ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 11, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

IN VITRO EVALUATION OF ANTIDIABETIC AND CYTOTOXICITY POTENTIALS OF THE RHIZOME EXTRACT OF *DRYNARIA QUERCIFOLIA* (L.) J. SMITH

PRASANNA G*, DEVI R, ISHWARYA G

Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Thiruvarur, Tamil Nadu, India.
Email: gprasannakeerthi@gmail.com

Received: 27 August 2019, Revised and Accepted: 16 September 2019

ABSTRACT

Objective: In the present study, an attempt has been made to evaluate the *in vitro* antidiabetic and cytotoxic potentials of the rhizome extract of *Drynaria quercifolia* (L.) J. Smith.

Methods: *In vitro* antidiabetic activity was determined by two different assays such as alpha-amylase inhibition assay and glucose uptake assay. The plant extract with three different concentrations was used for this assay. L6 rat myogenic cells were selected and subjected to glucose uptake assay. The cytotoxic activity of the different concentrations of the plant extract on HepG2 cell line was also investigated *in vitro* through 3-(4,5, dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Results: The findings of the study provide evidence that the rhizome extract of *D. quercifolia* possesses significant anti-diabetic activity. In MTT assay, the significant cytotoxic effect of plant extract was observed by measuring the percentage of cell viability on the HepG2 cell line.

Conclusion: The findings indicated that rhizome extracts of *D. quercifolia* have potential as a medicinal drug against diabetes mellitus (DM) and liver cancer. Further, studies with *in vivo* and clinical trials need to be conducted to establish rhizome extract as a safe agent for DM and liver cancer therapy.

Keywords: Anticancer, Antidiabetic, Cytotoxicity, *Drynaria quercifolia,* HepG2, L6 rat myogenic cells, 3-(4,5, dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, Rhizome extract.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i11.35078

INTRODUCTION

Diabetes mellitus (DM) is one of the metabolic and endocrinological disorders [1-3] which is a resulting from an irregularity in insulin secretions and insulin actions or both. Absence or reduced insulin secretion leading to persistence of high glucose level and glucose intolerance [4]. Diabetes is generally accepted as a major challenging health problem all over the world and especially in the developing countries. 3.5 million deaths occur globally by diabetes [5]. Insulin therapy, oral hypoglycemic agents, restricted diet, exercises either singly or in combination constitute a major regimen of therapy available for the present day diabetics. Although in modern medicine, there is no satisfactory effective therapy available to cure Diabetes Mellitus. In a many numbers of cases, treatment with traditional medicine in the form of plant extracts has been reported to give remarkable good results [6]. Plant and plant products played an important role in the treatment of DM and had been used throughout the world since ancient times. Folk and traditional medicine healing system has been used globally as an antioxidant, antihyperglycemic, and antidiabetic properties from plants [7]. Moreover, since the herbal products are coming from biological origin, they can subside the limitations arising from the use of conventional hypoglycemic.

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, and it may cause death worldwide. According to a recent estimate, about 782,000 people were diagnosed with liver cancer, out of which 746,000 people died [8]. A total of 30,640 new liver and intra hepatic bile duct cancer were estimated in 2013 in addition to the 21,670 deaths [9]. The efficacies of chemotherapy, radiotherapy, hormonal therapy, or surgery, which are mainly used for the treatment of cancer, are well-known for side effects [10]. Hence, the identification of novel natural products that possess better effectiveness against cancer, but less harmful effects have become desirable [11], and therefore, natural products are continuously being explored worldwide. Keeping the above states in mind, present study has been designed to assess the antidiabetic and cytotoxicity potentials of the rhizome extract of medicinal fern, *Drynaria quercifolia* L (J.) Smith by *in vitro* assays.

