993-5003. http://dx.doi.org/10.1039/C4RA12784F.

BAYNES, J.W. and THORPE, S.R., 1999. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*, vol. 48, no. 1, pp. 1-9. http://dx.doi.org/10.2337/diabetes.48.1.1. PMid:9892215.

BENALLA, W., BELLAHCEN, S. and BNOUHAM, M., 2010. Antidiabetic medicinal plants as a source of alpha glucosidase inhibitors. *Current Diabetes Reviews*, vol. 6, no. 4, pp. 247-254. http://dx.doi.org/10.2174/157339910791658826. PMid:20522017.

BHARTI, S.K., KRISHNAN, S., KUMAR, A., RAJAK, K.K., MURARI, K., BHARTI, B.K. and GUPTA, A.K., 2012. Antihyperglycemic activity with DPP-IV inhibition of alkaloids from seed extract of Castanospermum australe: investigation by experimental validation and molecular docking. *Phytomedicine*, vol. 20, no. 1, pp. 24-31. http://dx.doi.org/10.1016/j.phymed.2012.09.009. PMid:23063145.

CARVALHO, E.N., CARVALHO, N.A.S. and FERREIRA, L.M., 2003. Experimental model of induction of diabetes mellitus in rats. *Acta Cirurgica Brasileira*, vol. 18, no. spe, pp. 60-64. http://dx.doi.org/10.1590/S0102-86502003001100009.

CHANTIRATIKUL, P., MEECHAI, P. and NAKBANPOTEC, W., 2009. Antioxidant activities and phenolic contents of extracts from Salvinia molesta and Eichornia crassipes. *Research Journal of Biological Sciences*, vol. 4, pp. 1113-1117.

CHAUDHRY, S.R., AKRAM, A., ASLAM, N., ASIF, M., WAJID, M., KINFE, T., JABEEN, Q. and MUHAMMAD, S., 2016. Antidiabetic and antidyslipidemic effects of Heliotropium strigosum in rat models of Type I and Type II diabetes. *Acta Poloniae Pharmaceutica*, vol. 73, no. 6, pp. 1575-1586. PMid:29634112.

CHUN, O.K., KIM, D.-O. and LEE, C.Y., 2003. Superoxide radical scavenging activity of the major polyphenols in fresh plums. *Journal of Agricultural and Food Chemistry*, vol. 51, no. 27, pp. 8067-8072. http://dx.doi.org/10.1021/jf034740d. PMid:14690398.

DAVID, J., AFOLABI, E., OLOTU, P., OJERINDE, S., AGWOM, F. and AJIMA, U., 2017. Phytochemical analysis, antidiabetic and toxicity studies of the methanolic leaf extract of Detarium microcarpum guill and perr in wistar albino rats. *Journal of Chemical and Pharmaceutical Research*, vol. 9, no. 11, pp. 55-60.

DEEPASHREE, B. and PRAKASH, J., 2007. A study on the nutritional status of diabetics and associated risk factors. *Journal of Human Ecology (Delhi, India)*, vol. 21, no. 4, pp. 269-274. http://dx.doi.org/10.1080/09709274.2007.11905984.

DWIVEDI, C. and DASPAUL, S., 2013. Antidiabetic herbal drugs and polyherbal formulation used for diabetes: a review. *J Phytopharmacol*, vol. 2, no. 1-3, pp. 44-51. http://dx.doi.org/10.31254/phyto.2013.21308.

ELOSTA, A., GHOUS, T. and AHMED, N., 2012. Natural products as anti-glycation agents: possible therapeutic potential for diabetic complications. *Current Diabetes Reviews*, vol. 8, no. 2, pp. 92-108. http://dx.doi.org/10.2174/157339912799424528. PMid:22268395.

EMDIN, C.A., RAHIMI, K., NEAL, B., CALLENDER, T., PERKOVIC, V. and PATEL, A., 2015. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. *Journal of the American Medical Association*, vol. 313, no. 6, pp. 603-615. http://dx.doi.org/10.1001/jama.2014.18574. PMid:25668264.

