Evaluation of Antidiabetic and Antioxidant potential of *Nephrolepis unduranta* leaf extract in Streptozotocin Induced Diabetic Wistar Rats.

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ABSTRACT

Introduction:
Diabetes Mellitus (DM) is a disease associated with severe derangement of carbohydrates, protein and lipids metabolism. The growing global incidence of diabetes mellitus in adults as well as young people continues to challenge the health sector, especially the Type-2 DM. Management of DM via the orthodox means continues to struggle globally due to increasing human population, poverty and gene modifications from inter-racial marriages. In local settings, plants with purported antidiabetic characteristics have been employed over time in managing DM. This informed the desire to carry out this research to investigate the plant; *Nephrolepsis undulate*.

Materials and Methods:A Fresh leaves of *Nephrolepis undulate* (NU) collected washed air-dried and grounded and soaked in methanol for 48 hours and thereafter filtered using Whatman's No. 1 filtered paper. Extracts obtained were subjected to liquid-liquid fractionation using (Chloroform, Ethyl acetate, and n-hexane). The resultant fractions (stock solution) were allowed to air dry into a paste, stored in universal bottles and kept in the fridge to be used for the experiment. NU fractions were administered at a dose of 250mg/kg bw. 500mg of the drug for comparison (metformin) was dissolved in 10ml of distilled water and administered at a dose of 50mg/kgbw. DM was induced in the rats using STZ at a dose of 60mg/kgbw. At the end of the treatment period (21 days), the rats were sacrificed via cervical dislocation and samples of some tissues collected (blood, brain, pancreas, small intestine, liver and testes). The analyses were conducted using documented standard methods.

Results: Research findings from this study demonstrated significant reduction in serum glucose concentration following treatment with NU, a decrease in the elevated level of MDA and an increase in the depressed levels of SOD and Catalase.

Conclusion: NU possesses both anti-diabetic and antioxidant properties, and the probable mechanism of action could be its inhibitory effect on glucose hydrolyzing enzymes and its ability to facilitate cellular detoxification.

Key words: *Nephrolepis unduranta, Streptozotocin, Metformin, Anti-diabetic, Antioxidant.*

INTRODUCTION

Diabetes Mellitus (DM) is a disease associated with severe derangement of carbohydrates, protein and lipids metabolism[1,2,3]. It ranks high amongst chronic debilitating diseases marked by sustained hyperglycemia resulting from an anomaly in insulin secretion and/or insulin action[3].The growing global incidence of diabetes mellitus in adults as well as young people continues to challenge the health sector, especially the T2DM which constitutes most of the reported cases[2]. The World Health Organization (WHO) report estimated that more than 422 million people worldwide were diagnosed with diabetes in 2014 and this is likely to double by 2030, with India, China and United States predicted to having the largest number of affected individuals[2]. In order to survive, organisms most rely on defense mechanism that permit them to escape or repair oxidative damage of hydrogen peroxide (H_2O_2) resulting from metabolic disease like diabetes. One of such defense mechanism involves production of the enzyme catalase which facilitates cellular detoxication. As far back as the nineteenth century, John W. (1928) and George P. (1947) discovered that catalase neutralizes the bactericidal effects of hydrogen peroxide[4,5]. Its concentration in bacteria has been correlated with pathogenicity[6]. As part of the survival strategy in organisms, superoxide dismutase (SOD), a group of enzymes that catalyzes the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide are also synthesized to provide cellular defense against reactive oxygen species. Additionally is the biomonitoring of malondialdehyde (MDA) levels, which is indicative both in in-vivo and in-vitro studies of the extent of oxidative stress taking place within the organism[7]. It has been employed as a biomarker for various diseases, diabetes mellitus inclusive.