Drynaria, commonly known as basket ferns, is a genus of ferns in the family of *Polypodiaceae*. It contains around 16 species. They are found in wet tropical environments, usually in rainforests [12]. *D. quercifolia* contains various phytochemicals such as phenols, tannins, alkaloids, proteins, xanthoproteins, carboxylic acid, coumarins, saponins, catechin, flavonoids, steroids, and triterpenes [13]. Phytochemicals such as friedelin, epifriedelinol, beta-amyrin, beta-sitosterol, beta-sitosterol 3-beta-D-glucopyranoside, 3, 4 di hydroxyl benzoic acid, acetyl lupeol, aglycone naringenin, and flavones glycoside naringin were identified in dried rhizomes of *D. quercifolia*. Many pharmacological activities have been reported such as antimicrobial [14], antidermatophytic [15], antioxidant [16-18], hepatoprotective [19], anti-inflammatory and antipyretic [20], analgesic [21], and anti-ulcer [22] activities.

METHODS

Collection of plant material

The rhizomes of *D. quercifolia* (L.) J. Smith was collected from Kollimalai, Namakkal district, Tamil Nadu, India. The collected rhizomes were carefully baggage in polythene bags. These plant samples were authenticated by Dr. S. John Britto, Director, RAPINAT Herbarium, St. Joseph's College, Tiruchirappalli, and a voucher specimen was deposited in the Department of Biochemistry, S. T. E. T. Women's College, Mannargudi (Voucher No.:001).

Extract preparation

10 g of the powdered sample was extracted with 85% methanol using Soxhlet extraction method. The filtrate was condensed using rotary vacuum evaporator at 45°C and the extract was stored at 4°C until used.

Antidiabetic assay

L6 rat myogenic cells

L6 rat myogenic cells were cultured in hypotonic buffer (20 mM Tris-HCl, pH 8, 1 mM ethylenediaminetetraacetic acid, 0.2 mM EGTA, 50 mM NaF, 0.7 μg/ml pepstatin, 10 mM sodium orthovanadate, and 50 mM benzamidine, 0.5 μg/ml leupeptin, 4 μg/ml aprotinin, and 2 mM phenylmethylsulfonyl fluoride). The cell suspension was centrifuged at 20,000×g for 15 min and homogenate obtained. The supernatant was collected as the soluble fraction for further experiments.

Preparation of test solutions

The methanol extract of *D. quercifolia* was separately dissolved in distilled dimethyl sulfoxide (DMSO) and the volume was made up with medium supplemented with 2% inactivated fetal bovine serum (FBS) to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. From this stock solution, four different lower dilutions (5.0, 10.0, 20.0, and 40.0 μg/ml) were prepared and subjected to glucose uptake assay.

Alpha-amylase inhibition assay

The plant extract with three different concentrations (25, 50, and 100 μg/ml) were used for this assay. Plant extract a total of 500 μl and 0.02 M sodium phosphate buffer of 500 μl of pH 6.9 with 0.006 M sodium chloride containing α-amylase solution (0.5 mg/ml) were incubated for 10 min at 25°C. After pre-incubation, 500 μl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5 s intervals. This reaction mixture was then incubated for 10 min at 25°C. 1 ml of DNSA reagent was added to stop the reaction. After that, all the test tubes were incubated in a boiling water bath for 5 min and cooled at room temperature. Finally, this mixture was again diluted with 10 ml distilled water the absorbance was measured at 540 nm [23].

Glucose uptake assay

L6 rat myogenic cells were selected and seeded into 96-well plate with six wells left as blank wells and let growing to confluence; then, cells were fully differentiated in DMEM with 2% FBS for 5 days. Before tests, the medium was replaced by RPMI1640 (2 g/L glucose) supplemented with 0.2% BSA. The medium was removed after 2 h, and the same medium containing plant extract (5.0, 10, 20, and 40 μg/ml), metformin (0.01 mM) the standard, and DMSO was added to all wells in the absence or presence of insulin (1 μmol/L). Blank was also performed in the same manner. The glucose in the medium was determined by the glucose-oxidase method after 48 h treatment [24]. The amount of glucose uptake by muscle cells was calculated.

Cytotoxicity assay

HepG2 cell lines

Cell lines Hepatic carcinoma (HepG2 cell line) was used in this study and procured from National Centre for Cell Science, Pune, India. The liver cancer cell line HepG2 preserved in RPMI-1640 medium tissue culture flasks at 37°C under a humidified 5% CO_2 and 95% air.

