International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 3 Issue 9, August 2016

ISSN: 2394-4404

Evaluation Of Hypoglycemic Potential Of Extracts Of Balanites Aegyptiaca Parts

Mhya DH

Anigo, K.M.

Umar, I.A.

Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria

Alegbejo, J.O

Department of Pediatric, Ahmadu Bello University Teaching Hospital Zaria, Nigeria

Abstract: ***This study was aimed to evaluate hypoglycemic potential of extracts of Balanites aegyptica leaves, stem-bark and fruit-mesocarp. Balanites aegyptiaca leaves, stem-bark and fruit-mesocarp were extracted using ethanol followed by solvent-solvent fractionation with ethyl acetate and water. The solvent-fractions thus obtained were subjected to acute oral toxicity study on wistar rats. Phenolics and flavoniods content of the fractions were assayed. Solvent-fractions were evaluated in vivo in pursuit for those with hypoglycemic property. The solvent-fractions were orally administered at a dose of 400 mg/kg body wt. (i.e.1/10th of lethal dose) to normoglycemic/diabetic rats, positive control were given metformin (200 mg/kg body wt.) and negative controls received 10 % dimethyl sulfurdioxide (DMSO). Results obtained showed no death or lethal effect in the acute oral toxicity study up to a dose of 4000 mg/kg body wt. therefore, the LD_{50} value was considered to be greater than 4000 mg/kg body wt. Phenolics and flavoniods content showed varying levels; phenolics was highest compared to flavonoids in all the solvent-fractions. Acute effect of solvent-fractions on fasting blood sugar and oral glucose loaded test in normoglycemic/diabetic rats showed hypoglycemic effects. In sum, these results indicated that phenolics and flavonoids in Balanites aegyptiaca leaves, stem-bark and fruit-mesocarp exhibit antidiabetic effect; leaves and fruit mesocarp showed the better activity.***

Keywords: Balanite aegyptiaca, parts, antidiabetic property

I. INTRODUCTION

Medicinal plants have been employed in the management of diabetes and are well recognized as important source of new drug (Newman and Cragg, 2007). Polyphenols are molecules from plants that represent a wide range of substances with various structures. Evidence from several *in vitro*, animal model and human studies have provided the rationale for the use of polyphenols in disease interventions (Sasidharan *et al*., 2011; Atawodi *et al*., 2011; Wang *et al*., 2013; Tain *et al*., 2013).

The plant *'Balanites aegyptiaca Del.*', also known as 'desert date' in English, a member of *Zygophyllaceae* family, is a common plant species of the dry land areas of Africa and Asia (Hall and Waijer, 1991; Hall, 1992). In Nigeria, it is found in abundant in the Northern region. It is known as '*Aduwa*' in Hausa, '*Utazi'* in Igbo, and '*Teji'* in Yoruba. Literature survey revealed that *B. aegyptiaca* has a long history of traditional uses for wide ranges of disease including diabetes (Daya and Vegahasiya, 2011). Phytochemical investigation of *Balanites aegyptiaca* parts revealed the presence of various polyphenols; coumarins and quercetins were found in the leaves, alkaloids and coumarins were seen in the stem-bark while rutins was found in the fruit among others (Salwa *et al*., 1988; Sarker *et al*., 2000) [27,28]. In a bid to identify the part that exhibit better antidiabetic activity and hence promote the use and acceptance of the plant as antidiabetic remedy, this study attempt to identify *Balanites aegyptiaca* parts with better antidiabetic activity.

ISSN: 2394-4404

II. MATERIALS AND METHODS

A. PLANT COLLECTION

Balanites aegyptiaca leaves, stem-bark and fruit-mesocarp were collected from Gubi village in Bauchi, Bauchi state. It were identified and assigned a voucher number 900175 at the Herbarium Unit of the Department of Biological Science, Ahmadu Bello University Zaria.

B. EXTRACTION

Extraction was done as described by Jung *et al* (2002) and Govorko *et al* (2007). Plant sample powdered (50 g) was defatted twice for 2 hrs with 80 mL hexane on a mechanical shaker. The hexane solvent was discarded, the defatted sample powder was air-dried and then, 10 g of the defatted powdered sample of the *Balanite aegyptiaca* was heated to 80 °C with 100 ml of 80 % ethanol for 2 hrs. The extraction was continued for an additional 10 hrs at 20 °C. The extract was filtered through a cheese cloth and concentrated by evaporation to 10 ml using a rotary evaporator. The extract was then partitioned with 10 ml of water and 10 ml of ethyl acetate. The solvent fractions were concentrated using a rotary evaporator and air dried.

C. CHEMICALS/REAGENTS

All chemicals/reagents used were of analytical grade and were obtained from Sigma Aldrich, USA. Reagent kits were purchased from Randox Laboratory, UK.

