International Journal of Phytopharmacology
Research Article
www.onlineijp.com
e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

NOVEL *DIOSCOREA OPPOSTIFOLIA* TABLETS AND ITS ANTI DIABETIC POTENTIAL AGAINST STREPTOZOTOCIN-INDUCED DIABETIC RATS

Jhansi Rani M*, Hari Bhaskar V[1] Chandra Sekhar KB[2]

*Research Scholar, Ratnam Institute of Pharmacy, Nellore, Andhra Pradesh, India
[1]Dept. of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy, Nellore, Andhra Pradesh, India
[2]Director Foreign Affairs and Alumni matters Jawaharlal Nehru Technological University, Anantapuram, Andhra Pradesh, India.

ABSTRACT

Present study focused on development of herbal tablets using *Dioscorea oppostifolia* pure compounds and evaluate the anti diabetic activity of *Dioscorea oppostifolia* tablet formulation using streptozotocin induced diabetic rats. Isolate the pure compounds from *Dioscorea oppostifolia* root using column chromatography. Pure compounds were used for herbal tablet formulation by direct compression method and performed quality control tests. Developed formulation was used to evaluate anti diabetic activity on streptozotocin and alloxan-induced diabetic rats. Stigmasterol and ursolic acid were isolated from the roots of *Dioscorea oppostifolia* and confirmed by analytical data. Developed herbal tablet formulation was found in an acceptable range of thickness, hardness, weight variation and friability. DSC study revealed stability of the drug in the presence of excipients. *Dioscorea oppostifolia* tablet showed significant decrease in blood glucose and improved antioxidant activity on diabetic rats. Anti diabetic activity of tablet formulation due to the sterol and triterpenoid nature of Stigmasterol, Ursolic acid. Developed *Dioscorea oppostifolia* tablets exhibited unique anti-diabetic activity against streptozotocin and alloxan induced diabetic rats.

Key words: *Dioscorea oppostifolia*, *Dioscorea oppostifolia* tablets, Anti diabetic, Antioxidant, Stigmasterol, Ursolic acid.

Corresponding Author: **Jhansi Rani M** Email: jhansirani0008@gmail.com

INTRODUCTION

Dioscorea oppostifolia a medicinal and edible plant locally known as belonging to the family Dioscoreaceae, A deciduous perennial creeping and twinning vine reaching 3–4 m high with large, elongated sparsely hairy roots. Stem slender, terete, glabrous, purplish, clockwise (left to right) bearing bulbils in the leaf axils. Leaves alternate in basal stem, opposite above the middle, rarely 3 in a whorl, on long petioles, simple, entire margin, broad ovate-triangular, with cordate base, and acuminate apex, 7–9 nerved 3–9 cm long, 2–7 cm wide. Flowers are small, white (greenish-yellow), 4 mm, unisexual with a cinnamon fragrance in paniculate or spicate inflorescences (Hitchcock CL and Cronquist A, 2018). *Dioscorea oppostifolia* has been highlighted the use of its roots, stems and leaves as Traditional Medicine for the treatment of numerous ailments diabetes, diarrhoea and premature ejaculation (Hu SY, 2005). The root has been eaten for the treatment of poor appetite, chronic diarrhoea, asthma, dry coughs, frequent or uncontrollable urination, diabetes and emotional instability (Tu M, 2002). The leaves, flowers, tender shoots and roots of *Dioscorea oppositifolia* are used for cooling and demulcent; they are used in the form of decoction for

Access this article online		
DOI: http://onlineijp.com/ DOI: http://dx.doi.org/10.21276/ijp.2020.11.1.4	Quick Response code	
Received:16.09.19	**Revised**:22.10.19	**Accepted**:20.11.19

leprosy and cancerous lesions (Felix R *et al*., 2009). The root is chewed to cure toothache and aphthae.

The chemical composition and efficacy of extracts from genus *Pavetta* were intensively investigated in previous studies. The plant *D. oppostifolia* has been reported to contain several phytoconstituents including 6,7-dihydroxy-2-methoxy-1,4- phenanthrenedione, and four known compounds, chrysoeriol 4′- *O* -β- D - glucopyranoside, chrysoeriol 7- *O* -β- D - glucopyranoside, alternanthin and daucosterol(5), 3,5-dihydroxy-4-methoxybibenzyl; 3,3′,5-trihydroxy-2′-methoxybibenzyl; 10,11-dihydro-dibenz[*b*,*f*]oxepin-2,4-diol, 10,11-dihydro-4-methoxy-dibenz[*b* , *f*]oxepin- 2- ol (Yang MH *et al*., 2009), A phenanthrene glycoside, 3,4,6-trihydroxyphenanthrene-3-O-β- D -glucopyranoside, and five known compounds, soyacerebroside I, adenosine, β-sitosterol, palmitic acid and palmitoyloleoylphosphatidylcholine (Sautour M *et al*., 2004).

Various pharmacological activities has been reported so far i.e., antioxidant (Nagai T e*t al*., 2006), anti-inflammatory (Zheng KY *et al*., 2014), anticancer (Chan YS and Ng TB, 2013; Zhao G *et al*., 2003), ant diabetic (Gao X *et al*., 2007), neuroprotective (Zhang L *et al*., 2011), gastroprotective (Jeon JR *et al*., 2006), anti-hyperlipidaemic (Shujun W *et al*., 2008), antihypertensive (Nagai T and Nagashima T, 2006) and antimutagenic (Okabe Y *et al*., 1996).

As the drug is endowed with colossal exploitation and utilization value, it is medicinally vital to know precisely and comprehensively about its characteristics of pharmacognosy. Here in we made a detailed investigation on pharmacognostic, phytochemical, physicochemical, fluorescence analysis and thin layer chromatography of this plant to help in its identification and standardization and research was performed to be able to analyze the therapeutic potential of the plant with regards to its formulation, evaluation and pharmacological activity.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh plants of *Dioscorea oppostifolia* (DS) was collected from Tirumala, Tirupati, Andhra Pradesh, identified and authenticated by Prof. K. MadhavaChetty, Plant Taxonomist (IAAT:357), Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Preparation of sample

Transverse section

T.S of root of *D. oppostifolia* was made manually, that have been immobilized in FAA solution (formalin: glacial acetic acid: 70% ethyl alcohol; [5:5:90] for macro-and microscopic observations (Johansen DA, 1940; Shah G *et al*., 2013; Gokhale MS and Kokate C, 2008; Raaman N, 2006).

Powders

The powder was separately processed with glycerine and chloral hydrate through the heating for microscopic and macroscopic investigation.

Preparation of various extracts

The root of *D. oppostifolia* was collected, cleaned; dried in the shade at room temperature and chopped into small pieces. Dried pieces of roots and whole plant were powdered and then filled in the airtight bottle. The coarse powders of both plant materials subjected to maceration for 24 hr sequentially with solvents based on polarity, i.e., petroleum ether, chloroform, ethyl acetate, n-butanol, methanol, and water. Thereafter, the extracts were concentrated by using rotary vacuum evaporator (50 ^{o}C).

