Paper 3

Antidiabetic and Hypolipidaemic Effects of *Citrus aurantifolin* Leaves on Hyperglycaemic and diabetic Rats

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Abstract

Objectives: This research aimed to study the hypoglycaemic and hypolipidaemic effects of aqueous and methanolic extracts of *Citrus aurantifolin* leaves, in hyperglycaemic (type II) and Streptozotocin diabetic rats (type I).

Methodology: This plant was phytochemically screened using the standard methods for determination of its chemical composition., The hypolipidaemic and hypoglycaemic effects were determined following the Glucose tolerance test (GTT) model and the results were compared to the control and the reference drugs Insulin and Glibenclamide for types I and II diabetes mellitus respectively.

Results: Phytochemical screening of *C.aurantifolin* revealed presence of sterols (++), alkaloids (+), fla64rfc 64rfc 4wsazThe results of this research in type II

showed that both extracts of *C.aurantifolin* exhibited an early persistent hypoglycaemic effect in type II hyperglycaemic rats as compared to the control. Both doses of the aqueous extract and dose 400 mg / kg of the methanolic extract reduced blood glucose significantly ($P < 0.001$) throughout the experiment, while dose 200 mg / kg of the methanolic extract reduced blood glucose significantly ($P < 0.05$) throughout the experiment. In type I diabetic rats, the hypoglycaemic effect was slow but highly significant ($P < 0.001$) as it only started at the 4th hour post dosing. Regarding the effect of C.*aurantifolin* on blood cholesterol, the aqueous extract showed an earlier, less persistent and highly significant ($P < 0.001$) hypocholesterolaemic effect in type II hyperglycaemic rats than the methanolic extract as its effect occured at the 1st hour only while dose 400 mg/kg of the methanolic reduced cholesterol level significantly ($P < 0.05$), at the 2nd and 4th hours and dose 200 mg/kg at the 1st and 2nd hours post dosing. In type I diabetic rats, dose 400 mg/kg and Insulin,showed a significant ($P < 0.05$) hypocholesterlaemic effect at the 4th hour post dosing, the effect of the extract continued to the 8th hour. The highest significant reduction ($P < 0.001$) was exhibited by dose 200mg/kg of the methanolic extract at the 4th hour post dosing. Concerning blood triglycerides, both the aqueous and methanolic extracts of *C.aurantifolin*, reduced blood triglycerides significantly ($P < 0.001$) and ($P < 0.05$),at the 2nd and 4th hours post dosing, respectivly.In Type I diabetic rats,the effect of the aqueous extract was highly significant ($P < 0.001$) since the 1st hour and continued throughout the experiment. The methanolic extract, reduced blood triglycerides significantly ($P < 0.05$) 8 and 12 hours post dosing while the effect of Insulin was significant ($P < 0.05$) since the 1st hour and continued throughout the experiment.

In **conclusion** the leaves of *C.aurantifolin* confirmed its traditional use in herbal medicine as a hypoglycaemic and hypolipidaemic agent which can be very effective.

Introduction

Diabetes is world-wide in distribution and its incidence is rising. In the year 2000, 150 million people world-wide had diabetes, and this is expected to double by 2010 [(1)]. In spite of the introduction of synthetic hypoglycaemic agents, diabetes and the related complications continue to be a major medical problem [(2)]**.**Therefore the search for more effective and safer hypoglycaemic agents has continued to be an important area of active research [(3)]**.** Many medicinal plants have been found to be successful in management of diabetes [(4)]**.** However, search for new antidiabetic drugs continue**.**

Citrus aurantifolin (Family Rutaceae) is a spine scent tree up to 6 m in height. Its cultivated in various areas of the world. It contains volatile oils,terpeins,hesperidin and vitamin B,citric acid,vitamin C, potassium and calcium citrate and flavinoides. In folk medicine,the stem is used as an antiseptic for mouth, the juice is used for treatment of rhinitis and cold and can be added to coffee and tea for abdominal pain[(5)]**.**

Materials and Methods

Plants

The leaves of *Citrus aurantifolin* are brought from a home garden in Omdurman city.

Preparation of the aqueous extract

50 grams of the leaves of *C.aurantifolin* were weighed, immersed in cooled boiling distilled water and then incubated in a water bath at 60°c for four hours after which they were filtered. The filtrate was freeze dried [6].

Preparation of the methanolic extract

60 grams of the bark of *C. aurantifolin* were weighed and packed in Soxhlet apparatus using 500 ml of petroleum ether followed by chloroform, as solvents to separate lipids and terpenoids. The sample was then extracted using methanol as a solvent to get the polar constituents of the plants. The extract was evaporated till dryness using a rotatory evaporator [6].