FATIMA, S., AKHTAR, M.F., ASHRAF, K.M., SHARIF, A., SALEEM, A., AKHTAR, B., PEERZADA, S., SHABBIR, M., ALI, S. and ASHRAF, W., 2019. Antioxidant and alpha amylase inhibitory activities of Fumaria officinalis and its antidiabetic potential against alloxan induced diabetes. *Cellular and Molecular Biology*, vol. 65, no. 2, pp. 50-57. http://dx.doi.org/10.14715/cmb/2019.65.2.8. PMid:30860471.

FRIEDEWALD, W.T., LEVY, R.I. and FREDRICKSON, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, vol. 18, no. 6, pp. 499-502. http://dx.doi.org/10.1093/clinchem/18.6.499. PMid:4337382.

GORDON, M. (1990). The mechanism of antioxidant action in vitro. In: B.J.F. HUDSON, eds. *Food antioxidants*. Dordrecht: Springer, pp. 1-18. http://dx.doi.org/10.1007/978-94-009-0753-9_1.

GOUGEON, R., MORAIS, J.A., CHEVALIER, S., PEREIRA, S., LAMARCHE, M. and MARLISS, E.B., 2008. Determinants of whole-body protein metabolism in subjects with and without type 2 diabetes. *Diabetes Care*, vol. 31, no. 1, pp. 128-133. http://dx.doi.org/10.2337/dc07-1268. PMid:17921356.

GUPTA, R. and GUPTA, R., 2009. Protective role of Pterocarpus marsupium in diabetes-induced hyperlipidemic condition. *Journal of Complementary & Integrative Medicine*, vol. 6, no. 1, pp. 6. http://dx.doi.org/10.2202/1553-3840.1288.

HALLIWELL, B. (1999). Antioxidant defenses. In: B. HALLIWELL and J.M.C. GUTTERIDGE, eds. *Free radicals in biology and in medicine*. New York: Clarendon Press.

HASANI-RANJBAR, S., LARIJANI, B. and ABDOLLAHI, M., 2009. A systematic review of the potential herbal sources of future drugs effective in oxidant-related diseases. *Inflammation & Allergy Drug Targets*, vol. 8, no. 1, pp. 2-10. http://dx.doi.org/10.2174/187152809787582561. PMid:19275687.

HEMA, R., KUMARAVEL, S. and SIVASUBRAMANIAN, C., 2010. GC-MS study on the potentials of Syzygium aromaticum. *Researcher*, vol. 2, pp. 1-4.

IVORRA, M.D., PAYA, M. and VILLAR, A., 1989. A review of natural products and plants as potential antidiabetic drugs. *Journal of Ethnopharmacology*, vol. 27, no. 3, pp. 243-275. http://dx.doi.org/10.1016/0378-8741(89)90001-9. PMid:2693840.

JADHAV, J., MASIRKAR, V. and DESHMUKH, V., 2009. Antihyperglycemic effect of Diospyros melanoxylon (Roxb.) bark against alloxan-induced diabetic rats. *International Journal of Pharm Tech Research*, vol. 1, pp. 196-200.

JÖRNS, A., MUNDAY, R., TIEDGE, M. and LENZEN, S., 1997. Comparative toxicity of alloxan, N-alkylalloxans and ninhydrin to isolated pancreatic islets in vitro. *The Journal of Endocrinology*, vol. 155, no. 2, pp. 283-293. http://dx.doi.org/10.1677/joe.0.1550283. PMid:9415063.

JYOTHI, S., CHAVAN, S.C. and SOMASHEKARAIAH, B., 2012. In vitro and in vivo antioxidant and antidiabetic efficacy of Cassia auriculata L. flowers. *Global Journal of Pharmacology*, vol. 6, pp. 33-40.

KAKKAR, R., MANTHA, S.V., RADHI, J., PRASAD, K. and KALRA, J., 1998. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. *Clinical Science*, vol. 94, no. 6, pp. 623-632. http://dx.doi.org/10.1042/cs0940623. PMid:9854460.