Isolation Of Major Phytoconstituents

Isolation of PC-01

Petroleum ether extract (PEE) of *D. oppostifolia* was subjected to silica-gel (100–200 mesh) column (length 100 cm and diameter 3 cm) chromatography and eluted with Petroleum ether and ethyl acetate in different proportions. At a proportion of Pet. ether: Ethyl acetate (92:8), a white crystalline compounds separates out. That isolated compound responds positive with Libermann burchard and Salkowaski test, which has a steroid moiety.

Isolation of PC-02

Ethyl acetate extract was dissolved in alcohol. Reflux for ten minutes and cooled to room temperature. Then adsorbed with silica and run the column with Pet. Ether and ethyl acetate and at a ratio of 60:40, where a white, needle like crystals were obtained. Repeated crystallization from methanol was done.

Phytochemical Analysis

Various extracts of *D. oppostifolia* had been subjected to qualitative chemical evaluation of various primary and secondary phytoconstituents according to methods of Khandelwal.

Physicochemical parameters analysis

The physicochemical parameters analysis of the powders, including ash value, moisture content, extractive values, fluorescence, and TLC analysis were determined as per the standard guidelines (Khandelwal KR, 2008; WHO, 2011; Kokate C, 1994).

Fluorescence analysis

Fluorescence evaluation was carried out by ultrasonic processing the powder drug with different reagents and then detected at 254 nm, 366 nm within an

ultraviolet chamber and visible light (Khandelwal KR, 2008; WHO, 2011; Kokate C, 1994).

Formulation of Herbal Tablet

PC-01 (Stigmasterol) and PC-02 (Ursolic Acid) obtained from the fractionation of herbal plant *Dioscorea oppostifolia* are used as it is. Lactose Monohydrate and Micro Crystalline Cellulose were used as diluents, Sodium Starch Glycollate as super disintegrant, Magnesium stearate as lubricant, talc as Anti adherent. The formula is given in Table No.1.

Preparation Method of Herbal Tablet

Herbal tablets of *Dioscorea oppositifolia* were prepared by utilizing direct compression method. Pure compound-1 & 2, Micro Crystalline Cellulose, Lactose Monohydrate, Sodium Starch Glycollate were weighed as per the formula given in Table 1 and are passed through sieve no.40. Then all ingredients were mixed following geometric dilution technique for 15min. Later talc and magnesium stearate are passed through sieve No.100 and are mixed to the above powder blend. The final powder blend is compressed into 100mg tablets using rotary tablet compression machine (Cadmach, Ahmedabad).

Evaluation of Herbal Tablets

Pre-compressional studies of powder blend

In development of dosage forms pre-formulation study is the initial step in the formulation development.

It is the principal investigation in the formulation development to obtain information on the properties of compounds. So, this pre-formulation investigation may merely confirm that there are no significant barriers to formulation development. Following pre-compressional parameters were studied like angle of repose, bulk density, tapped density, compressibility indices (Puri D *et al*., 2018; Pal AK *et al*., 2014).

Post-compressional studies of prepared herbal tablets

The herbal tablets were evaluated for various parameters after consideration of preformulation to overcome errors during formulation preparation. These are like appearance, thickness, weight variation, hardness and friability, Disintegartion Time (Puri D *et al*., 2018; Pal AK *et al*., 2014).

Differential Scanning Calorimetry Study

DSC for pure drugs and polymers were performed individually and in formulation using DSC Q20 from TA instruments. Approximately, 2 mg of the sample was placed in the aluminium pan and sealed with the lid. And the sealed aluminium pan is heated from room 35 to 350° C at a heating rate of 10°C /min under nitrogen atmosphere. DSC data were analyzed using the TA Universal analysis software, (TA Instruments, Inc.) (Hani U *et al*., 2016).

Experimental design for study anti diabetic activity on STZ induced diabetic rats

Diabetes mellitus was induced in male wistar rats by a single injection of Streptozotocin (STZ 40 mg/kg, i.p.) after overnight starvation. Three days after the injection, fast blood glucose level was measured using a glucometer (Onetouch, Johnson & Johnson, New Jersey, USA) by tail vein blood sampling. Six rats with blood glucose level >200 mg/dl were consider as diabetic rats and selected in this study.

Rats were randomly divided into four groups (n = 6): diabetic rats treated with vehicle (normal saline, p.o.) and

Group I: Normal rats (NC)
Group II: Diabetic rats (DC)
Group III: Metformin (100 mg/kg B.wt.)
Group III: Formulation (20 mg/kg B.wt.)

Experimental design for study anti diabetic activity on alloxan induced diabetic rat

Diabetes mellitus was induced in male wistar rats by a single injection of alloxan (100 mg/kg, i.p.) after overnight starvation. Three days after the injection, fast blood glucose level was measured using a glucometer (Onetouch, Johnson & Johnson, New Jersey, USA) by tail vein blood sampling. Six rats with blood glucose level >200 mg/dl were consider as diabetic rats and selected in this study (El-Missiry M, El Gindy A, 2000).

Rats were randomly divided into four groups (n = 6): diabetic rats treated with vehicle (normal saline, p.o.) and

Group I: Normal rats (NC)
Group II: Diabetic rats (DC)
Group III: Metformin (100 mg/kg B.wt.)
Group III: Formulation (20 mg/kg B.wt.)

Blood was collected from tail vein for estimation of glucose levels using Accu check active meter. The treatment was continued for 6 weeks from 2^{nd} to 8^{th} week. At the end of 8 weeks rats were euthanized, isolated pancreas for estimation of antioxidant activity using following methods.

Pancreatic antioxidant enzyme activity

For biochemical assays using pancreatic tissue, 10 % homogenate of the pancreas was prepared in 0.1M Tris-HCl (pH 7.4). Briefly, the tissue was rinsed in normal saline, weighed and homogenized in 0.1M Tris-HCl buffer (pH 7.4). The tissue homogenate was centrifuged at 1,000 x g for 20 minutes, at 4^{o}C, and the supernatant collected for assessment of antioxidant enzymes activities, total glutathione (GSH) level (Ellman GL, 1959), catalase (CAT) (Abei H, 1963).

Statistical analysis

Graph Pad prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) was used to analyze the data obtained in this study. One-way analysis of variance (ANOVA)

was employed, followed by TUKEY test. Data are presented as mean ±standard deviation (SEM) and values of $p<0.05$ were considered significant.

RESULTS

Root

The transverse section of the root of *D .oppostifolia* showed the presence of epidermal cells shows thick walled parenchymatous cells filled with brown content; followed by tangentially elongated parenchymatous cells. The endodermis showed the presence of phloem and xylem. Radially arranged vascular bundles were present in which, Phloem is well developed and shows the presence of phloem fibers, which are non-lignified. It also showed the presence of phloem parenchyma. The xylem region was similar to phloem region and was also surrounded by uniseriate to triseriate medullary rays. Xylem tissue consists of spiral xylem vessels, xylem fibers and xylem parenchyma (Fig. 1 and 2).

Powder Microscopy

The powder microscopy of root revealed the presence of Cork Cells, Xylem Fibres, Xylem vessels and Parenchyma cells, which displayed in figure 3.

Phytochemical Analysis

Qualitative phytochemical examination of successive extracts with petroleum ether, chloroform, ethyl acetate, methanol, n-butanol and water extracts of *D. oppostifolia* root powder was carried out, and outcomes are shown in below table 2.