Animals

Adult male Wistar albino rats weighing 70-300 grams were used in this study. Rats were obtained from the Faculty of Pharmacy, University of Khartoum. They were divided into groups of tens among the controls, standards and subgroups of samples. They were supplied with a standard pellet diet and tap water *ad libitum*.

Experimental type II diabetes mellitus:

(Glucose tolerance test)

Rats were divided into groups of tens among the controls, standards and samples. After eighteen hours - fast, blood samples were obtained from the retro orbital plexus of rats [7], using heparinized capillary tubes. The (0) time sample was collected and then all groups of animals were over- loaded with (2g/kg) of 50% glucose intraperitoneally; the control was given distilled water, the standard was given (10 mg/kg) of Glibenclamide while the tested groups were given (400 and 200 mg /kg) of the aqueous and methanolic extracts orally. The 1, 2 and 4 hours samples, were

collected and the plasma obtained after centrifugation was estimated for glucose, cholesterol and triglycerides.

Experimental type I diabetes mellitus:

(Streptozotocin – induced diabetes)

In this experiment, induction of diabetes in rats was achieved by destruction of the pancreatic cells using an intraperitoneal injection of Streptozotocin (STZ), at a dose of (60 mg / kg b wt), dissolved in citrate buffer at a concentration of (20 mg / ml) to provide a pH of 4.5 [8] and [9]. Soluble insulin at a dose of (3U/kg diluted 100 times) was used as standard (reference drug) and samples were collected at 0, 4, 8 and 12 hours [10] Samples were then analysed biochemically for glucose [11] cholesterol [12] and triglycerides [13].

Statistical analysis

Data were expressed as means ± standard error of means using paired student's t – test [14].

Results

Phytochemical screening revealed presence of triterpenes alkaloids, tannins and saponin (Table 1).

Both doses of the aqueous extract and dose 400 mg ⁄ kg of the methanolic extract reduced blood glucose significantly ($P < 0.001$) throughout the experiment, while dose 200 mg ⁄ kg of the methanolic extract reduced blood glucose significantly ($P < 0.05$) throughout the experiment. In type I diabetic rats, the hypoglycaemic effect of both extraxts was slow but highly significant ($P < 0.001$) as it only started at the 4th hour

post dosing. Regarding the effect of C.*aurantifolin* on blood cholesterol, the aqueous extract showed a significant ($P < 0.001$) hypocholesterolaemic effect at the 1st hour in type II hyperglycaemic rats. The effect of the methanolic extract occured at the 1st hour only while dose 400 mg/kg of the methanolic reduced cholesterol level significantly ($P<0.05$), at the 2nd and 4th hours and dose 200 mg/kg at the 1st and 2nd hours post dosing. In type I diabetic rats, dose 400 mg/kg and Insulin,showed a significant ($P < 0.05$) hypocholesterlaemic effect at the 4th hour post dosing, the effect of the extract continued to the 8th hour. The highest significant reduction ($P < 0.001$) was exhibited by dose 200mg/kg of the methanolic extract at the 4th hour post dosing. Concerning blood triglycerides, both the aqueous and methanolic extracts of *C.aurantifolin*, reduced blood triglycerides significantly ($P < 0.001$) and ($P<0.05$),at the 2nd and 4th hours post dosing, respectivly.In Type I diabetic rats, the effect of the aqueous extract was highly significant ($P < 0.001$) since the 1st hour and continued throughout the experiment. The methanolic extract, reduced blood triglycerides significantly ($P<0.05$) 8 and 12 hours post dosing while the effect of Insulin was significant ($P<0.05$) since the 1st hour and continued throughout the experiment.

Table 1

General Phytochemical screening of *Citrus aurantifolin* leaves,

Chemical Ingredient	***Citrus aurantifolin***
Sterols	++
Triterpenes	-
Alkaloids	+
Flavonoides	+
Tannins	+
Anthraquinones	-
Saponin	+
Cyanogenic glycosides	+
Coumarins	+

Table 2

Effects of the aqueous extract of *C.aurantifolin* on the blood glucose, cholesterol and triglycerides of hyperglycaemic rats:

Name of Group	Blood Glucose (mg/dl)			
	Time (hours)			
	0	1	2	4
Control (water)	110±8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide (10 mg/k	105±5.8	141.7±32.8	87.38±2.89	86.7±10.45*
C.aurantifolin (400 mg/kg	90±10.6	149.5±9.1*	76±10.5**	75.9±6.9**
C.aurantifolin (200mg/kg)	87.8±2.7	127±11.3*.	87.2±4.8**	100.9±0.73**
Cholesterol (mg/dl)				
Control (water)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide (10 mg/kg)	88±8.87	87±8.2	83.6±2.8*	86.4±21.2
C.aurantifolin (400mg/kg)	66.6±4.8	67.8±9.6**	107.2±13.2	83.5±6.4
C.aurantifolin (200mg/kg)	57.4±4.5	71.8±5.6**	68.6±4.4**	67.7±6.8**
Triglycerides (mg/dl)				
Control (water)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide (10 mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4

C.aurantifolin (400mg/kg)	11.4 ± 16.7	135.2±19.4	87±22.4**	74±20**
C.aurantifolin (200mg/kg)	131±10.5	115.2±9.7	118.4±5.3	108.5±14.9

(Data are expressed in mean ± standard error of mean)

***** = (P<0.05),

** = (P<0. 001)

Table 3

Effects of the methanolic extract of *C.aurantifolin* on the blood glucose, cholesterol and triglycerides of hyperglycaemic rats

Name of Group	Blood Glucose (mg/dl)			
	Time (hours)			
	0	1	2	4
Control(water)	110±8.9	166.8±7.51	129.4±17	110.4±8.5
Glibenclamide(10mg/kg)	105±5.8	141.7±32.8	87.38±2.89	86.7±10.45*
C.aurantifolin 400mg/kg)	100.2±2.4	82.2±15**	76.4±3.6**	88.5±8.8**
C.aurantifolin (200mg/kg)	105±8.7	138.2±2.1*	119.1±6.7*	91.9±1.6*
Cholesterol (mg/dl)				
Control (water)	75.8±6.7	104.2±16.8	107.2±6.4	83.5±6.4
Glibenclamide(10mg/kg)	88±8.87	87±8.2	83.6±2.8*	86.4±21.2
C.aurantifolin (400mg/kg)	72.6±5.1	104.2±16.8	93.2±21.6*	75.8±4.6*
C.aurantifolin (200mg/kg)	65±3.7	79.8±21.6*	88.2±6.4*	80±6
Triglycerides (mg/dl)				
Control (water)	108±13.6	118.5±11.2	135.2±19.4	122.2±16.5
Glibenclamide(10mg/kg)	132.2±10.5	140.6±10.2	134.8±16.3	144.4±9.4
C.aurantifolin (400mg/kg)	123.8±9.7	131.2±5.6	120.8±9.5	114.4±7.5

C.aurantifolin (200mg/kg)	125.4±19.2	114±22.4	101.4±11.8*	99.4±16.3*

(Data are expressed in mean± standard error of mean)

***** = (P<0.05),

** = (P<0. 001)

Table 4:

Effects of the aqueous extract of *C.aurantifolin* on the blood glucose, cholesterol and triglycerides of diabetic rats:

Name Of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	4	8	12
Control(water)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide(10mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2	222±6.6

C.aurantifolin (400mg/kg)	210.4±19.5	249.6±20.2	184.8±2.2	130.8±2.2**
C.aurantifolin (200mg/kg)	314±44	247.2±52.3	180.6±14.4	160.6±5**
Cholesterol (mg/dl)				
Control (water.)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide(10mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble insulin (3U/kg)	40.4±2.5	31.4±3*	34.2±.8	42 ±2.1
C.aurantifolin (400mg/kg)	75.8±12.7	51±4.7*	39.6±3.3*	42.8±1.1
C.aurantifolin (200mg/kg)	65.6±13.7	87.4±13	48.8±.8	47.2±.7
Triglycerides (mg/dl)				
Control (water)	247.2±35.7	227.8±20.3	187±15.3	284±54.3

Glibenclamide(10mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5
Soluble insulin (3U/kg)	57.8±12.8	33.2±4.8*	33.6±2.5*	42±6.5*
C.aurantifolin (400mg/kg)	122.2±14.3	55.8 ±12.8**	43.4 ± 8**	27.4 ± 0.8*
C.aurantifolin (200mg/kg)	143.2 ± 36.6	24.3 ± 37**	32.4 ±37**	44.4 ± 5.3**

(Data are expressed in mean± standard error of mean)

* = ($P<0.05$),

** = ($P<0.001$).

Table 5

Effects of the methanolic extract of *C.aurantifolin* on the blood glucose, cholesterol and triglycerides of diabetic rats:

Name of Group	**Blood Glucose (mg/dl)**			
	Time (hours)			
	0	4	8	12
Control (water)	286.2±18.2	226.6±12.5	202.8±56.5	277±56.5
Glibenclamide(10mg/kg)	311.8±46.3	221.2±55	259.8±64.9	266.6±49.4
Soluble insulin (3U/kg)	273.6±18.3	286.8±6.6	226±2	222±6.6

C.aurantifolin (400mg/kg)	277.8±22.7	233.2±14.7	179.8±16.6	168.4±8.6**
C.aurantifolin (200mg/kg)	224.8±10	223.6±6.8	201±41.6	171.4±29.8**
Cholesterol (mg/dl)				
Control (water. 10ml/kg)	66±10.2	61±3.7	53±5.4	56.8±5.9
Glibenclamide(10mg/kg)	98.6±18.6	112±17.4	97.4±15	56.8±4.9
Soluble insulin (3U/kg)	40.4±2.5	31.4±3*	34.2±.8	42±2.1
C.aurantifolin (400mg/kg)	58.8±5.9	33±2.7*	49.2±5.9	41±5.1
C.aurantifolin (200mg/kg)	63.4±11	48±5.4**	69±5.4	51.2±5
Triglycerides (mg/dl)				
Control (water)	247.2±35.7	227.8±20.3	187±15.3	284±54.3

Glibenclamide(10mg/kg)	123.2±22.5	222.4±52.3	247±55.9	230.4±61.5
Soluble insulin (3U/kg)	57.8±12.8	33.2±4.8*	33.6±2.5*	42±6.5*
C.aurantifolin (400mg/kg)	210.2 ± 51.2	383.4 ±16.2	165.2 43.7*	156.8±43.7**
C.aurantifolin (200mg/kg)	179.8 ± 6.5	195 ± 57.9	165.2±61.4*	163.2±43.1**

(Data are expressed in mean± standard error of mean)

***** = ($P<0.05$),

** = ($P<0.001$)

Discussion:

In accordance to the recommendations of the WHO Expert Committee[16], on diabetes mellitus, it is important to investigate the hypoglycaemic action for plants which were traditionally used in traditional medicine [17]

The limited efficacy and the draw back of the currently used hypoglycemic agents prompted the scientists world-wide to search for more effective phytomedicenes. [18]

More than 1200 species of plants have been used ethno-pharmacologically or experimentally to treat symptoms of diabetes mellitus. They represent more than 725 genera in 183 families. The most frequently sited families are *Asteraceae, Fabaceae, Poaceae*, *Laminaceae* and *Liliaceae* [19]**.** According to the taxonomy of [20] *C.aurantifolin* belongs to the family Rutaceae.

The biologically active components of plants with hypoglycaemic action include; flavonoides, alkaloids, glycosides, polysaccharides, peptidoglycans, steroids and terpenoides [21].

In this study, phytochemical screening of *C.aurantifolin revealed* presence of sterols, alkaloids, flavonoids,tanninsm, saponin, cyanogenic glycosides and coumarins. In addition [20] reported presence of Volatile oil, and terpenoides.

Thus components, such as alkaloid, salicylic may be responsible for the hypoglycemic activity of hypoglycaemic plants [22]. Coumarin, an active constituent of *Trigonella*, was found to have a profound hypoglycemic activity in normal and alloxan –diabetic rats [23]. Thus presence of alkaloids and coumarin in *C.aurantifolin,* are probably responsible for its hypoglycaemic activity.

Many plants are recently studied for their hypoglycaemic effects The leaf alcohol extract of the plant *Annona squamosa* was investigated for its anti-diabetic activity in diabetic rats. The findings showed the significant anti diabetic potential of the extract in monitoring the diabetes rats [24]. In studying the antihyperglycemic effect of the ethanol extract of *G. montanum* leaves to Diabetic rats, the results indicatated a positive role of *G. montanum* as atherapeutic agent for Diabetes[25]

The results of this current study, showed that the extracts of the bark of *C. verum* possess blood glucose lowering effect in both hyperglycaemic (tables 1 and 2) rats and in diabetic rats (tables 4 and 5).. Thus the folk use of this plant may be validated by this study. The bark of this plant seems to have a promising value for the development of potent phytomedicine for diabetes.

It can be concluded that *C.aurantifolin* can be promising a antidiabetic and hypolipidaemic This verifies the traditional use of this plant as a hypoglycaemic agents.