Preparation of test solutions

The plant extract was separately dissolved in distilled DMSO. To obtain a stock solution of 1mg/ml concentration, the content was made up with 2% inactivated FBS and then sterilized by filtration. From this stock solution, five different lower dilutions (25, 50, 100, 200, and 400 μg/ml) were prepared.

3-(4,5, dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [25]

MTT was reduced by mitochondrial succinate dehydrogenase which can be calorimetrically measured in MTT assay. HepG2 cells were seeded at the density of 2×105 cells/well and were placed on into 6 well plates and treated with extract for 24 h. The cells were permitted to adhere for 24 h, and the growth medium (Minimal Essential Media [MEM]) removed using micropipette and the monolayer of cells washed twice with MEM without FBS to remove dead cells and excess FBS. 1 ml of medium (without FBS) containing different dilution of drugs wa added in respective wells; 200 μl of MTT (5mg/ml in PBS) were added to each well, and the cells incubated for a further 6-7 h in 5% CO_2 incubator. After removal of the medium, 1 ml of DMSO was added to each well. The effect of extracts on cell growth inhibition was assessed as percent cell viability, where vehicle-treated cells were taken as 100% viable. The cells were then exposed to the medium alone (as positive control). Concentrations of the plant extract ranging 25–400 μg/ml were used. The supernatant was removed and 50 μl of propyl alcohol was added and therefore the plates were gently agitated to solubilize the formed formazan. The MTT enters the cell and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product.

The cells are then solubilized with an organic solvent (e.g., isopropanol) and the released, solubilized formazan reagent. Since, if cells are in metabolically active, reduction of MTT can only occur and the level of activity is a measure of the viability of the cells. The cells were incubated with the extract for 24 h and 48 h and the cell mortality was checked. The plates were placed on a shaker for 15 min and the absorbance was read on an enzyme-linked immunosorbent assay reader at 570 nm.

Statistical analysis

Values are expressed as mean±standard deviation for three determinations. For statistical analysis of data, multiple comparisons were performed using one-way analysis of variance followed by the least significant difference test for *post hoc* analysis. Data were analyzed using SPSS (version 11). $p<0.001$ was considered to indicate statistical significance.

RESULTS AND DISCUSSION

DM is a leading metabolic disorder in worldwide and India is the capital for diabetes. Diabetes is a condition which is caused by defective synthesis or defective function of insulin, the body does not produce enough insulin or properly respond to insulin. Insulin is a hormone which is produced in β cells of the pancreas and it stimulates the body cells to absorb glucose from the blood. DM is one of the most common endocrine and metabolic disorders which cause various microvascular complications such as retinopathy, neuropathy and nephropathy, and macrovascular complications such as heart attack, stroke, and peripheral vascular diseases. [26]. Nearly 2.8% of the world's population affected by diabetes and it is expected to increase up to 5.4% in the year 2025 [27]. Various kinds of antidiabetic therapies such as sulfonylureas, biguanides, and glinides are available, but it causes various adverse effects. Hence, the researchers are investigating and try to find more effective and safer hypoglycemic agent without causing any side effects [28].

Nowadays, people are turning toward herbal remedies due to fewer side effects with comparing to the oral hypoglycemic agents for the treatment of DM [29]. It is estimated that more than a thousand plant species are being used as folk medicine for diabetes [30]. Biological actions of the plant products used as alternative medicines to treat diabetes are in relevance to their phytoconstituents. Phytocomponents such as flavonoids, phenolic compounds, coumarins, terpenoids, and other constituents help to reduce blood glucose levels [31].

D. quercifolia (L.) J. Smith belongs to *Polypodiaceae* is distributed widely in the evergreen forests of India. The rhizome is reported to be used by different cultural groups in India for the treatment of diarrhea, typhoid, cholera, chronic jaundice, fever, and headache, and skin diseases [32,33]. The whole plant is anthelmintic, expectorant, tonic and used in the treatment of chest and skin disease. With this background, the present study has been designed with an aim to justify traditional claims of using *D. quercifolia* as a source of antidiabetic agents with scientific tools. In the present study, we have selected two methods such as alpha-amylase inhibitory assay and glucose uptake

assay, to evaluate the antidiabetic activity of rhizome of *D. quercifolia*. Obtained results were recorded and discussed here.