Many plants and their active chemical compounds have been employed locally in the management DM[8]. According to ethnobotanical information, more than 800 plants have been employed traditionally in the management of diabetes[9,10,11,12]. The management of diabetes without any side effects is still a challenge[13]; hence, plants continue to play an important role in the discovery of new compounds for the treatment of this disease[14]. Drugs derived from natural products have played a major role in the development of potent pharmaceutical agents for management of diabetes. Metformin, a frequently prescribed agent for the treatment of diabetes[13], was discovered from herbal product, ‘ galesine’ a plant-derived anti-diabetic agent which was isolated from *Galega officinalis*,[15,16]. Experimental and clinical trials provided the pharmacological and chemical basis for the subsequent discovery of metformin[15,17]. 1-deoxynojirimycin (DNJ), a potent α-glucosidase Inhibitor, was also isolated from the water extract of leaves of the mulberry tree (*Morus alba* L.)[14]. *Nephrolepis unduranta* (annual sword fern), belongs to the tracheobionta kingdom, a wood fern plant of the pteridophyta division. The part use for medicinal purposes is the leaves and the roots and it has demonstrated usefulness as an antimicrobial agent comparable to the commercial anti-microbial agents such as gentamicin and ketoconazole. It has also shown antiviral, anti-inflammatory, antitussive and antitumor properties[18,19]. Traditionally, the rhizome juice of *Nephrolepis unduranta* was used to cure stomach disorders as well as peptic ulcer[20], and in treatment of cuts and wounds [21]. However, not much literature on its anti-diabetic activities is available.

MATERIALS AND METHODS

Chemicals and Drugs

Sodium Citrates (BDH chemicals LTD Pools England). Streptozotocin (STZ) (Batch 1378) Aldrich Sigma Co.3050, Spruce Str, St. Louis Mo 63103 U.S.A Ethyl Acetate (1502 batch 1356o517) Gato Perez, 33-P.1. Masden Gsa 08181 sentmenat Spam. Metformin (Glucophage) Tablet. All the chemicals and drugs used were of analytical grade.

Plant Collection

Fresh leaves of *Nephrolepis unduranta* (annual swordfern) were collected from its growing habitant in Obinomba, Ukwuani LGA, of Delta State, Nigeria, identified and authenticated in Forestry Research Institute of Nigeria, Ibadan with a voucher specimen FHI 110387 allocated to it at the herbarium of National Research Centre, Ibadan, Nigeria.

Preparation of Extract

The extract of *Nephrolepis unduranta* was prepared by the method used by Ojieh et al. (2016). Fresh leaves of *Nephrolepis undulate* collected, washed, air-dried and grounded into a fine paste. Wet weight of the paste was measured with Mettler weighing balance instrument (S/N 754550, Zurich, Switzerland). The weight of 1500g of the paste was soaked in 6 litres of methanol for 48 hours. The mixture was filtered using Whatman’s No. 1 filtered paper. The extracts obtained were allowed to air dry into a

paste and stored in universal bottles and kept in the fridge to be used for the experiment.

Fractionation of Crude Methanolic Extract

The plant was fractionated by the method used by Ojieh et al. (2016) rude methanolic extracts of *N. unduranta* was fractionated using a serial liquid-liquid separation method (Chromatography). 200ml of the crude extract of *N. unduranta* leave filtrates was measured using a measuring cylinder into a separating funnel (500ml), held in place by the aid of a retort stand and clamp. 200ml of n-hexane was added to it, shaken properly and allowed to stand for 30 minutes. The mixture was then separated into two layers; one layer containing hexane soluble constituents of *N. unduranta* which was collected into a beaker, and the other layer comprising of non-hexane soluble filtrate. The resultant residue was left to stand in an open beaker in order for the n-hexane to evaporate. After drying, 200ml of the residue was measured into a separating funnel and 200ml of chloroform was added to it, agitated appropriately and allowed to stand for separation. The mixture parted in two layers, a chloroform soluble phase and non-chloroform soluble residue. The non-chloroform soluble phase was collected, left to stand for evaporation of the chloroform to take place, it was then poured into another separating funnel for the next extraction phase using Ethyl acetate; the mixture separated into two phases and collected as above. 200ml of the residue from ethyl acetate fractionation was allowed to air-dry. The filtrate was slowly evaporated to dryness in a water bath at 40-50 °C, yielding a semisolid substance with a percentage yield of ethyl acetate 9.35g; chloroform 8.64g and n-hexane 12.30g; this was stored in a refrigerator to prevent bacterial decomposition, contamination and possible loss of efficacy before use