Characterization of isolated compounds from D. oppostifolia

PC-01

IR Spectrum: IR (KBr) $\nu_{max}cm^{-1}$: 3418 (-OH strech), 2934 (C-H strech in CH_2 and CH_3), 2866 (=C-H strech), 2339, 1602 (C=C assymetricstrech), 1566, 1461 (C-H deformation in gem dimethyl), 1409, 1383, 1251, 1191, 1154, 1109, 1089, 1053 (Cycloalkane), 1020, 791. **^{1}H NMR Data (400 MHz, $CDCl_3$)** δ : 7.25 (1H, s, OH-2), 5.34-5.35 (1H, d), 5.12-5.18 (1H, m), 4.99-5.05 (1H, m), 3.48-3.56 (1H, m), 2.18-2.31 (2H, m), 1.93-2.09 (3H, m), 1.82-1.87 (2H, m), 1.66-1.75 (1H, m), 1.37-1.54 (13H, m), 1.05-1.31 (m, 7H), 0.99-1.01 (m, 8H), 0.90-0.98 (m, 2H), 0.78-0.85 (m, 9H), 0.66-0.70 (3H, t), which are characteristics for stigmasterol.**^{13}C NMR Data (400 MHz, $CDCl_3$)** δ: 140.85 (C-5), 138.31 (C-22), 129.40 (C-23), 121.72 (C-6), 77.34 (C-3), 71.86 (C-14), 56.95 (C-17), 56.09 (C-24), 51.29 (C-9), 50.29 (C-13), 42.41 (C-4), 42.30 (C-20), 40.46 (C-12), 39.77 (C-10), 37.35 (C-1), 36.59 (C-7), 32 (C-8), 31.96 (C-2), 31.91 (C-27), 31.77 (C-16), 28.91 (C-15), 25.41 (C-28), 24.41 (C-29), 21.24 (C-19), 21.14 (C-11), 21.06 (C-21), 19.42 (C-18), 19.03 (C-25), 12.23 (C-26), which are characteristic for stigmasterol. **Mass Spectrum:** m/z: 409.2, 395.3, 335, 161, 144, 121.1, 105.1, 97.1, 85.1, 69, 67.2, 65, 50.2. The above spectral data (MASS, NMR) is analyzed for molecular formula $C_{29}H_{48}O$ and is similar with that of stigmasterol (Fig. 4).

PC-02

IR Spectrum: IR (KBr) $\nu_{max}cm^{-1}$: 3450 (-OH strech), 2925 (-CH strech), 2869, 2339, 1556 (-C=O, carbonyl), 1456 (-CH bend), 1387, 1247, 1157 (C-O strech), 822.**^{1}H NMR Data (400 MHz, $CDCl_3$)** δ: 11.91 (1H, s), 5.14 (1H, s), 4.27 (1H, s), 3.01 (1H, s), 2.51 (1H, s), 2.10-2.13 (1H, d) 1.85-1.93 (4H, t), 1.26-1.32 (4H, t), 1.05 (1H, s), 0.91-0.92 (8H, d), 0.88 (1H, s) 0.82-0.83 (4H, d), 0.76 (3H, s), 0.69 (4H,s); **^{13}C NMR Data (400 MHz, $CDCl_3$)** δ: 178.16 (C-29), 138.17 (C-13), 124.58 (C-12), 76.86 (C-3), 56.01 (C-5), 54.82 (C-18), 52.40 (C-14), 47.05 (C-9), 46.82 (C-17), 41.64 (C-8), 40.41 (C-4), 40.21 (C-19), 40 (C-1), 39.79 (C-10), 39.58 (C-22), 39.37 (C-7), 39.16 (C-21), 38.96 (C-2), 38.49 (C-15), 38.46 (C-16), 38.36 (C-24), 38.28 (C-23), 36.53 (C-11), 36.31 (C-30), 32.73 (C-8), 30.2 (C-28), 28.24 (C-25), 27.55 (C-26), 26.99 (C-15). PC-02 was identified as ursolic acid; **Mass Spectrum:** m/z: 455.2 (M^+), 456.2, 457.3

The above spectral data (MASS, NMR) is analyzed for molecular formula $C_{30}H_{48}O_3$ and is similar to that of ursolic acid (Fig. 5).

Physicochemical analysis

The physicochemical parameters of root powder of *D. oppostifolia* had been determined and tabulated in table 3.

Fluorescence analysis

Fluorescence analysis of powder and different extracts of *D. oppostifolia* with different reagents were carried out to observe the color reactions (table 4).

Pre- compression studies of powder blend

The powder blend was evaluated for various Pre compression parameters and their results are shown in Table 6. The evaluation parameters such as angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio were found to be 21.12±0.11(θ), 0.4649±0.12g/ml, 0.4262±0.08g/ml, 12.19±0.14, 1.14±0.16 respectively. After evaluation of pre-formulation parameters it showed that there is no presence of moisture in powder and showed uniformity of powder blend. After study of flow rate it is concluded that powder blend exhibits maximum flow rate (Table 5).

Post-compression study of powder blend

The result from different physical parameters like thickness, hardness, weight variation, and friability of tablets was shown in table 7. The presence of active pharmaceutical ingredients, filler, glidant and lubricant is sufficient for provided bulk to the tablet which decrease

risk during punching. The thickness, hardness, weight variation, and friability of herbal tablet were founded to be in acceptable limit. It shows that the herbal drugs containing herbal tablets have satisfactory disintegration profile due to their hardness within range of standard limit. The values are presented in table 6.

Physical appearance

The general appearance of tablet was found to be round in shape, white in color, smooth texture.

Thickness

The thickness of herbal tablet was found to be 3.00± 0.1cm. It depends upon the size of die and punches or a function of die fill and compression force. The

Weight variation

The weight of 20 tablets was measured and it was found to be 1.996±0.004 g . All the herbal tablets passed weight variation test as the average percentage weight variation was within the USP limits of ±5%.

Hardness

The hardness of herbal tablet was found to be 5.2±0.21kg/cm^2. It depends upon the compression force of punching machine and showed that it is sufficient for tolerating mechanical stress.

Friability

Friability of formulation was found to be 0.15±0.02 %.The friability herbal tablet was found to be in acceptable limit i.e. less than 1%. There is no capping problem in the tablet.

Disintegartion Time: The Disintegration time of the formulation was found to be 2.2±0.5 Minutes.

Differential Scanning Calorimetry Study

DSC for pure drugs and polymers were performed individually and in formulation using DSC Q20 from TA instruments. Approximately, 2 mg of the sample was placed in the aluminum pan and sealed with the lid. And the sealed aluminum pan is heated from room 35 to 350° C at a heating rate of 10°C /min under nitrogen atmosphere. DSC data were analyzed using the TA Universal analysis software (TA Instruments, Inc.)

The pure compound 1 exhibited a sharp melting point at 140.98°C and the pure compound 2 exhibited sharp melting point at 312.85°C. MCC exhibited no sharp melting point. Lactose monohydrate exhibited two melting points at 152.30°C and 212.39°C. Talc exhibited flat melting endothermic graph. SSG exhibited broad endothermic curve at 75°C and Exothermic curve at 300°C which correspond to adsorbed moisture and degradation at high temperature respectively. Magnesium stearate exhibited a small endothermic curve at 87.97°C and another melting curve at 118.71°C. The DSC thermogram of formulation exhibited a diminished melting point of pure compound 1 and 2 at 150.23°C and 305.94°C which indicates stability of the drug in the presence of excipients (Fig. 6).