One-way of controlling Type 2 DM (T2DM) is to limit intestinal carbohydrate digestion. Although the gastrointestinal tract does not play a major role in the pathogenesis of T2DM, modification of its physiological activities can be used to decrease post-prandial hyperglycemia through inhibition of the carbohydrate hydrolyzing enzymes, α-amylase, and α-glucosidase [34,35]. In our study, different concentrations (25, 50, and 100 µg/ml) of methanol extract of selected plant parts were subjected to alpha-amylase inhibition assay. Metformin was used as standard. Inhibition percentage of plant extract was found to be 40.1±2.6, 65.7±4.1, and 79.8±3.5 at concentration of 25, 50, and 100 mg/ml, respectively. For standard drug, it was 85.07±7.6 (Table 1).

In general, i*n vitro* antidiabetic assays help to assess the effects of compounds on the inhibition of the two key enzymes involved in carbohydrate metabolism, namely, α-amylase and α-glucosidase, which has been described by several authors [36,37]. Inhibition of these enzymes results in a delay in the degradation of starch and oligosaccharides, which decreases the absorption of glucose and consequently inhibits post-prandial hyperglycemia [38]. In humans and other mammals, the maintenance of plasma glucose concentration for the long term is one of the most important and closely regulated processes observed under a variety of dietary conditions [39].

In the present study, there was a dose-dependent, alpha-amylase inhibition was observed in plant extract-treated cells. That the concentration was increased, inhibition of activity of alpha-amylase was also significantly ($p<0.001$) increased. By comparing with standard, plant extract produced moderate inhibition. Metformin is one popular drug used in the treatment of DM. It causes a variety of side effects such as gastrointestinal upset, lactic acidosis, and loss of weight [40]. On the other hand, medicinal plants have much less side effects and are thus being preferred over synthetic drugs in our modern health-care system.

Adipose tissue, skeletal muscle, and the liver are the three major target tissues of insulin. T2DM is characterized by insulin resistance and an insulin secretion deficiency [41,42]. The latter result in a reduction of glucose uptake in adipose tissue and skeletal muscle, as well as it increases gluconeogenesis in the liver. In *in vitro* assay, cell lines, such as L6 (rat myoblasts), C2C12 (mouse myoblast), 3T3-L1 (mouse fibroblast), HepG2 (human hepatocarcinoma), and Chang liver cells (human liver) are commonly used to assess glucose uptake. Glucose uptake assays assess the ability of the experimental compounds to enhance glucose uptake in the major target tissues of insulin, represented by the cell lines. In the present study, insulin sensitization effect of selected plant extract was examined in L6 rat skeletal muscle cells through glucose uptake action to confirm the possible antidiabetic mechanism of plant extract.

In this assay, incubation of *D. quercifolia* extract (5.0, 10, 20, and 40 µg/ml), and metformin (0.01mM) in muscle cells in the presence of insulin (1µmol/L) showed significant ($p<0.001$) glucose uptake action when compared to vehicle control. In the presence of insulin, glucose uptake was 6.13±0.28, 6.23±0.19, 6.39±0.54, and 6.73±0.41 at a concentration of 5.0, 10, 20, and 40 µg/ml of plant extract, respectively. Similarly, in the absence of insulin, it was found to be 5.02±0.32, 4.98±0.27, 4.06±0.16, and 3.16±0.21. Standard metformin has the glucose consumption of 7.07±0.30 and 4.81±0.31 in the presence and absence of insulin, respectively (Table 2).