Chemical and Drug Preparation

Citrate Buffer and Streptozotocin (STZ)

Two grams (2g) of Sodium Citrate was dissolved in 100ml of water to yield 2% of citrate buffer and 0.6g of streptozotocin (STZ) was dissolved in 10ml of citrate buffer to yield 60mg of stock solution (Diabetic agent).

Metformin Diamet Tablet

500mg of metformin (Glucophage) was dissolved in 10ml of distilled water, to give a solution of anti-diabetic drugs.

Animal Use

Thirty six (36) male Wistar rats weighing 150-200 g were used for this study. The animals were housed in the Animal facility of Department of Human Physiology, Faculty of Basic Medical Sciences, Delta State University, (DELSU), Abraka, Nigeria. The animals were randomly divided into experimental and control groups and were housed in metabolic cages. They were maintained on standard animal feeds growers mash diet and water ad libitum. The protocol for this study was examined and approved by the Ethical Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria and the experiment performed in accordance with the ethical standards laid down in the 1964 Helsinki declaration.

Induction of Diabetes Mellitus [Akbarzadeh et al. (2007)]

After 12 hours fast, the animals were weighed with the aid of an electronic weighing balance, and the fasting blood glucose level checked using a glucometer (Accu-Chek). The rats were then randomly selected into six groups of six rats each. Group one represents the positive control; hence diabetes was not induced and treatment given. Induction of diabetes was done on rats in Group two to Group six. 1ml of the resultant solution was injected intramuscularly into the animals through the lateral tail at a dose of 60mg/kg body weight and 72hours after induction, a 50% increase in pre-induction fasting blood glucose level was considered to be diabetic.

Animals Grouping and Treatment

Group 1 (n= 6) – Positive control, rats were not induced and not treated within the period of the study

Group 2 (n=6) – Negative control, diabetes was induced but not treated

Group 3 (n=6) – Diabetes induced and treated with 50mg/kg of metformin drugs

Group 4 (n=6) – Diabetes induced and treated with 250mg/kg body weight of *Nephrolepis unduranta ethyl acetate extract*

Group 5 (n=6) – Diabetes induced and treated with 250mg/kg body weight of *Nephrolepis unduranta n-hexene extract*.

Group 6 (n=6) – Diabetes induced and treated with 250mg/kg body weight of *Nephrolepis unduranta chloroform extract.*

Sample Collection

At the end of the 4 weeks of administration, rats were sacrificed by cervical decapitation and each rat was placed on its dorsal surface, and a laparotomy was carried out to expose the internal organs. Blood was collected by cardiac puncture, using 5ml syringes and 21G needle into blood sample containers. The blood samples were centrifuged at a rate of 4000 rpm for 10 minutes and serum collected and stored in a refrigerator at a temperature of 4^{o}C for analysis. The different organs, Brain, liver, Kidney, Testis, pancreas, Duodeum, Jejunum and Ileum were harvested for antioxidant studies [Superoxide Dismutase (SOD), Catalase, Lipid peroxidase and Malondialdehyde (MDA)].

Assay for Superoxide Dismutase (SOD)

The activity of SOD in the tissue homogenates was estimated using the method described by Misra and Fredorich (1972).

Assay for Catalase

The activity of catalase was determined in the tissue homogenates by the method described by Cohen et al. (1996).

Determination of Lipid Peroxidation

A breakdown product of lipid peroxidation thiobarbitoric acid reactive substance (TBARS) was measured in the tissue homogenates by the method described by Gutteridge and Wilkins (1982).