Hypoglycemic activity

To determine the effect of our interventions on glycemic state of diabetic rats, we found that the significant increase ($p<0.001^{***}$) in all the STZ and alloxantreated rats compared to normal rats at the end of 6th week of the study. Metformin treated rats shows the blood glucose levels were significantly ($p<0.001^{***}$) decreased in STZ and alloxan induced diabetic rats, similarly Formulation treated rats blood glucose levels were significantly ($p<0.01^{**}$, p<0.001***) decreased in STZ and alloxan induced diabetics rats, respectively.

At the end treatment, Pancreatic homogenate antioxidants such as reduced glutathione and catalase activity were significantly ($p<0.001^{***}$) decreased in STZ and alloxan treated rats compared to normal rats. GSH and catalase activity was significantly ($p<0.01^{**}$) increased in metformin treated rats, similar finding was shown in formulation in STZ induced diabetes rats.

GSH and catalase activity was significantly ($p<0.01^{**}$) increased in metformin treated rats, similar finding was shown in formulation (p<0.001***) in alloxan induced diabetes rats (Figure 6-11 and Table 7-10).

Table 2: Phytochemical analysis of Roots of *D. oppostifolia*

Phytoconstituents	Method	Pet. ether Extract	Chloroform Extract	Ethyl acetate Extract	Methanolic Extract	n-butanol Extract	Aqueous Extract
Flavonoids	Shinoda Test	-	-	+	+	-	+
	Zn+HCl test	-	-	+	+	-	+
	Lead acetate Test	-	-	+	+	-	+
Volatile oil	Stain test	-	-	-	+	-	+
Alkaloids	Wagner Test	-	+	-	+	-	+
	Hager's Test	-	+	-	+	-	+
Tannins & Phenols	$Fecl_3$ Test	-	-	+	+	-	+
	Potassium dichromate test	-	+	+	+	-	+
Saponins	Foam Test	-	-	-	+	+	+
Phytosterols	Libermann's test	+	+	-	+	-	-
Carbohydrates	Molish test	-	-	-	+	-	-

Acid compounds	**Litmus test**	-	-	-	-	-	-
Glycoside	**Borntragers test**	-	-	-	+	-	+
Amino acids	**Ninhydrin test**	-	-	-	+	-	+
Proteins	**Biuret test**	-	-	-	+	-	+
Fixed oils & fats	**Spot test**	+	-	-	+	-	+

+ : Present and - : Absent

Table 3. Physicochemical analysis of *D. oppostifolia*

Parameters	**%w/w Values**
Moisture content (Loss on drying)	8.52 ± 1.32
Total ash	7.98 ± 0.63
Acid insoluble ash	3.26 ± 0.25
Water soluble ash	2.25 ± 0.12
Petroleum ether soluble extractive value	1.38 ± 0.15
Chloroform soluble extractive value	2.08 ± 0.12
Ethyl acetate soluble extractive value	5.23 ± 0.28
Alcohol soluble extractive value	9.83 ± 1.86
Water soluble extractive value	13.68 ± 0.22

Table 4. Fluorescence analysis of *Dioscorea oppostifolia*

Solvent used	**Visible light**	**UV light**	
		254nm	**366nm**
Distilled water	Buff	Dark Brown	Black
1 N NaOH	Buff	Brown	Pale yellow
1N Hcl	Dark Brown	Buff	Dark brown
50% HNO_3	Pale brown	Black	Reddish brown
$FeCl_3$	Yellowish brown	Blue	Dark blue
Picric acid	Brownish yellow	Brown	Golden Yellow

Table 5. Pre compression studies of *Dioscorea oppostifolia* Herbal Tablets

Pre-Compression Parameters	Formulation
Angle of repose (θ)	21.12±0.11
Bulk Density (g/ml)	0.4649±0.12
Tapped Density (g/ml)	0.4262±0.08
Carr's Index	12.19±0.14
Hausner's Ratio	1.14±0.16

Table 6. Post- compression studies of *Dioscorea oppostifolia* Herbal Tablets

Post- Compression Parameters	Formulation
Thickness (mm)	3.00±0.1
Hardness (kg/cm2)	5.2±0.2
% Weight Variation	99.8±0.2
% Friability	0.15±0.02
Disintegartion Time (min)	2.2±0.5

Table 7. Effect of formulation on blood glucose levels in STZ induced diabetic rats

	Blood glucose (mg/dl)			
Treatment	**0 day**	**2 week**	**4 week**	**6 week**
Normal control (NC)	98.5±3.84	101.83±4.19	102±2.93	108.6±3.09
Diabetic control (DC)	258±12.14	269.33±11.87	284.3±10.39	295.5±9.05***
Metformin (100 mg/kg B.wt.)	266.2±9.18	207.7±3.68	167.8±4.19	128.7±2.22***
Formulation (20 mg/kg B.wt.)	260.2±3.54	234.0±3.16	195.3±4.45	168.7±4.84 **(35.17%)

Values are mean ± SEM, n = 6. *** p<0.001 DC Vs NC; *** p<0.001 Metformin Vs NC; ** p<0.01 Formulation Vs DC.

Table 8. Effect of formulation on blood glucose levels in alloxan induced diabetic rats

Treatment	Blood glucose levels (mg/dl)			
	0 day	2 weeks	4 weeks	6 weeks
Normal control (NC)	98±3.8	101±4	102±3	108±4
Diabetic control (DC)	158±12	178±9	217±12	239±10***
Metformin (100 mg/kg B.wt.)	152±9	142±12	139±12	128±9***
Formulation (20 mg/kg B.wt.)	161±10	164±7	148±8	127±9 ***(21.1%)

Values are mean ± SEM, n = 6. [a]*** p<0.001 DC Vs NC; *** p<0.001 Metformin Vs NC; *** p<0.001 Formulation Vs DC.

Table 9. Effect of formulation on stress parameters in STZ Induced diabetic rats

Treatment	GSH level (nmolGSHEq./mg protein)	CAT activity (unit/mg protein)
Normal control (NC)	3.21±0.8	45.67±3.5
Diabetic control (DC)	0.86±0.02***	12.6±1.2***
Metformin (100 mg/kg B.wt.)	2.8±0.08**	34.5±2.1**
Formulation (20 mg/kg B.wt.)	2.1±0.06**	29.8±1.9**

Values are mean ± SEM, n = 6. *** p<0.001 DC Vs NC; ** p<0.001 Metformin Vs NC; Formulation Vs DC.

Table 10. Effect of formulation on stress parameters in alloxan Induced diabetic rats

Treatment	GSH level (nmolGSHEq./mg protein)	CAT activity (unit/mg protein)
Normal control (NC)	2.82± 0.76	46.8±3.5
Diabetic control (DC)	1.9± 0.01***	25.7±2.8***
Metformin (100 mg/kg B.wt.)	1.43± 0.12**	34.5±3.1**
Formulation (20 mg/kg B.wt.)	1.64± 0.23**	32.9±2.7***

Values are mean ± SEM, n = 6. *** p<0.001 DC Vs NC;
Significant change in GSH levels in between ** p<0.001 Metformin Vs NC; Formulation Vs DC.
Significant change inCAT levels in between ** p<0.001 Metformin Vs NC; ***p<0.001 Formulation Vs DC.