Two main important features observed in type 2 diabetes and they are peripheral insulin resistance and impaired insulin secretion from pancreatic β-cells. Liver, skeletal muscle, and adipose tissue are the peripheral tissues in which resistance of insulin generally occurred. Hyperglycemia and dyslipidemia are interlinked disorders in type 2 diabetic patients commonly cause the occurrence of cardiovascular diseases which mainly due to insulin resistance. Hyperlipidemia is one

Table 1: Alpha-amylase inhibition of *Drynaria quercifolia* rhizome

Treatment	Concentration (µg/ml)	% of inhibition
Control	0	0±0
Plant extract	25	40.1±2.6*
	50	65.7±4.11*
	100	79.8±3.5*
Metformin	0.1	85.07±7.6*

Values are expressed as mean±standard error of mean (n=3); values not sharing common superscript were statistically significant with from each other ($p<0.001$)

Table 2: Glucose uptake assay of *Drynaria quercifolia* rhizome on L6 rat muscle cells

Treatment	Concentration (µg/ml)	Glucose consumption (µg/ml)	
		Absence of insulin	Presence of insulin
Control	0	1.18±0.06[b]	5.67±0.50**
Plant extract	5.0	5.02±0.32[a]	6.13±0.28*
	10	4.98±0.27[a]	6.23±0.19*
	20	4.06±0.16[a]	6.39±0.54*
	40	3.14±0.21[a]	6.73±0.41*
Metformin	0.1 µM	4.81±0.31[a]	7.07±0.30*

Values are expressed as mean±standard error of mean (n=3); values not sharing common superscript were statistically significant with from each other ($p<0.001$)

of such common complication of diabetes, which is characterized by increase in serum total cholesterol, triglycerides, low-density lipoprotein (LDL), and very LDL [43]. Drugs such as metformin diminishes insulin resistance as well as effectively control hyperglycemia and ameliorate lipid metabolism in type 2 diabetes, hence it prevents diabetes-mediated cardiovascular complications [44]. This class of drugs has adverse effects such as lactic acidosis, gastrointestinal disturbance, liver toxicity, and cardiovascular risk [41]. Thus, for long-term treatment in type 2 diabetes, drugs which improve insulin sensitivity without adverse effects were reported to be useful.

In the present study, we examined glucose uptake action of rhizome extract of *D. quercifolia* in L6 rat skeletal muscle cells; the results revealed that standard drug metformin significantly ($p<0.001$) enhanced glucose uptake activity which was higher than plant extract in the presence and absence of insulin. Plant extract did not exhibit higher glucose uptake action like standard drug metformin in the absence and presence of insulin. However, results confirmed that plant extract enhanced glucose uptake activity in the presence of insulin than the absence of insulin when compared to vehicle control and also plant samples produced dose-dependent glucose uptake action. The plant drug effectively prevents hyperglycemia and thereby it controls lipid metabolism.

This data have given clear evidence that the selected plant part possibly acts to improve the glucose uptake in the skeletal muscle in the presence of insulin, and hence it has potential to reverse insulin resistance in type 2 diabetes. It also suggests that the plant extract has insulin-mimetic properties and could be a potential antidiabetic drug, which could alleviate hyperglycemia by enhancing glucose uptake.

MTT assay

Primary liver cancer (PLC) has two major forms which are of HCC and intrahepatic cholangiocarcinoma, accounting for approximately 90% and 5%, respectively [45]. HCC is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis. HCC is now the third leading cause of cancer deaths worldwide, with over 500,000 people affected. The induction of HCC is preceded by the occurrence of hepatocellular damage through reactive

Table 3: MTT assay of *Drynaria quercifolia* rhizome against Hep G2 cell lines

S. No.	Treatment	Conc. (µg/ml)	Absorbance at 570 nm	% cell viability
1	HepG2 cells untreated	-	0.28	100.0±7.5**
2	Plant extract treated	25	0.24	85.8±5.4*
3		50	0.22	76.7±6.9*
4		100	0.19	68.1±5.3*
5		200	0.17	58.3±4.9*
6		400	1.10	35.6±2.8*
7	Doxorubicin treated	100	0.07	24.3±105*

Values are expressed as mean±standard error of mean (n=3); Values not sharing common superscript were statistically different from each other (p<0.001). MTT: 3-(4,5, dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide

oxygen species (ROS) and the generation of chronic inflammation related to hepatic carcinogenesis [46].