Principle

Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acid (PUFA) served as a convenient index for the determination of the extent of peroxidation reaction

Statistical Analysis

Data were analyzed by comparing values of different treatment groups with the values of individual controls, differences between means of the groups were analyzed using ANOVA and results was expressed as mean ± standard (Mean ± SD). *P*-values of less than 0.05 ($P<0.05$) were considered statistically significant. SPSS version 23 software was employed.

RESULTS

Figure 1 shows the effect of various fractions of *Nephrolepis unduranta* (NU) extract on diabetic Wistar rats. The base-line blood glucose was below 80mg/dl across the 6 groups. Day 0 represents the blood glucose level 72hours after induction with the blood glucose of the rats increasing to between 300-450mg/dl. After 7 days of treatment with various extracts of NU and metformin, there was a sustained elevation in blood glucose in the negative control group, but a gradual reduction across the treatment groups with the metformin group showing the most reduction. After 14 days of treatment, the metformin group still showing the most reduction. At day 21 of treatment, rats in the metformin group had blood glucose level similar to the base-line value. Amongst the rats treated with different extracts of NU, there was a significant reduction in blood glucose level, with those treated with ethyl acetate fraction of NU demonstrating a blood glucose reduction comparable to that of metformin. Malondialdehyde is a biomarker of oxidative stress. Results from this study showed an elevation in MDA levels following induction of diabetes and this was obvious in all the tissue samples analysed. After treatment for 21 days, there was a significant reduction in the MDA level across all groups. The ethyl acetate extract of Nephrolepis unduranta showed the most reduction amongst the extract groups. Also following 21 days treatment, there were no significant changes in the SOD concentration across the groups in the tissue samples analysed. The only finding of note is the sharp reduction in the brain tissue SOD level in the negative control group. Result from this study also showed a moderate reduction in catalase concentration in the diabetic group but these were observed to increase slightly following the 21 days treatment.

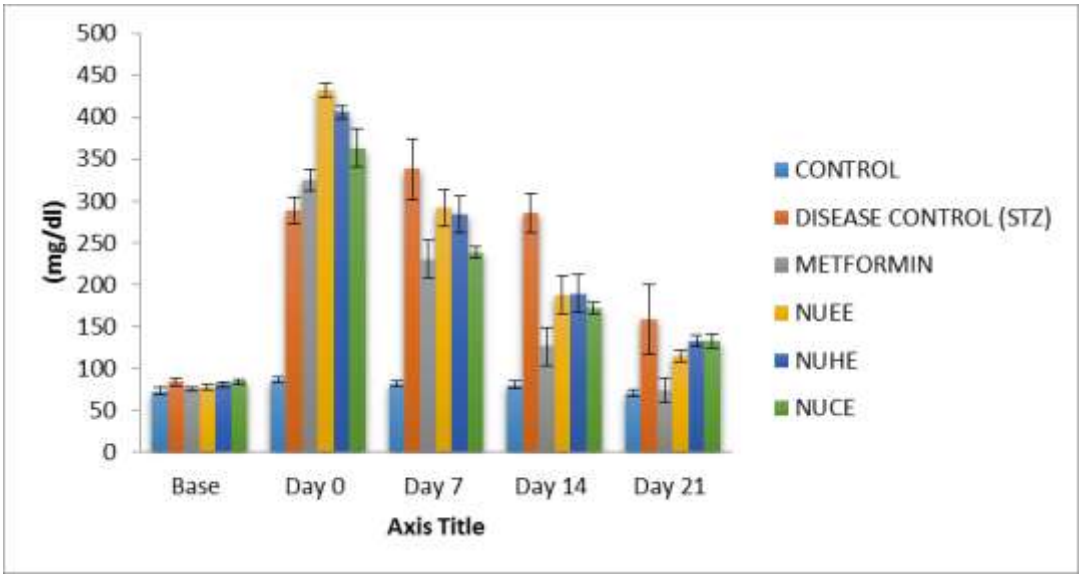

Figure 1: Effect of various fractions of NU extract on glucose level in diabetic rats.