Fig 1. Transverse Section of *D. oppostifolia* root. RH: Root Hairs; Epi: Epiderims; Par: Parenchyma; CX: Cortex; XV: Xylem Vessels; XP: Xylem Parenchyma.

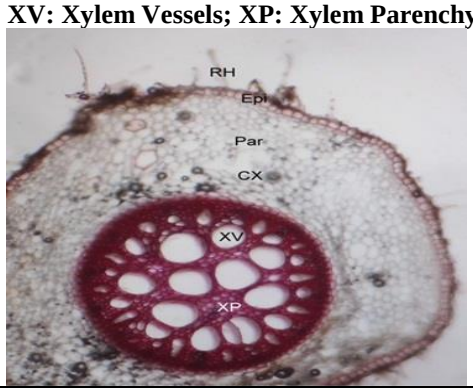


Fig 2. Root hairs, Epidermis and Ground Tissue of *D. oppostifolia*Root (b) Vascular region of *D. oppostifolia*root at 40X.

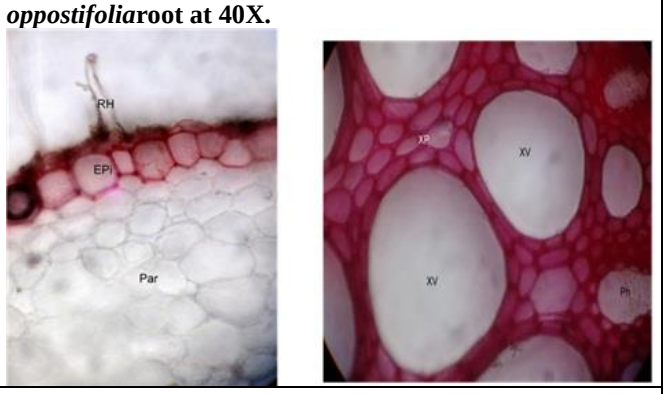


Fig 3. Powder Microscopy of *D. oppostifolia*Roots. *CC: Cork Cells; XF: Xylem Fibres; Xv: Xylem vessels;Par: Parenchyma cells;*

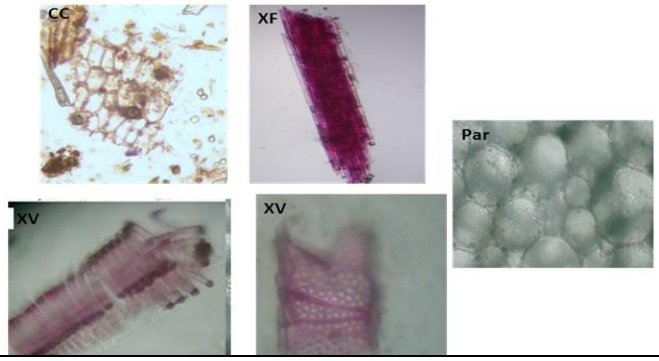


Fig 4. Structure of stigmasterol

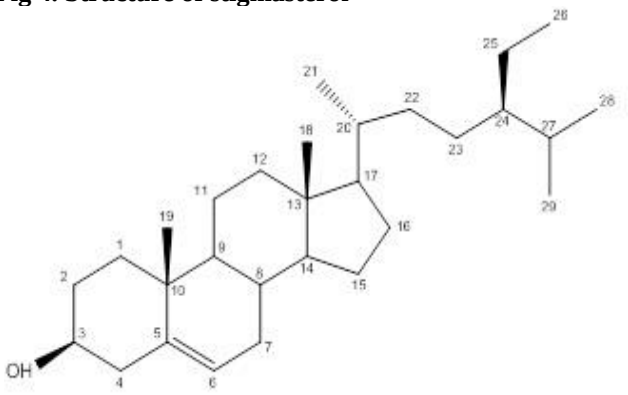


Fig 5. Structure of ursolic acid (PC-02)

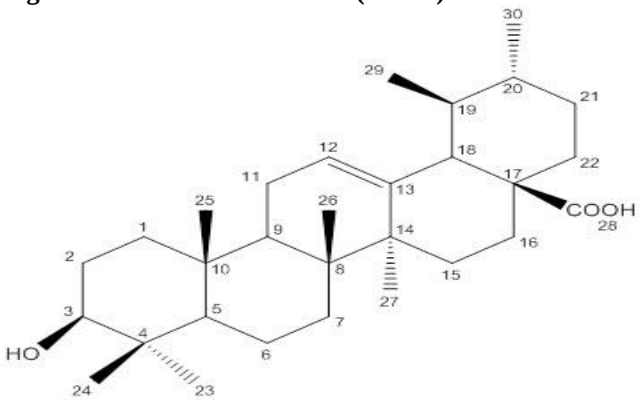


Fig 6. DSC thermograms PC1 (Stigmasterol), PC2(Ursolic Acid), MCC(Micro Crysatlline Cellulose, Lactose, Talc, SSG (Sodium Starch Glycollate), Magnesium Stearate, Formulation (Tablet)