References:

1- Christopher Haslett, Edwin R. Chilvers, Nicholas A. Nicki R. Colledge John A.A. Hunter (2002). Davidson's Principles and Practice of Medicine 19 edition Illustrated by Robert Britton,. (50th anniversary edition) in slipcase, $ 79.95, 642-682. ISBN 0-443-07111-X, New York, NY, Churchill Livingstone,. jama.ama-assn.org/cgi/content/full/290/20/2743.

2- Satyanarayana T, Sarita T, Balaji M, Ramesh A and Murthy K.(2004) Antihyperglycaemic and Hypoglycaemic Effect of *Thespesia populena* Fruit in Normal and Alloxan – Induced Diabetes in Rabbits. Saudi Pharmaceutical Journal, Vol. 12, No. 2 -3 July 2004; 107 – 111. informahealthcare.com/doi/pdf/10.1080/13880200701214631.

3-The WHO Expert Committee on Diabetes Mellitus (1980). Technical Report Series World Health Organization, Geneva..

4- Subramanian A, Pushpangadan P, Rajasekharan S, Evans DA, Latha PG, Valsaraj R (1996). Effects of *Artimisia pallens* wall on blood glucose levels in normal and alloxan – induced diabetic rats.J Ethnopharmacol; 50: 13-17. *www.ingentaconnect.com/content/els/03788741/1996/.../art01329*.

5- Alam K.R (2003).Diabetic Support Formula – Diabetes Care. Agricultural University in Peshawar, Pakistan. 26:3080-3086. American Diabetes Association: Standards of medical care in diabetes (Position Statement). Alam K.R (2003).Diabetic Support Formula – Diabetes Care. Agricultural University in Peshawar, Pakistan. 26:3080-3086. American Diabetes Association: Standards of medical care in diabetes (Position Statement). care.diabetesjournals.org/content/28/2/260.

6- Elghazali E.B.Jamal, Khalid-SH-Eltohami, S.Mahjoob, Abdalla.S.Wail and S.M.Yagi (1998). Common Medicinal Plants in Khartoum State. National Center for Researches – Khartoum Sudan.. Sudan Medical Journal 25-27. www.sudan-health.net/bibliography/hs190.htm.

7- Bruneton J (1995). Pharmacognacy, Phytochemistry, Medicinal plants. 2nd ed., (Paris: Lavoisier Publishing 1993). 218-219. 5. Blumenthal M, Goldberg A, Brinckmann J (eds). www.medicinehunter.com/artichokeleaf.htm Paris.

8- Leung A and Foster S (1996). Encyclopedia of Common Natural Ingredients used in foods drugs and cosmetics, 2nd edition. New York: John Wiley and Sons, Inc. 50-53350–2469-72. PDR for Herbal Medicines, 2nd edition. Montvale, NJ: Medical Economic. www.gaiaherbs.com/pdf_files/Astragalus%20Sup.pdf.

9- Harborne J-B. (1973) Phytochemical Methods. Second edition.Choman and Hall, London and New York. ISBN 0-412-25550-2. 1984. 288 pp. $36 (cloth). www.springerlink.com/index/G2PQ5XU78Q648270.pdf.

10- Khanna, A.K, Chander, R. Kapoor, N, K Singh, C. and A, K. Sryvastava (1992.) Hypoglycaemic Activity of *T.chebuain* in rats. Fitoterapia 1992: (4) 315 – 356. Med1992;57:29-33. 179 . Liu J. Expert Opinion on Drug Discovery, Mar 2009, Vol. 4, No. 3, Pages 315-356.www.informahealthcare.com/doi/abs/10.../17460440902911433.

11- Rakieten, N; Rakieten, M.L.and Nadkarni, M.V.(1963). Streptozotocin as an Anti – Tumor and Antidiabetic Agent. Cancer Chemo. Ther. Journal of comparative pathology. Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemotherapy Reports, 29, 91-98.

12- Heor-RR and Jahuke- HK (1967). Hydrocarbon Chemistry (Second Edition). J.Am.Chem.Soc. (89) 480- 488.doi.wiley.com/10.1002/0471433489.ch6.

13- Suba V.T Nurugefan and R. Phafkarao (2004). Antidiabetic Potential of *Barleria lupulina* Extract in Rats.Fitoterapia 75 1-4.Fitoterapia, 75: 1-4,2004. ... Fitoterapia. 74: 372-374,2003. 8. www.dukesmoking.com/files/userfiles/R_Murugesan_CV-2_08.pdf.