Data are expressed as mean ± SD (n=3). **NUEE = *Nephrolepis unduranta ethyl acetate extract,* NUHE = *Nephrolepis unduranta n-hexene extract,* NUCE = *Nephrolepis unduranta* chloroform extract.**

Table 1: Effect of *Nephrolepis unduranta* leaves extract on MDA levels in diabetic rats

SAMPLE	***MDA (nmol/MDA)***						
	BRAIN	**Kidney**	**Iluem**	**Duodenum**	**Jejunum**	**Liver**	**Testis**
CONTROL	0.91±0.06	0.47±0.06	0.67±0.02	0.43±.04	0.55±0.02	0.47±0.04	0.47±0.03
DIABETIC CONTROL (STZ)	3.90±0.23	2.49±0.09	2.49±0.10	1.49±.09	1.89±0.30	3.09±0.40	1.28±0.05
METFORMIN	0.88±0.16	0.57±0.11	0.66±0.09	0.61±.100	0.61±0.12	0.92±0.06	0.62±0.08
NUEE	0.93±0.42	0.53±0.04	0.67±0.07	0.60±0.06	0.48±0.06	1.87±0.60	2.47±0.35
NUHE	2.41±0.77	0.60±0.04	0.77±0.02	0.65±.004	0.53±0.08	0.95±0.07	0.95±0.06
NUCE	1.33±0.22	0.79±0.20	0.82±0.05	0.63±0.04	0.44±0.03	1.02±0.14	1.02±0.13

Data are expressed as mean ± SD (n=3). NUEE = *Nephrolepis unduranta ethyl acetate extract*, NUHE = *Nephrolepis unduranta n-hexene extract*, NUCE = *Nephrolepis unduranta* Chloroform extract

Table 2: Effect of *Nephrolepis unduranta* leaves extract on SOD levels in diabetic rats

SAMPLE	*SOD (U/mg protein)*						
	BRAIN	**Kidney**	**Iluem**	**Duodenum**	**Jejunum**	**Liver**	**Testis**
CONTROL	32.23±0.60	34.07±0.23	33.47±0.08	34.65±0.14	34.10±0.05	33.82±0.36	31.11±1.61
DIABETIC CONTROL (STZ)	19.39±1.99	33.80±0.38	33.75±0.38	33.95±0.38	33.50±0.38	33.34±0.38	29.30±2.74
METFORMIN	32.67±0.52	34.55±0.13	33.79±0.12	34.94±0.06	34.11±0.07	33.89±0.15	33.38±0.70
NUEE	32.52±1.51	34.10±0.16	34.13±0.09	33.85±0.21	34.30±0.23	29.44±2.17	30.72±1.67
NUHE	30.45±2.52	34.01±0.14	33.40±0.12	33.47±0.16	34.48±0.32	32.61±0.22	32.47±0.68
NUCE	31.32±0.75	33.34±0.50	33.19±0.20	33.84±0.11	34.55±0.14	32.32±0.48	33.63±0.16

Data are expressed as mean ± SD. (n=3). NUEE = *Nephrolepis unduranta ethyl acetate extract*, NUHE = *Nephrolepis unduranta n-hexene extract*, NUCE = *Nephrolepis unduranta*. Chloroform extract

Table 3: Effect of *Nephrolepis unduranta* leaves extract on Catalase levels in diabetic rats

SAMPLE	*CAT (U/mg protein)*						
	BRAIN	**Kidney**	**Iluem**	**Duodenum**	**Jejunum**	**liver**	**Testis**
CONTROL	25.24±0.39	18.89±2.00	17.19±2.02	26.12±1.17	14.96±2.08	20.71±2.94	15.78±1.96
DIABETIC CONTROL (STZ)	18.47±2.29	19.45±1.99	18.06±1.48	17.17±4.36	19.38±2.43	27.67±1.62	12.84±1.64
METFORMIN	22.39±1.58	16.18±1.75	17.95±1.38	23.67±3.41	15.23±1.55	20.71±3.57	15.40±2.25
NUEE	26.84±4.08	18.74±1.34	15.34±1.00	22.84±0.96	18.84±2.26	19.26±2.79	16.47±1.14
NUHE	20.64±1.36	16.97±0.86	14.23±0.86	16.16±1.69	16.36±2.56	20.02±1.95	15.78±1.59
NUCE	23.06±2.41	18.02±.84	16.37±2.13	20.83±2.74	17.41±1.89	18.19±2.43	12.72±2.97