Over the years, different approaches have been employed and are still in use, individually or in combination, in the treatment of cancer such as chemotherapy, radiotherapy, surgery, and immunotherapy. Chemotherapeutic agents are cytotoxic to other than cancer cells and proliferate them into cancer cells, including those localized in the gastrointestinal tract, hair, and bone marrow, thus eliciting the gastrointestinal side effects such as nausea and vomiting, alopecia, and myeloid suppression. Anticancer cells have limitations in their efficacy due to insolubility and instability, the low rate at which the tissue absorbs them, and tumor drug resistance [47]. In addition, antitumor drugs have also been associated with the development of secondary malignancy. All the drawbacks presently associated with available chemotherapeutic agents are the impetus for the search for newer, more efficacious, and better-tolerated drugs.

Plants have a long history of use in the treatment of cancer [48,49] and the interest in nature as a source of potential chemotherapeutic agents continues. Recently, researchers put steps forward at herbal plants for the discovery of new antiproliferative agents with safety and efficacy. Hence, in the present study, we evaluate *in vitro* cytotoxicity potential of medicinal plant *D. quercifolia* on HeoG2 cell line by MTT assay.

Different concentrations of 25, 50, 100, 200, and 400 µg/ml of rhizome extract of *D. quercifolia* were subjected to evaluate anticancer activity against HepG2 cell lines by MTT assay. Standard doxorubicin was also evaluated to compare the efficacy of anticancer activity of selected plant extract. Percentage of viability was found to be 85.8±5.4 at 25 µg/ml followed by 76.7±6.9 at 50 µg/ml, 68.1±5.3 at 100 µg/ml, 58.3±4.9 at 200 µg/ml, and 35.6±2.8 at 400 µg/ml (p<0.001). For standard drug, it was 24.3±1.5 µg/ml. The percentage viability was increased with decreasing concentration of test compounds (Table 3). Thus, cancer cell growth was inhibited maximum at high concentration of plant extract, but it was less than that of standard.

The MTT assay is a sensitive, quantitative, and reliable colorimetric assay that measures viability, proliferation, and activation of cells. The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate MTT into a dark blue formazan product that is insoluble in water. Viable cells are able to reduce the yellow MTT under tetrazolium ring cleavage to a water-insoluble purple-blue formation, which precipitates in the cellular cytosol and can be dissolved after cell lysis, whereas cells were dead following toxic damage, cannot transform MTT. This formation production is proportionate to the viable cell number and inversely proportional to the degree of cytotoxicity. In the present study, percentage of viability was increased when the concentration of plant extract was decreased. That is cell viability was increased at the concentration of 25 µg/ml then it was significantly decreased with increased concentration of extract.

Several reports described that the anticancer activity of the medicinal plants which may be due to the presence of antioxidants (namely, vitamins, carotene, enzymes, minerals, polysaccharides, polyphenols, flavonoids, lignins, and xanthones). In addition, it was reported that these plants may promote host resistance against infection by re-stabilizing body equilibrium and conditioning the body tissues [50]. Our result suggests that *D. quercifolia* may be a potential chemotherapeutic or a chemopreventive agent based on its ability to induce apoptosis in cancer cells with relatively low toxicity to normal cells.

CONCLUSION

From the present work, it was concluded that the selected medicinal fern *D. quercifolia* possesses remarkable pharmacological activities toward DM and HCC, which will claim their folk-lore uses as antidiabetic and cytotoxic agents. Further, studies on *in vivo* action and isolation of the principle bioactive constituent(s) are also needed.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Management, Sengamala Thayaar Educational Trust Women's College, Sundarakkottai, Mannargudi, Thiruvarur Dt., Tamil Nadu, India, for their support to carry out research work successfully.