14- Tinder P (1969). Determination of blood glucose using an oxidase- peroxides system with a non – carcinogenic chromogen. J Clin. Pathol; 22: pp.158 – 161. linkinghub.elsevier.com/retrieve/pii/S000399690300102X.

15- Richmond. (1973). Cholesterol-enzymatic colorimetric tests (CHOD-PAP).Clin. Chem.19, 1350 – 1356 Cat.No. 101-0440. www.chemoquip.com/html/downloads/13.pdf.

16- Vad, B.G. (1960) Place of Momordica charantia in the treatment of diabetes. Maharasthra Medical Journal 6:733. Published in: Maharashtra Medical Journal indianmedicine.eldoc.ub.rug.nl› ABIM › V › 135007. *www.nutritionjrnl.com/article/PIIS0899900703002843/ 194*

17- Mendenhall, W. (1971).Introduction to probability andstatistics.3th ed p.669-683 Wadsworth Publishing Company Inc. Belmont, California, U.S.A. journals.tubitak.gov.tr/veterinary/issues/.../vet-28-3-20-0302-24.pdf - www.jstor.org/stable/2344620.

18- Lemela M, Cadavid I, Gato A, Calleja JM (1985). Effect of *Lythrum saricaria* in normoglycaemic rats. J Ethnophaemacol: 41:83 - 91. faculty.ksu.edu.sa/hisham/Documents/SPJ/122306.pdf .

19. Expert Committee (2002). Report on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care, Vol. 25, suppl.1, January, pp. S5-S20. Report of a WHO study group. Diabetes Care 2003;26:S5-S20. External Resources Am J Hum Genet 2002;71:964-968. content.karger.com/produktedb/produkte.asp?typ=fulltext&file.

20. Alarcon-Aguilara FJ, Rornan-Ramos, Perez- Guti errez (1998); . Study of the anti-hyperglycaemic effect of plants used as antidiabetics.J.Ethnopharmicol, 61 (Pt 2):101-110). www.naturalstandard.com/monographinfo.asp.

21. RahmanAU and ZamanK (1989). Medicinal plants with hypoglycaemic activity. 26:1-55J. Ethnopharmacol. 26, 1-55. informahealthcare.com/doi/pdf/10.3109/13880209309082925

22. Thorne, R.F. (1981) .Phytochemistry and angiosperm, phylogeny. Summary statement. In: (D.A. Young D.S.Seigler, Eds), PP.233-295.Praeger Scientific New York, www.publish.csiro.au/?act=view_file&file_id=SB9940057.pdf

23. Elghazali.B., Eltohami, M, Abdalla SW and.Yagi SM (1998). Common medicinal plants in Khartoum state. National Center for Researches (MAPRI) Khartoum Sudan.

24. Grover JK, Yadav S, and Vats V (2002). Medicinal plants of India with anti-diabetic potential J Ethnopharmacol; 81 : 81-100. 6. Ivorra MD Paya.. PDF/Adobe Acrobat www.icmr.nic.in/ijmr/2007/september/0910.pdf.

25. Oliver-Bever, B. and Zahnd G.R. (1979) Plants with oral hypoglycemic action. Quart. J.Crude Drug Res.17:139-196 informahealthcare.com/doi/abs/10.1076/phbi.34.1.41.13186.

26.Shani,J.A.;Goldschmied,A.;Joseph,B.;Ahronson,Z.andSulman,F.G. (1974).Hypoglycaemic effect of Trigonella foenum graceum and Lupinus termnis seeds and their major alkaloids in alloxan diabetic and normal rats. Arch Int. Pharmacodyn Ther. 210:27-37. J . Appl. Sci. Res., 3 (10): 1073-1083, 2007. 1082. 32. Indian J. Physiol Pharmacol. www.insipub.com/jasr/2007/1073-1083.pdf .

27. Annie Shirwaikar, K. Rajendran and C. Dinesh Kumar (2004). Oral Antidiabetic Activity of Annona squamosa Leaf Alcohol Extract in NIDDM Rats. Pharmaceutical Biology Formerly International Journal of Pharmacognosy) Volume 42, Number 1,

February 2004 , pp. 30-35(6)
www.ingentaconnect.com/content/tandf/phbi/2004/00000042/art00006

28. Ramkumar, K.M., Rajaguru, P., Latha, M. and Ananthan, R. (2007). Ethanol extract of *Gymnema montanum* leaves reduces glycoprotein components in experimental Diabetes. Nutrition research, Nutr Res 27: 97-103.