Data are expressed as mean ± SD (n=3). NUEE = *Nephrolepis unduranta ethyl acetate extract*, NUHE = *Nephrolepis unduranta n-hexene extract*, NUCE = *Nephrolepis unduranta*. Chloroform extract

DISCUSSION

Blood glucose in experimental animals was assessed at every 7-day interval of the investigation to assess the of NU extract on circulating serum glucose levels in the diabetic animals (Fig 1). Also samples of different tissues were analysed for presence of oxidative stress, by assaying some biomarkers (SOD, MDA and CAT) at the 21st day of the study (Tab 1-3). Treatment with NU extract (at 250 mg/kg), significantly reduced serum glucose level in this study (Fig 1). This finding shows a similar anti-diabetic activity as previously studied anti-diabetic plants[2,22]. Established pathogenesis of diabetes mellitus includes amongst others, destruction of the pancreatic β-cell[27], which can be managed by transplantation of islet cells plus bone marrow cell via portal vein[28]. The resistance to insulin at cellular membranes and inhibition of glucose metabolizing enzymes[29,30]. One effective therapeutic approach in managing hyperglycaemia that has been described is the inactivation of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase, enzymes involved in the production and subsequent absorption of glucose from GIT. This action has the ability of lowering postprandial hyperglycemia and its potential challenges[31,32,33,34]. The mechanism by *Nephrolepsis unduranta* caused reduction

in serum glucose levels in this study was not established, however it may be via inhibition of the actions of glucose hydrolyzing enzymes such as α-amylase and α-glucosidase, as STZ is known to cause irreversible damage to pancreatic β-cell [27]. The deleterious effect of metabolic diseases, have been reported to occur through their ability to generate free radical oxygen molecule which brings about oxidative cell damage [35]. And these oxidative damages are reported to occur more rapidly in the face of depleting cellular antioxidants [36, 37, 38]. In order to survive, cells most rely on defense mechanism which allows them to either escape or repair oxidative damages [4,5,7]. SOD, MDA and Catalase are antioxidants that are employed as biomarkers in estimating the level of oxidative stress in a living tissue[7]. In this study, treatment with *Nephrolepsis unduranta* increased catalase concentration in tissues from diabetic animals. In their review titled role of antioxidant in management of diabetes and its complications, Rahimi et al. (2005) stated the oxidative stress counteracting property of antioxidant contained in plants. The present study equally demonstrated a mild increase in SOD concentration amongst the tissues however, this was not statistically significantly. This study also showed an elevation in MDA level following induction of DM across all the tissues analyzed, a similar report has earlier been documented by Seven et al. (2004) in their study titled the effect of vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats. In this study treatment resulted in reductions in MDA levels in the tissues studied, with the ethyl acetate fraction of *Nephrolepsis unduranta* demonstrated the most reduction.

Conclusion

The results from this study propose that fraction of NU extract possesses anti-diabetic potential and the probable mechanism of its hypoglycaemic action could be via blockage of glucose hydrolyzing enzymes with in vitro anti-diabetic activity. The plant extract equally demonstrated significant antioxidant potentials which could also enhance its anti-diabetic properties. However, further research is necessary to isolate and estimate the specific components present in NU extract that may be responsible for these beneficial properties.