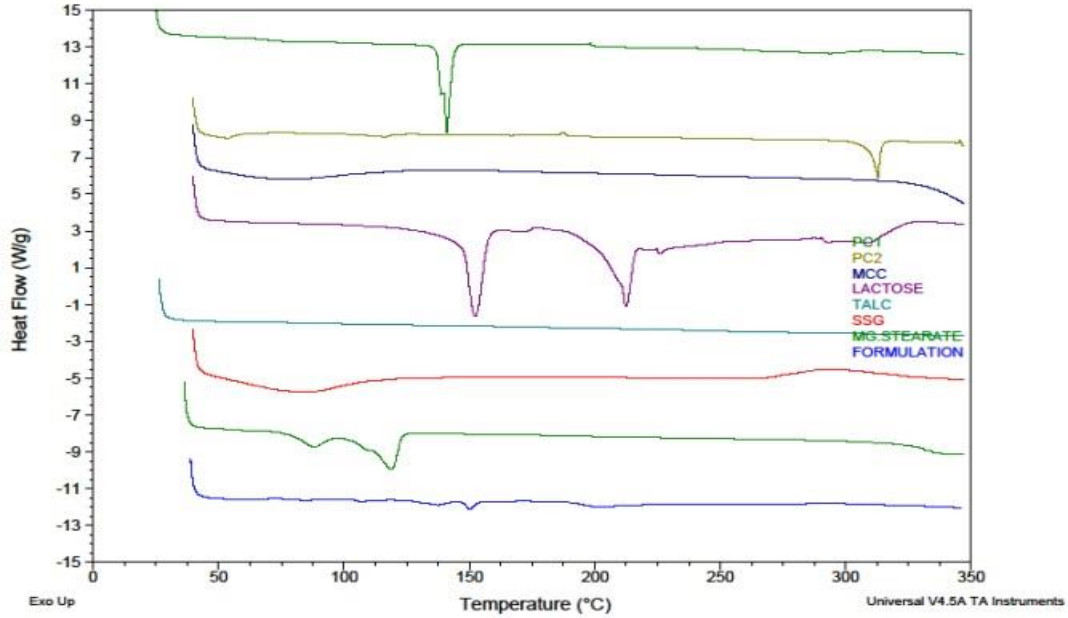


Fig 6. Effect of formulation on glucosein STZ diabetic rats

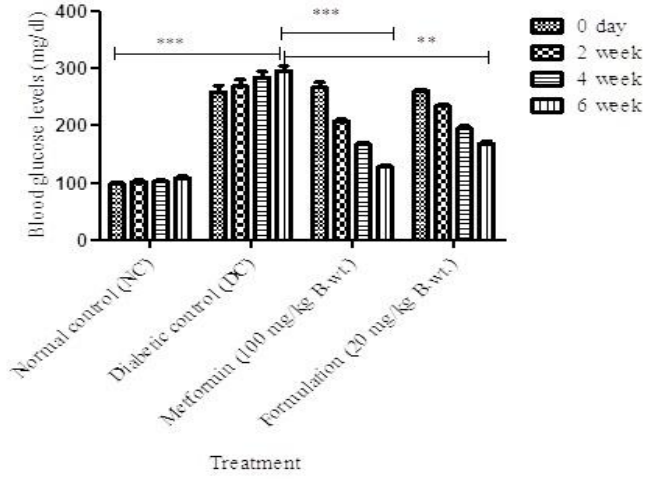


Fig 7. Effect of formulation on GSH level in STZ diabetic rats

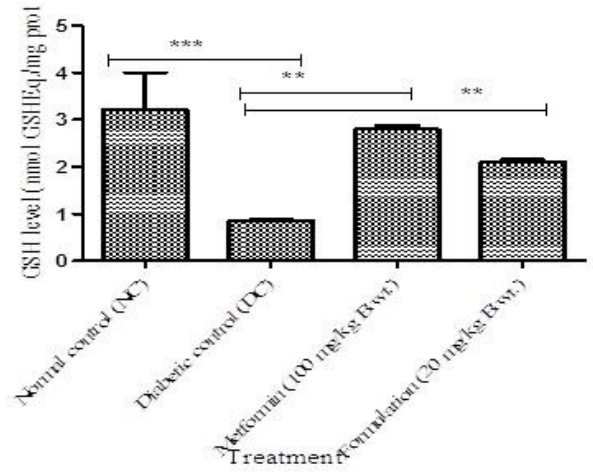


Fig 8. Effect of formulation on CAT activity in STZ diabetic rats

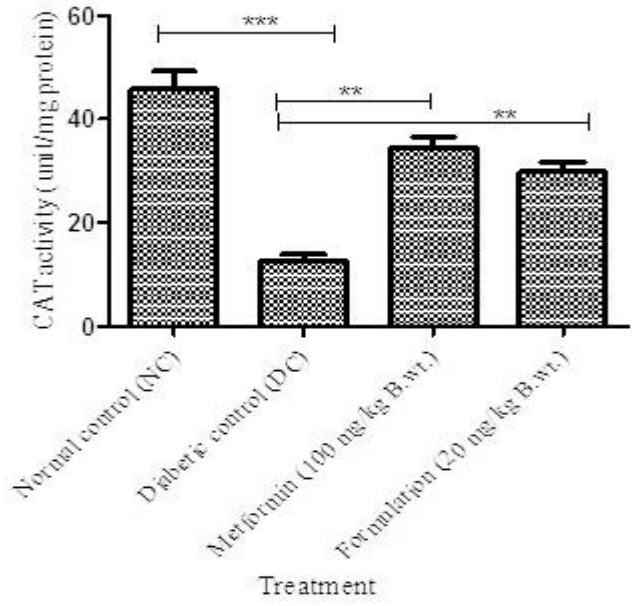


Fig 9. Effect of formulation on glucose in alloxan diabetic rats

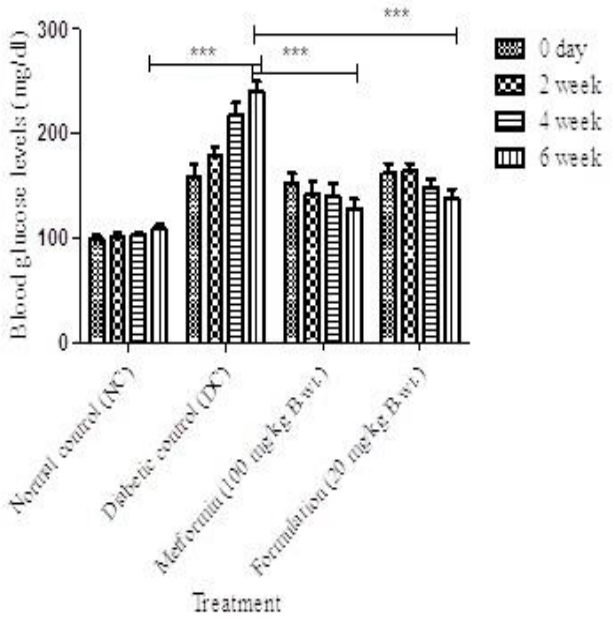


Fig 10. Effect of formulation on GSH level in alloxan diabetic rats

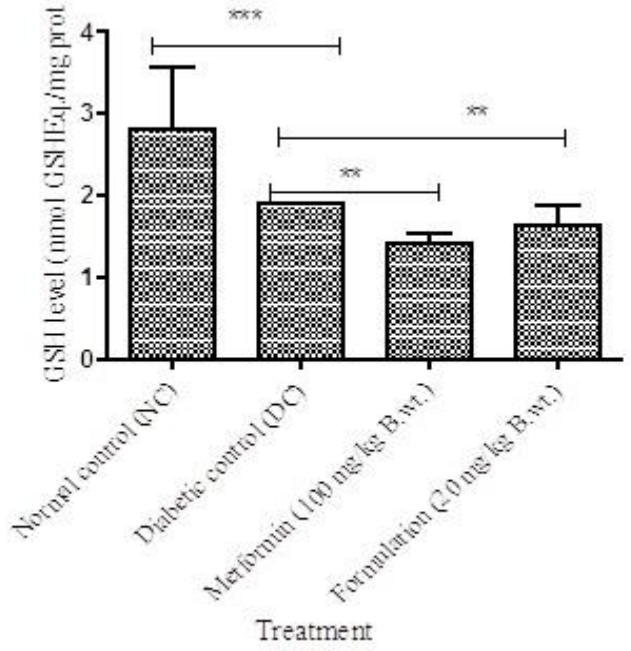
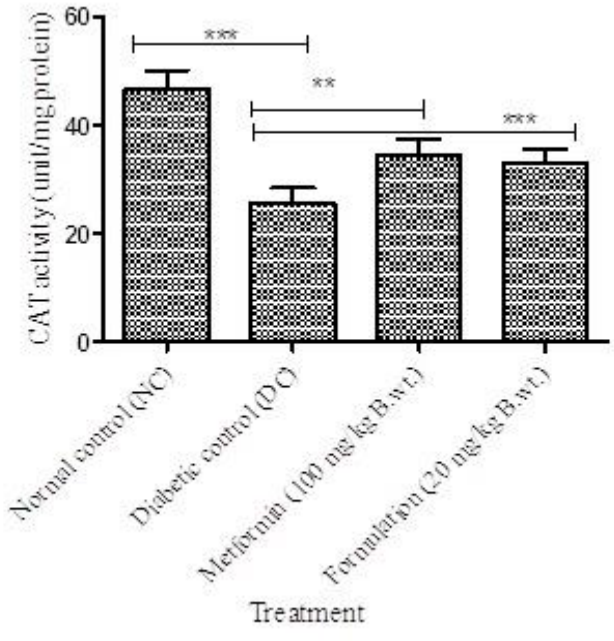