KAMALAKKANNAN, N. and PRINCE, P.S.M., 2003. Hypoglycaemic effect of water extracts of Aegle marmelos fruits in streptozotocin diabetic rats. *Journal of Ethnopharmacology*, vol. 87, no. 2-3, pp. 207-210. http://dx.doi.org/10.1016/S0378-8741(03)00148-X. PMid:12860309.

KARASU, Ç., 1999. Increased activity of H2O2 in aorta isolated from chronically streptozotocin-diabetic rats: effects of antioxidant enzymes and enzyme inhibitors. *Free Radical Biology & Medicine*, vol. 27, no. 1-2, pp. 16-27. http://dx.doi.org/10.1016/S0891-5849(99)00028-3. PMid:10443915.

KESARI, A.N., GUPTA, R.K., SINGH, S.K., DIWAKAR, S. and WATAL, G., 2006. Hypoglycemic and antihyperglycemic activity of Aegle marmelos seed extract in normal and diabetic rats. *Journal of Ethnopharmacology*, vol. 107, no. 3, pp. 374-379. http://dx.doi.org/10.1016/j.jep.2006.03.042. PMid:16781099.

KUMAR, S., KUMAR, V. and PRAKASH, O., 2011. Antidiabetic, hypolipidemic and histopathological analysis of Dillenia indica (L.) leaves extract on alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*, vol. 4, no. 5, pp. 347-352. http://dx.doi.org/10.1016/S1995-7645(11)60101-6. PMid:21771674.

LAI, J.H., WANG, M.T., WU, C.C., HUANG, Y.L., LU, C.H. and LIOU, J.T., 2020. Risk of severe hypoglycemic events from amiodarone-sulfonylureas interactions: a population-based nested case-control study. *Pharmacoepidemiology and Drug Safety*, vol. 29, no. 8, pp. 842-853. http://dx.doi.org/10.1002/pds.5034. PMid:32483856.

LAL, S.S., SUKLA, Y., SINGH, A., ANDRIYAS, E.A. and LALL, A.M., 2009. Hyperuricemia, high serum urea and hypoproteinemia are the risk factor for diabetes. *Asian Journal of Medical Sciences*, vol. 1, pp. 33-34.

LENZEN, S., 2008. The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia*, vol. 51, no. 2, pp. 216-226. http://dx.doi.org/10.1007/s00125-007-0886-7. PMid:18087688.

LI, G., HE, D., QIAN, Y., GUAN, B., GAO, S., CUI, Y., YOKOYAMA, K. and WANG, L., 2012. Fungus-mediated green synthesis of silver nanoparticles using Aspergillus terreus. *International Journal of Molecular Sciences*, vol. 13, no. 1, pp. 466-476. http://dx.doi.org/10.3390/ijms13010466. PMid:22312264.

MA, Q., GUO, Y., SUN, L. and ZHUANG, Y., 2017. Anti-diabetic effects of phenolic extract from rambutan peels (Nephelium lappaceum) in high-fat diet and streptozotocin-induced diabetic mice. *Nutrients*, vol. 9, no. 8, pp. 801. http://dx.doi.org/10.3390/nu9080801. PMid:28933738.

MARLES, R.J. and FARNSWORTH, N.R., 1995. Antidiabetic plants and their active constituents. *Phytomedicine*, vol. 2, no. 2, pp. 137-189. http://dx.doi.org/10.1016/S0944-7113(11)80059-0. PMid:23196156.

MASUKO, T., MINAMI, A., IWASAKI, N., MAJIMA, T., NISHIMURA, S.-I. and LEE, Y.C., 2005. Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. *Analytical Biochemistry*, vol. 339, no. 1, pp. 69-72. http://dx.doi.org/10.1016/j.ab.2004.12.001. PMid:15766712.

MBIRI, W., KASILI, S., KISANGAU, D.P., MUSILA, N., PIERO, N. and MBINDA, W.M., 2016. Antinociceptive properties of methanolic bark extracts of Terminalia brownii in wistar rats. *Journal of Pain & Relief*, vol. 5, no. 5, pp. 261. http://dx.doi.org/10.4172/2167-0846.1000261.