REFERENCES

1. World Health Organization: Geneva. Definition and diagnosis of diabetes mellitusand intermediate hyperglycaemia: Report of a WHO/IDF consultation. Google Scholar.2006.
2. Ojieh, AE; Ugorji AE; Ovuakporaye IS; Ewhre OL; Ossai NR. "Comparative Evaluation of Hypoglycemic Properties of Raw and Boiled *Allium cepa* in Alloxan-Induced Diabetes Mellitus Rats. UK Journal of Pharmaceutical and Biosciences. 2016; 4(3), 38-44
3. Ozougwu, J.C, Obimba, K.C, Belonwu, C.D, Unakalamba, C.B. "The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus," Journal of Physiology and Pathophysiology. 2013; 4(4): 46–57.
4. John W. The decomposition of hydrogen peroxide by liver catalase. Journal of General Physiology. 1928; 11(4): 309-337
5. George P. Reaction between catalase and hydrogen peroxide. Nature. 1947; 160: 41-43
6. Mahon CR, Lehman DC and Manuselis G. "Textbook of diagnostic microbiology, 4th ed. WB Saunders CO, Philadelphia, PA. 2011
7. Singh Z, Karthigesu IP, Singh P and Kaur R. "Use of malondialdehyde as a biomarker for assessing oxidative stress in different disease pathologies: a review. Iranian Journal of Public Health. 2014; 43(3): 7-16.
8. Mohammed A, Ibrahim MA, Islam MS. African medicinal plants with antidiabetic potentials: A review. Plant Medicine .2014; 80: 354-377
9. Gupta PD and De A. "DM and its herbal treatment". International Journal of Research in Pharmaceutical and Biomedical Science. 2012; 3:706-721.
10. Grover JK, Yadav S, Vats V. Medicinal plants with hypoglycemic potentials. Journal of Ethnopharmacology. 2002;81:81-100
11. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ.Leads from Indian medicinal plants with hypoglycemic potentials. Journal of Ethnopharmacology. 2006; 106: 1-28
12. Twari BK, Pandey KB,Abidi AB, Rizvi SI. Therapeutic potential of Indian medicinal plants in diabetic condition. Annual Phytomedicine. 2013; 2: 37-43
13. Philippe J, Raccah D. Treating type 2 diabetes: How safe are current therapeutic agents?. International Journal of Clinical Practice. 2009;63:321-332
14. Newman DJ, Cragg GM. Natural products as source of new drugs over the last 25 years. Journal of Natural Product. 2007;70:461-477
15. Bailey C, Day C. "Metformin: its botanical background." Practical Diabetes International. 2004; 21(3):115–117.
16. Day C. "Are herbal remedies of use in diabetes? Diabetes Medicine. 2005; 22(1):10–12.
17. Howlett HCS, Bailey CJ, editor Galegine and antidiabetic plants. Chichester, UK: Wiley. 2007
18. Chang HC, Gupta SK., Tasay HS. "Studies on Folk Medicinal Fern: An Example of "Gu-Sui-Bu", 285-304 p. In: Fernández H, Kumar A, Revilla MA (Eds.). Working with Ferns, Issues and Applications, Springer, New York, Dordrecht, Heidelberg, London. 2011