AUTHORS' CONTRIBUTIONS

All the authors contributed equally to the paper.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

1. Kumar PJ. Clark M. Textbook of Clinical Medicine. London: Saunders; 2002. p. 1099-121.
2. Ahmed AM. History of diabetes mellitus. Saudi Med J 2002;23:373-8.
3. Ford ES, Li C, Zhao G. Prevalence and correlates of metabolic syndrome based on a harmonious definition among adults in the US. J Diabetes 2010;2:180-93.
4. Jahan S, Fariduddin M, Sultana N, Aktar Y, Hasan M. Predictors of post-partum persistence of glucose intolerance and its association with cardio-metabolic risk factors in gestational diabetes mellitus. J Diabetes Metab 2015;6:609.
5. Das A, McGuire PG, Monickaraj F. Novel pharmacotherapies in diabetic retinopathy: Current status and what's in the horizon? Indian J Ophthalmol 2016;64:4-13.
6. Ghosh S, Suryawanshi SA. Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. Indian J Exp Biol 2001;39:748-59.
7. Majid DG, Iqbal M. Antihyperglycemic and antioxidative effects of *Lygodium microphyllum* in alloxan induced diabetic rats. Int J Pharm Pharm Sci 2018;10:75-9.
8. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, *et al*. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359-86.
9. Crissien AM, Frenette C. Current management of hepatocellular carcinoma. Gastroenterol Hepatol (N Y) 2014;10:153-61.
10. Stopeck AT, Thompson PA. Breast Cancer Treatment and Management. WebMD Health Professional Network; 2012. Available from: http://www.emedicine.medscape.com/article/1947145-treatment.
11. Lachenmayer A, Alsinet C, Chang CY, Llovet JM. Molecular approaches to treatment of hepatocellular carcinoma. Dig Liver Dis 2010;42 Suppl 3:S264-72.
12. Cumming R, Sked J. *Drynaria* and *Platycerium*: Interesting fern genera. Soc Growing Aust Plants 2006;27:2011.
13. Prasanna G, Chitra M. Phytochemical screening and GC-MS analysis of *Drynaria quercifolia* rhizome. Am J Adv Drug Deliv 2015;3:72-8.
14. Prasanna G, Chitra M, Suvitha N. *In vitro* antimicrobial activity of *Drynaria quercifolia* L. rhizome. Asian J Biochem Pharm Res 2014;4:342-9.
15. Nejad BS, Deokule SS. Anti-dermatophytic activity of *Drynaria quercifolia* (L.) J. Smith, Jundishapur. J Microbiol 2009;2:25-30.
16. Mohanta MC, Ganguly A, Begum SM, Rahman A. Evaluation of anti-nociceptive properties of *Drynaria quercifolia* rhizome in Swiss-albino