Fig 11. Effect of formulation on catalase level in alloxan diabetic rats



DISCUSSION

The standardization of natural drugs is of crucial significance in building their appropriate identification, likewise performs an essential part in understanding its composition, biology, botanical quality, and medical effectiveness because of the often obtaining of alternative or counterfeit natural supplies available in the market (32). The evaluation of macroscopic and microscopic characters, chemical and physical variables and genetic details would be the confirmatory assessments concerning standardization. Thus, it is recommended to research pharmacognostical characters of a medicinal plant. Nevertheless, the herb *D. oppostifolia* is not yet having much-confirmed evidence for its standardization in the

contemporary approach to medication regardless of its extensive applications amongst locals for the treating numerous illness, as seldom pharmacognostical or anatomical analysis is on record to rationalize their authenticity. Taking into consideration all these information, authors made an effort to finger out pharmacognostical characteristics which can be useful in building the identification and then standardization of the herb.

The research offered some fundamental details about the authentic crude drug. The macroscopic characteristics consisting of taste, sight, smell, and touch had been noticed to provide an initial indication regarding the level of quality variance. The distinguished diagnostic attributes of microscopy may be regarded as differentiating feature for identifying the anatomical structures and establishing the proper identity of the herb. Fluorescence evaluation is a required variable that may be unique to the herb and shows the indication of the chromophore in the drug, which can be needed in its first line standardization (Prasanth D *et al*., 2016). A few herbal constituents display fluorescence in the ultraviolet or visible light when they might often be changed into fluorescent derivatives by making use of diverse regents, which can be very useful to distinguish from dubious specimens as fast approaches.

The physicochemical tests were carried out (Table 2). The extractive values are undoubtedly an essential component to physicochemical evaluation, and these types of quantitative values will be determined for further standardization of the flower powder. They are indicative weights of active ingredients from the crude drug. High extractive values show the desirable extraction of phytochemicals from the herb materials. These values additionally promote the choice of best solvent that will even more help in having maximum yield. The ash values for the powdered plant will be computed to determine the quantity of siliceous material remaining in the residue and to determine the extraneous elements adhered to the herb while it's gathering. A herb which has not gone through garbling procedure generally reveals greatest ash values, and it is made as of low quality. However, loss on drying shows them relating to the moisture content within the dried or gathered herb material, as the existence of unwanted moisture when compared to mentioned, contributes to microbe degradation. The presence of extreme moisture is additionally among the prime cause of the deterioration of the constituents within the herb since it results in the hydrolysis of the constituents (Chanda S, 2014). Lower levels of moisture assists with the prevention of microbe contamination (Madhav NS *et al*., 2011). Loss on drying is calculated to estimate the moisture content (Gong X *et al*., 2014).

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels resulting due to deficiency in insulin release, insulin action, or both. Many mechanisms are involved in anti diabetic and hypoglycemic activity. One of these might involve the modulating insulin secretion or insulin action, or related with extrapancreatic and pancreatic effects (Stanely P *et al*., 2000). Other mechanisms may involve an antioxidant system (Maroo J *et al*., 2003) or may include enhancement of cell glucose metabolism or activation of enzyme system via generation of cyclic AMP or phospholipid derived messenger (Hawley *et al*., 2002), Streptozotocin (STZ) 69%, alloxan (31%) was most frequently used as diabetic inducers and this model has been useful for screening the anti diabetic activity (Frode T and Medeiros Y, 2008). Streptozotocin enters the pancreatic cell via a glucose transporter GLUT2 causes deoxyribonucleic acid (DNA) alkylation and also activation of poly adenosine diphosphate ribosylation and nitric oxide release, resulting pancreatic cells are destroyed by necrosis (Mythili MD *et al*., 2004).

Present study was reported that the administration of single dose of STZ (4 mg/kg.) and alloxan (100 mg/kg) was produced significant hyperglycemia in normal rats and hyperglycemia was significantly ameliorated by treatment of formulation activity was compared with standard drug glibenclamide. Glibenclamide was regulated hyperglycemia by increased releasing of insulin from pancreatic beta cells (Gregorio F *et al*., 1989). STZ and alloxan induced diabetes is associated with increased oxidative stress resulting in diminished enzymatic levels such as catalase and nonenzymatic antioxidants such as reduced glutathione. Hyperglycemia is a known cause for elevation of free radical levels, results increased lipid peroxidation (Balasubashini MS *et al*., 2004) decreased levels of GSH, catalase. The restoration of antioxidative defensive system was significantly improved by the treatment of formulation in diabetic rats, due to presence of sterols and triterpinoids in formulation. number of scientific studies are indicated that certain sterols, triterpenoids have protective effect against pancreatic cell damage of due to its antioxidant properties (Alqasoumi SI and Abdel-Kader MS, 2012).

CONCLUSION

Generally, information resulted in this research might be beneficial to additional progress *D. oppostifolia*, which might become reference information and create a solid basis for appropriate recognition, authentication, collection and analysis of the herb material. Even more, it can be useful to retrieve the interest of pharmacologist to explore this herb in the line of scientific study.

From the above study, we conclude that the herbal tablets were prepared by direct compression method and gave satisfactory and acceptable result. The exhibiting antioxidant and anti diabetic activity of formulation could be due to the existing of sterols and terpenoids compounds.

AKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

Nil

REFERENCES

Alqasoumi SI, Abdel-Kader MS. Terpenoids from *Juniperus procera* with hepatoprotective activity. *Pakistan Journal of Pharmaceutical Sciences*, 25(2), 2012.

Abei H. Methods of enzymatic analysis: Bergmeyer Ed: catalase. New York, Academic Press, 1963.

Akbar S, Hanif U, Ali J, Ishtiaq S. Pharmacognostic studies of stem, roots and leaves of *Malva parviflora* L. *Asian Pacific journal of tropical biomedicine*, 4(5), 2014, 410-415.

Balasubashini MS, Rukkumani R, Viswanathan P, Menon VP. Ferulic acid alleviates lipid peroxidation in diabetic rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 18(4), 2004, 310-314.

Chan YS, Ng TB. A lectin with highly potent inhibitory activity toward breast cancer cells from edible tubers of Dioscorea opposita cv. nagaimo. *PloS one*, 8(1), 2013, e54212.

Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. *Journal of Pharmacognosy and Phytochemistry*, 2(5), 2014.

El-Missiry M, El Gindy A. Amelioration of alloxan induced diabetes mellitus and oxidative stress in rats by oil of *Eruca sativa* seeds. *Annals of Nutrition and Metabolism*, 44(3), 2000, 97-100.

Ellman GL. Tissue sulfhydryl groups. *Archives of biochemistry and biophysics*, 82(1), 1959, 70-77.

Felix R, Kumar NN, Raj T. Pharmacognostical Study of Dioscorea oppositifolia L. *Ethnobotanical Leaflets*, 9(1), 2009, 10.