19. Iyamu Mercy Itohan. "Antimicrobial and Phytoconstituent Analysis of the Leaf Extracts of Annual Swordfern *(Nephrolepis undulate).* current study design Comparative Education". Science and Technology. 2017; 4(2):216-229
20. JariaL R, Shard A, Thakur S, Sakinah M, Zularisam A.W, Shahabaldin Rezania. Characterization of flavonoids from fern *Cheilanthes tenuifolia* and evaluation of antioxidant, antimicrobial and anticancer activities. Journal of King Saud University - Science. 2018; 30(4):425-432.
21. Nath, K. Bhattacharya, M.K. Kar, S. "Antibacterial activity of rhizome extracts of four pteridophytes from Southern Assam, North East India. Asian Journal of Pharmaceutical and Clinical Research. 2016; 4 (1): 1-5.
22. Ojieh AE, Adegor EC, Asisuodionoe ME, Okunima A and Onyekpe CU. Evaluation of the ameliorative property of *Vernonia amygdalina* fractions in streptozotocin induced diabetes. European Journal of Medicinal Plants. 2016; 13(4): 1-10
23. Akbarzadeh A, Norouzia D, Mehrabi MR, Jamshidi SH, Farhangi A, Allah-Verdi A. "Induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistry. 2007; 22(2): 60-64.
24. Misra HP and Fridovich I. "The role of superoxide ion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biology and Chemistry. 1972; 247:3170–3175
25. Cohen, H.J., Betcher–Lange, Kessler, D.L. And Rajagopalan K.V. "Hepatic sulphite oxidase congruency in mitochondria of prosthetic groups and activity. Journal of. Biology and. Chemistry. 1972; 247(2):7759-7766.
26. Gutteridge JM and Wilkins S. "Copper-dependent hydroxyl radical damage to ascorbic acid: formation of a thiobarbituric acid-reactive product. FEBS Letters. 1982; 137(2):327-330.
27. Elias D, Prigozin H and Polak N. Autoimmune diabetes induced by the b-cell toxin STZ. Diabetes. 1994; 43: 992-998
28. Ikebukuro K, Adachi Y, Yamada Y, Fujimoto S, Seino Y and Oyaizu H. "Treatment of streptozitocin-induced diabetes mellitus by transplantation of islet cells plus bone marrow cells via portal vein in rats. Transplantation. 2002; 73(4):512-518.
29. Bhanderi MR, Jong-Anurakkun N, Hong G, Kawabata J. "Alpha-glucosidase ans alpha-amylase inhibitory activities of Nepalese medicinal herb pakhanbhed (Bergenia ciliate; Haw). Food Chemistry. 2008; 106: 247-252.
30. Ibrahim MA, Koorbanaly NA, Islam MS. "Antioxidant activity and inhibition of key enzymes linked to type-2 diabetes (α-glucosidase and α-amylase), by Khaja Senegalensis. Acta Pharmaceutica. 2014; 64: 311-324.
31. Singh LW. "Traditional medicinal plants of Manipur as anti-diabetics. Journal of Medicinal Plant Research. 2011; 5: 677-687.
32. Pate DK, Prasad SK, Kumar R, Hemalatha S. "An overview on anti-diabetic medicinal plants having insulin mimetic property. Asian Pacific Journal of Tropical Biomedicine. 2012; 2: 320-330.
33. Zaid H and Saad B. "State of the art of diabetes treatment in Greco-Arab and Islamic medicine. In; Watson RR, Preedy VR (eds). Bioactive food as dietary interventions for diabetes. Academic Press, San Diego/ London. 2013; 327-335.
34. Kadan S, Saad B, Sasson Y and Zaad H." In-vitro evaluation of anti-diabetic activity and cytotoxicity of chemically analysed *Ocimum basilicum* extracts. Food Chemistry. 2016; 196: 1066-1074.
35. Laszio G. " Catalase deficiency and type 2 diabetes. Diabetes Care. 2008; 31(12): e93-e93.
36. Fumiaki K, Goji H, Hiroshi O, Tetsuo A, Hirokazu H, Mitsuhiro O. "Serum extracellular superoxide dismutase in patients with Type 2 Diabetes. Diabetes Care. 2003; 26(4): 1246-1250.
37. Mamal K and Khadega A. "Evaluation of trace elements and malondialdehyde levels in type 11 diabetes mellitus. *Diabetes and Metabolic Syndrone:* Research and Review. 2009; 3(4): 214-218.
38. Parmar I and Rupasinghe H. "Antioxidant capacity and anti-diabetic activity of wild berry stem infusions. European Journal of Medicinal Plants. 2015; 8: 11-28.
39. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomedical Pharmacotherapy. 2005;59:365-373
40. Seven A, Guzel S, Seymen O, Civelek S, Bolayirli M, Uncu M *et al.* Effect of vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats: Investigation of liver and plasma.Yonsei Medical Journal. 2004;45:703-710