MESTRY, S.N., DHODI, J.B., KUMBHAR, S.B. and JUVEKAR, A.R., 2016. Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract. *Journal of Traditional and Complementary Medicine*, vol. 7, no. 3, pp. 273-280. http://dx.doi.org/10.1016/j.jtcme.2016.06.008. PMid:28725620.

MILIAUSKAS, G., VENSKUTONIS, P. and VAN BEEK, T., 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, vol. 85, no. 2, pp. 231-237. http://dx.doi.org/10.1016/j.foodchem.2003.05.007.

MORIKAWA, T., TOYAMA, T., KUDO, N. and KAWASHIMA, Y., 2007. Reducing effect of matrix metalloproteinase inhibitors on serum triacylglycerol in streptozotocin-induced diabetic rats and Zucker fa/fa rats. *Biological & Pharmaceutical Bulletin*, vol. 30, no. 8, pp. 1461-1467. http://dx.doi.org/10.1248/bpb.30.1461. PMid:17666804.

NABI, S.A., KASETTI, R.B., SIRASANAGANDLA, S., TILAK, T.K., KUMAR, M.V.J. and RAO, C.A., 2013. Antidiabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats. *BMC Complementary and Alternative Medicine*, vol. 13, no. 1, pp. 37. http://dx.doi.org/10.1186/1472-6882-13-37. PMid:23414307.

NUALKAEW, S., PADEE, P. and TALUBMOOK, C., 2015. Hypoglycemic activity in diabetic rats of stigmasterol and sitosterol-3-O--D-glucopyranoside isolated from Pseuderanthemum palatiferum (Nees) Radlk. leaf extract. *Journal of Medicinal Plants Research*, vol. 9, no. 20, pp. 629-635. http://dx.doi.org/10.5897/JMPR2014.5722.

OKON, E.B., CHUNG, A.W., ZHANG, H., LAHER, I. and VAN BREEMEN, C., 2007. Hyperglycemia and hyperlipidemia are associated with endothelial dysfunction during the development of type 2 diabetes. *Canadian Journal of Physiology and Pharmacology*, vol. 85, no. 5, pp. 562-567. http://dx.doi.org/10.1139/Y07-026. PMid:17632592.

OMALE, J. and OKAFOR, P.N., 2008. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissus multistriata. *African Journal of Biotechnology*, vol. 7, no. 17, pp. 3129-3133.

ONSA, G.H., BIN SAARI, N., SELAMAT, J. and BAKAR, J., 2004. Purification and characterization of membrane-bound peroxidases from Metroxylon sagu. *Food Chemistry*, vol. 85, no. 3, pp. 365-376. http://dx.doi.org/10.1016/j.foodchem.2003.07.013.

OYEDAPO, O., AKINPELU, B. and OREFUWA, S., 2004. Anti-inflammatory effect of Theobroma cacao, root extract. *Journal of Tropical Medicinal Plants*, vol. 5, pp. 161-166.

PARI, L. and LATHA, M., 2002. Effect of Cassia auriculata flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. *Singapore Medical Journal*, vol. 43, no. 12, pp. 617-621. PMid:12693765.

PRAMILA, D., XAVIER, R., MARIMUTHU, K., KATHIRESAN, S., KHOO, M., SENTHILKUMAR, M., SATHYA, K. and SREERAMANAN, S., 2012. Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (Mentha piperita: lamiaceae). *Journal of Medicinal Plants Research*, vol. 6, no. 3, pp. 331-335. http://dx.doi.org/10.5897/JMPR11.1232.

PREETHI, K.C. and KUTTAN, R., 2009. Hepato and reno protective action of Calendula officinalis L. flower extract. *Indian Journal of Experimental Biology*, vol. 47, no. 3, pp. 163. PMid:19405380.

RAGAVAN, B. and KRISHNAKUMARI, S., 2006. Antidiabetic effect ofT. arjuna bark extract in alloxan induced diabetic rats. *Indian Journal of Clinical Biochemistry*, vol. 21, no. 2, pp. 123-128. http://dx.doi.org/10.1007/BF02912926. PMid:23105628.