mice. J Pharm Res 2014;8:41-4.
17. Prasanna G, Chitra M. *In vitro* antioxidant activity of *Drynaria quercifolia* L. Rhizome. Int J Pharm Sci Res 2015;6:3061-6.
18. Prasanna G, Anuradha R. Evaluation of *in vitro* antioxidant activity of rhizome extract of *Drynaria quercifolia* L. Int J ChemTech Res 2015;8:183-7.
19. Devika V, Prasanna G. *In vitro* hepatoprotective activity of *Drynaria quercifolia* L. Rhizome. World J Pharm Pharm Sci 2016;5:512-21.
20. Janaranjani B, Prasanna G, Chitra M. Anti-inflammatoryand antipyretic activities of *Drynaria quercifolia* rhizome in rats. Int J Pharm Sci Rev Res 2014;29:57-61.
21. Anuja GI, Latha PG, Suja SR, Shyamal S, Shine VJ, Sini S, *et al.* Anti-inflammatory and analgesic properties of *Drynaria quercifolia* (L.) J. Smith. J Ethnopharmacol 2010;132:456-60.
22. Soni DK, Mohan AJ, Mantena VR, Raju K. Antiulcer activity of ethanolic extract of *Drynaria quercifolia* Linn. Leaves. J Pharm Res 2012;5:117-9.
23. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426-8.
24. Sundarrajan T, Velmurugan V, Srimathi R. Phytochemical evaluation and *in vitro* antidiabetic activity of ethanolic extract of *Alternanthera ficodia* Linn. Res J Pharm Technol 2017;10:2981-3.
25. Masters RW. Animal Cell Culture, Cytotoxicity and Viability Assays. 3rd ed. Oxford University Press: Oxford; 2000.
26. Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and *in vitro* antioxidant potential of *Hybanthus enneaspermus* (Linn) F. Muell in streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed 2011;1:316-22.
27. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed 2012;2:320-30.
28. Saxena A, Vikram NK. Role of selected Indian plants in management of Type 2 diabetes: A review. J Altern Complement Med 2004;10:369-78.
29. Bhalodi M., Shukla Saluja AK. *In vitro* antioxidant activity of the flowers of *Ipomoea aquatica* forsk. Pharmacogn Mag 2008;4:220-6.
30. Bhandari MR, Anurakkun NJ, Hong G, Kawabata J. Alpha glucosidase and alpha amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw). Food Chem 2008;106:247-52.
31. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK, *et al.* Antidiabetic agents from medicinal plants. Curr Med Chem 2006;13:1203-18.
32. Viswanathan MB. Industries Meet Cum Seminar on Biodiversity and Information on Medicinal and Aromatic Plants. New Delhi: NISCOM; 1995. p. 60.
33. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research India; 1956. p. 57, 102, 139, 198, 256.
34. Lebovitz HE. Alpha-glucosidase inhibitors as agents in the treatment of diabetes. Diabetes Rev 1998;6:132-45.
35. Scott LJ, Spencer CM. Miglitol: A review of its therapeutic potential in Type 2 diabetes mellitus. Drugs 2000;59:521-49.
36. Ali H, Houghton PJ, Soumyanath A. Alpha-amylase inhibitory activity of some malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. J Ethnopharmacol 2006;107:449-55.
37. Conforti F, Statti G, Loizzo MR, Sacchetti G, Poli F, Menichini F, *et al. In vitro* antioxidant effect and inhibition of alpha-amylase of two varieties of *Amaranthus caudatus* seeds. Biol Pharm Bull 2005;28:1098-102.
38. Loizzo MR, Tundis R, Menichini F, Saab AM, Statti GA, Menichini F, *et al.* Cytotoxic activity of essential oils from labiatae and lauraceae families against *in vitro* human tumor models. Anticancer Res 2007;27:3293-9.
39. Srividya AR, Dhanabal P, Bavadia P, Vishnuvarthan VJ, Kumar MN. Antioxidant and antidiabetic activity of *Curcuma aromatica*. Int J Res Ayurveda Pharm 2012;3:401-5.
40. Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS. Systematic review of herbs and dietary supplements for glycemic control in diabetes. Diabetes Care 2003;26:1277-94.
41. Rang HP, Dale MM, Ritter JM, and Flower RJ. The Endocrine Pancreas and the Control of Blood Glucose in Rang and Dales Pharmacology. 6th ed. Edinburgh: Church Livingstone 2007. p. 397-409.
42. Nolte M. Pancreatic hormones and antidiabetic drugs. In: Katzung BG, Masters SB, Trevor AJ, editors. Basic and Clinical Pharmacology. 11th ed. New Delhi: McGraw-Hill Education; 2009. p. 727-51.
43. Ramesh C, Rani AP. *In vivo* and *in vitro* evaluation of *Tephrosia calophylla* for anti-diabetic properties. Int J Pharm Pharm Sci 2018;10:138-44.
44. Reaven G, Abbasi F, McLaughlin T. Obesity, insulin resistance, and cardiovascular disease. Recent Prog Horm Res 2004;59:207-23.
45. Kumar M, Zhao X, Wang XW. Molecular carcinogenesis of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: One step closer to personalized medicine? Cell Biosci 2011;1:5.
46. Tanaka H, Fujita N, Sugimoto R, Urawa N, Horiike S, Kobayashi Y, *et al.* Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. Br J Cancer 2008;98:580-6.
47. Rao MR, Adagale UR, Shetty A, Namjoshi P, Gaitonde P, Jain P. Cancer Immunotherapy; 2007. Available from: http://www.pharmainfo.net/reviews/cancer immunotherapy.
48. Cragg GM, Newman DJ. Antineoplastic agents from natural sources: Achievements and future directions. Expert Opin Investig Drugs 2000;9:2783-97.
49. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 2003;66:1022-37.
50. Govind P, Sahni YP. Phytotherapy of malnutritional cancers in animals. Int J Pharm Sci Rev Res 2011;8:14-20.