Gokhale MS, Kokate C. Practical pharmacognosy: Editora Record, 2008.

Gong X, Li Y, Qu H. Removing tannins from medicinal plant extracts using an alkaline ethanol precipitation process: a case study of Danshen injection. *Molecules*, 19(11), 2014, 18705-18720.

Fröde T, Medeiros Y. Animal models to test drugs with potential antidiabetic activity. *Journal of Ethnopharmacology*, 115(2), 2008, 173-183.

Jeon JR, Lee JS, Lee CH, Kim JY, Kim SD, Nam DH. Effect of ethanol extract of dried Chinese yam (*Dioscorea batatas*) flour containing dioscin on gastrointestinal function in rat model. *Archives of pharmacal research*, 29(5), 2006, 348-353.

Hawley SA, Gadalla AE, Olsen GS, Hardie DG. The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. *Diabetes*, 51(8), 2002, 2420-2425.

Metformin potentiates B-cell response to high glucose: an *in vitro* study on isolated perfused pancreas from normal rats. *Diabete & metabolisme*, 15(3), 1989, 111-117.

Shujun W, Jinglin Y, Hongyan L, Weiping C. Characterisation and preliminary lipid-lowering evaluation of starch from Chinese yam. *Food Chemistry*, 108(1), 2008, 176-181.

Nagai T, Nagashima T. Functional properties of dioscorin, a soluble viscous protein from Japanese yam (*Dioscorea opposita* thunb.) tuber mucilage Tororo. *Zeitschrift für Naturforschung C*, 61(11-12), 2006, 792-798.

Okabe Y, Shinmoto H, Tsushida T, Tokuda S. Antimutagenicity of the extracts from four root crops on the Trp-p 2-induced mutagenicity to Salmonella typhimurium TA 98. *Journal of the Japanese Society for Food Science and Technology*, 43(1), 1996, 36-39.

Johansen DA. Plant microtechnique, 1940.

Kokate C. Practical Pharmacognosy, VallabhPrakashan, New Delhi, 1, 1994, 15-30.

Shah G, Chawla A, Baghel US, Rahar S, Singh PS, Dhawan RK. Pharmacognostic standardization of leaves of *Melaleuca leucadendron*. *Pharmacognosy Journal*, 5(4), 2013, 143-148.

Raaman N. Phytochemical techniques: New India Publishing, 2006.

Khandelwal KR. Practical pharmacognosy : techniques and experiments. Maharashtra: Niral Prakashan, 2008.

Hitchcock CL, Cronquist A. Flora of the Pacific Northwest: an illustrated manual: University of Washington Press, 2018.

Hu SY. Food plants of China. Hong Kong: The Chinese University of Hong Kong; 2005.

Tu M. Element Stewardship Abstract for Dioscorea oppositifolia L. syn. *Dioscorea batatas.* 2002.

Ma C, Wang W, Chen Y-Y, Liu R-N, Wang R-F, Du L-J. Neuroprotective and Antioxidant Activity of Compounds from the Aerial Parts of *Dioscorea o pposita. Journal of natural products*, 68(8), 2005, 1259-1261.

Sautour M, Mitaine-Offer A-C, Miyamoto T, Wagner H, Lacaille-Dubois M-A. A new phenanthrene glycoside and other constituents from *Dioscorea opposita*. *Chemical and pharmaceutical bulletin*, 52(10), 2004, 1235-1237.

Nagai T, Suzuki N, Nagashima T. Antioxidative activity of water extracts from the yam (*Dioscorea opposita* Thunb.) tuber mucilage tororo. *European journal of lipid science and technology*, 108(6), 2006, 526-531.

Gao X, Li B, Jiang H, Liu F, Xu D, Liu Z. Dioscorea opposita reverses dexamethasone induced insulin resistance. *Fitoterapia*, 78(1), 2007, 12-5.

Puri D, Bhandari A, Gaur PK, Yasir M, Kumar SS, Choudhary D, Saxena PK. Formulation of Herbal Fast Disintegrating Tablets and its Ex-vivo Study for Anti-histaminic Activity in Guinea Pig Ileum. *Current clinical pharmacology*, 13(2), 2018, 128-135.

Pal AK, Nagaich U, Bharti C, Gulati N. Formulation and evaluation of nutraceutical tablet using herbal drurs by direct compression method. *Journal of Drug Delivery and Therapeutics*, 4(2), 2014, 47-51.

Hani U, Shivakumar H, Osmani RAM, Srivastava A, Varma NSK. Development of a curcumin bioadhesive monolithic tablet for treatment of vaginal candidiasis. *Iranian journal of pharmaceutical research: IJPR*, 15(1), 2016, 23.

Prasanth D, Rao AS, Yejella RP. Assessment of pharmacognostic, phytochemical and physicochemical standards of *Aralia racemosa* (L.) root. *Ind J Pharm Edu Res*, 50(3), 2016, S225-S30.

Madhav NS, Upadhyaya K, Bisht A. Phytochemical screening and standardization of polyherbal formulation for dyslipidemia. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(3), 2011, 235-238.

Khan PMA, Farooqui M. Analytical Applications of Plant Extract as Natural pH Indicator: A Review. *Journal of Advanced Scientific Research*, 2(4), 2011.

Stanely P, Prince M, Menon VP. Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats. *Journal of ethnopharmacology*, 70(1), 2000, 9-15.

Maroo J, Ghosh A, Mathur R, Vasu VT, Gupta S. Antidiabetic efficacy of Enicostemma littorale methanol extract in alloxan-induced diabetic rats. *Pharmaceutical biology*, 41(5), 2003, 388-391.

Mythili MD, Vyas R, Akila G, Gunasekaran S. Effect of streptozotocin on the ultrastructure of rat pancreatic islets. *Microscopy research and technique*, 63(5), 2004, 274-81.

World Health O. Quality control methods for herbal materials Geneva: World Health Organization; 2011. Available from: http://whqlibdoc.who.int/publications/2011/9789241500739_eng.pdf.

Yang MH, Yoon KD, Chin Y-W, Park JH, Kim SH, Kim YC, et al. Neuroprotective effects of Dioscorea opposita on scopolamine-induced memory impairment in *in vivo* behavioral tests and in vitro assays. *Journal of ethnopharmacology*, 121(1), 2009, 130-4.

Yang MH, Yoon KD, Chin Y-W, Park JH, Kim J. Phenolic compounds with radical scavenging and cyclooxygenase-2 (COX-2) inhibitory activities from *Dioscorea opposita*. *Bioorganic & medicinal chemistry*, 17(7), 2009, 2689-2694.

Zhao G, Li Z, Chen Z. Structural analysis and antitumor activity of RDPS-I polysaccharide from Chinese yam. *Acta Pharmaceutica Sinica*, 38(1), 2003, 37-41.

Zhang L, Bai B, Liu X, Wang Y, Li M, Zhao D. α-glucosidase inhibitors from Chinese Yam (*Dioscorea opposita* Thunb.). *Food chemistry*, 126(1), 2011, 203-206.

Zheng KY-Z, Zhang Z-X, Zhou W, Cao H, Xiang F-J. New phenanthrene glycosides from *Dioscorea opposita*. *Journal of Asian natural products research*, 16(2), 2014, 148-152.