RAHIMI, R., NIKFAR, S., LARIJANI, B. and ABDOLLAHI, M., 2005. A review on the role of antioxidants in the management of diabetes and its complications. *Biomedicine and Pharmacotherapy*, vol. 59, no. 7, pp. 365-373. http://dx.doi.org/10.1016/j.biopha.2005.07.002. PMid:16081237.

RAJAKUMAR, G., GOMATHI, T., THIRUVENGADAM, M., RAJESWARI, V.D., KALPANA, V. and CHUNG, I.-M., 2017. Evaluation of anti-cholinesterase, antibacterial and cytotoxic activities of green synthesized silver nanoparticles using from Millettia pinnata flower extract. *Microbial Pathogenesis*, vol. 103, pp. 123-128. http://dx.doi.org/10.1016/j.micpath.2016.12.019. PMid:28025099.

RAJARAM, K., AISWARYA, D. and SURESHKUMAR, P., 2015. Green synthesis of silver nanoparticle using Tephrosia tinctoria and its antidiabetic activity. *Materials Letters*, vol. 138, pp. 251-254. http://dx.doi.org/10.1016/j.matlet.2014.10.017.

RAMU, R., SHIRAHATTI, P.S., NAYAKAVADI, S., VADIVELAN, R., ZAMEER, F., DHANANJAYA, B.L. and PRASAD, N., 2016. The effect of a plant extract enriched in stigmasterol and β-sitosterol on glycaemic status and glucose metabolism in alloxan-induced diabetic rats. *Food & Function*, vol. 7, no. 9, pp. 3999-4011. http://dx.doi.org/10.1039/C6FO00343E. PMid:27711824.

REITMAN, S. and FRANKEL, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, vol. 28, no. 1, pp. 56-63. http://dx.doi.org/10.1093/ajcp/28.1.56. PMid:13458125.

SENTHIL, J., RAMEASHKANNAN, M. and MANI, P., 2016. Phytochemical profiling of ethanolic leaves extract of Ipomoea sepiaria (Koenig Ex. Roxb). *International Journal of Innovative Research in Science Engineering and Technology*, vol. 5, pp. 3140-3147.

SEZIK, E., ASLAN, M., YESILADA, E. and ITO, S., 2005. Hypoglycaemic activity of Gentiana olivieri and isolation of the active constituent through bioassay-directed fractionation techniques. *Life Sciences*, vol. 76, no. 11, pp. 1223-1238. http://dx.doi.org/10.1016/j.lfs.2004.07.024. PMid:15642593.

SHARMA, A.K., KUMAR, S., CHASHOO, G., SAXENA, A.K. and PANDEY, A.K., 2014. Cell cycle inhibitory activity of Piper longum against A549 cell line and its protective effect against metal-induced toxicity in rats. *Indian Journal of Biochemistry & Biophysics*, vol. 51, no. 5, pp. 358-364. PMid:25630105.

SHARMA, B., SALUNKE, R., BALOMAJUMDER, C., DANIEL, S. and ROY, P., 2010. Anti-diabetic potential of alkaloid rich fraction from Capparis decidua on diabetic mice. *Journal of Ethnopharmacology*, vol. 127, no. 2, pp. 457-462. http://dx.doi.org/10.1016/j.jep.2009.10.013. PMid:19837152.

SHARMA, U.K., KUMAR, R., GUPTA, A., GANGULY, R., SINGH, A.K., OJHA, A.K. and PANDEY, A.K., 2019. Ameliorating efficacy of eugenol against metanil yellow induced toxicity in albino Wistar rats. *Food and Chemical Toxicology*, vol. 126, pp. 34-40. http://dx.doi.org/10.1016/j.fct.2019.01.032. PMid:30738991.

SIDHU, M.C. and SHARMA, T., 2014. Antihyperglycemic activity of petroleum ether leaf extract of Ficus krishnae L. on alloxan-induced diabetic rats. *Indian Journal of Pharmaceutical Sciences*, vol. 76, no. 4, pp. 323-331. PMid:25284930.

SINGH, L.W., 2011. Traditional medicinal plants of Manipur as anti-diabetics. *Journal of Medicinal Plants Research*, vol. 5, pp. 677-687.

SOSA, S., PELIN, M., CAVION, F., HERVÉ, F., HESS, P. and TUBARO, A., 2020. Acute oral toxicity of pinnatoxin G in mice. *Toxins*, vol. 12, no. 2, pp. 87. http://dx.doi.org/10.3390/toxins12020087. PMid:32012834.

TAFESSE, T.B., HYMETE, A., MEKONNEN, Y. and TADESSE, M., 2017. Antidiabetic activity and phytochemical screening of extracts of the leaves of Ajuga remota Benth on alloxan-induced diabetic mice. *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, pp. 243. http://dx.doi.org/10.1186/s12906-017-1757-5. PMid:28464813.

TAREKEGNE, W., SINTAYEHU, B., GEBRELIBANOS, M., HADIGU, A. and YARLAGADDA, R., 2016. Radical scavenging activity and preliminary phytochemical screening of the leaf extracts of T. brownii Fresen. *World J Pharm Sci*, vol. 4, pp. 296-301.

TIAN, Y.A., JOHNSON, G. and ASHCROFT, S., 1998. Sulfonylureas enhance exocytosis from pancreatic beta-cells by a mechanism that does not involve direct activation of protein kinase C. *Diabetes*, vol. 47, no. 11, pp. 1722-1726. http://dx.doi.org/10.2337/diabetes.47.11.1722. PMid:9792541.

VARADHARAJ, V., RAMASWAMY, A., SAKTHIVEL, R., SUBBAIYA, R., BARABADI, H., CHANDRASEKARAN, M. and SARAVANAN, M., 2019. Antidiabetic and antioxidant activity of green synthesized starch nanoparticles: an in vitro study. *Journal of Cluster Science*, vol. 31, no. 6, pp. 1257-1266. http://dx.doi.org/10.1007/s10876-019-01732-3.

WEI, Y., LI, P., LI, B., GAO, J., WANG, D., QIN, L., SUN, W., XU, Y., SHI, H., XU, T. and LIU, T., 2017. Study of the hypoglycemic activity of derivatives of isoflavones from Cicer arietinum L. *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, pp. 8746823. http://dx.doi.org/10.1155/2017/8746823. PMid:28421123.

YIN, Z., ZHANG, W., FENG, F., ZHANG, Y. and KANG, W., 2014. α-Glucosidase inhibitors isolated from medicinal plants. *Food Science and Human Wellness*, vol. 3, no. 3-4, pp. 136-174. http://dx.doi.org/10.1016/j.fshw.2014.11.003.

YUSUFOGLU, H., SOLIMAN, G.A., ABDEL-RAHM, R.F., ABDEL-KADE, M.S., GANAIE, M.A., BEDIR, E., BAYKAN, S. and OZTURK, B., 2015. Antihyperglycemic and antihyperlipidemic effects of Ferula duranii in experimental type 2 diabetic rats. *International Journal of Pharmacology*, vol. 11, no. 6, pp. 532-541. http://dx.doi.org/10.3923/ijp.2015.532.541.

ZAIN-UL-ABIDIN, S., KHAN, R., AHMAD, M., BHATTI, M.Z., ZAFAR, M., SAEED, A. and KHAN, N., 2018. Ethnobotanical survey of highly effective medicinal plants and phytotherapies to treat diabetes mellitus II in South-West Pakistan. *Indian Journal of Traditional Knowledge*, vol. 17, no. 4, pp. 682-690.

ZEB, A., KHAN, Y., YASEEN, T. and SHAH, S., 2020. Ethno botanical study of wild medicinal plants of Peerano Valley, District Malakand, Khyber Pakhtoonkhwa, Pakistan. *Asian Plant Research Journal*, vol. 6, no. 1, pp. 34-44. http://dx.doi.org/10.9734/aprj/2020/v6i130121.

ZEB, M., KHAN, S., RAHMAN, T., SAJID, M. and SELONI, S., 2017. Isolation and biological activity of β-Sitosterol and Stigmasterol from the roots of indigofera heterantha. *Pharmacy & Pharmacology International Journal*, vol. 5, pp. 139.