D. ANIMALS

Thirty (30) male wistar albino rats, approximately of the same age weighing between 200-230 g purchased from the Department of Biochemistry, University of Jos, Plateau state were used for the study. They were allowed free access to water and animal feed (Vital feeds, Jos).

E. EXPERIMENTAL DESIGN

Solvent-fractions of ethanolic extracts of *Balanite aegyptiaca* leaves, stem-bark and fruit-mesocarp were studied for acute oral toxicity, phenolics and flavonoids contents were quantified. Thereafter, acute hypoglycemic effects of the solvent-fractions of ethanolic extracts of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp were investigated in normoglycemic and Streptozotocin-induced diabetic rats. Blood samples were collected from the tail vein for fasting blood glucose estimation at 0, 30, 60, 90 and 120 minutes interval using Accu-chek glucometer (Roche Diagnostics Co., Germany).

F. DETERMINATION OF TOTAL FLAVONOIDS (TFC) AND PHENOLICS CONTENTS (TPC)

The TFC of the solven fractions of ethanolic extracts of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp were determined using the aluminium trichloride assay as done by Meda *et al* (1999). Folin-Ciocalteu method was used to determine the total phenolics content of the solvent-fraction of ethanolic extracts of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp (Singleton *et al* 1999).

G. ACUTE ORAL TOXICITY STUDY

The acute oral toxicity study was performed according to OECD 425 guidelines: up-and-down acute toxicity test (OECD, 1998). The extracts were administered in a single dose by using oral gastric tube. Animals were deprived of food 3 hours prior to dosing, after each extract administration animal was observed 30 minutes interval for 4 hours then after 24 hours for behavioral change or death. The Following dosage (5, 50, 500, 1000, 2000, 4000 mg/kg body wt) were determined and used according to the OECD 425 guidelines with limit at 2000-5000 mg/kg body wt. Bruce's (1985) up and down procedure for acute toxicity study was used to investigate acute toxicity of the plant extract on wistar albino rats. Lethal Median Dose (LD_{50}) was calculated using the formula below: LD_{50} = (the apparent least dose lethal to animals – [(a x b)/N])

Where, N = number of animal used, a = dose difference, and b = mean mortality.

NB: For the study, $1/10^{th}$ of LD_{50} was used.

H. ASSESSMENT OF HYPOGLYCEMIC ACTIVITY OF PLANT EXTRACT

The hypoglycemic activity of plant extract was assessed on overnight fasted rats. Rats were divided into 8 groups of 3 rats. Group I served as the negative control and received 10 % dimethyl sulfuroxide (DMSO) via oral gavage. Group II served as the positive control and was orally administered with metformin (200 mg/kg body weight). The animals of groups III (a & b), IV (a & b) and V (a & b) received 400 mg/kg body weight of various solvent-fractions of ethanolic extracts of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp via oral gavage. Blood samples were collected for fasting blood glucose estimation at 0, 30, 60, 90 and 120 minutes interval using Accu-chek glucometer (Roche Diagnostics Co., Germany).

I. ASSESSMENT OF ACUTE ANTI-HYPERGLYCEMIC ACTIVITY OF PLANT EXTRACT

The anti-hyperglycemic activity was assessed using an oral glucose loaded test performed in overnight-fasted normal and diabetic rats. Rats were divided into 8 groups of 3 rats for each of the separate experiment. Group I served as the negative control and received 10 % dimethyl sulfuroxide (DMSO) via oral gavage. Group II served as the positive control and was orally administered with metformin (200 mg/kg body weight). The animals of groups III (a & b), IV (a & b) and V (a & b) received 400 mg/kg body weight of various solvent-fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp ethanolic extracts via oral gavage. Blood samples were collected for fasting blood glucose estimation at 0, 30, 60, 90 and 120 minutes interval after

IJIRAS

ISSN: 2394-4404

glucose loading (3 g/kg body weight) using Accu-chek glucometer (Roche Diagnostics Co., Germany).

J. DATA ANALYSIS

Results of the experiments were pooled and expressed as mean ± standard deviation (SD). Data were presented in both tabular and graphical format. Means were analyzed by one way analysis of variance (ANOVA) and compared by Duncan's multiple range test (DMRT) (Duncan 1957). Significant difference was accepted at $P \leq 0.05$.

III. RESULTS

A. YIELD AND TOTAL PHENOLICS /FLAVONOIDS CONTENTS OF EXTRACTS OF BALANITES AEGYPTIACA

The yields of solvent-fractions derived from ethanolic extracts of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp is presented in Table 1. Six solvent-fractions namely; aqueous leaves fraction (ALF), ethyl acetate leaves fraction (ELF), aqueous fruit-mesocarp fraction (AFF), ethyl acetate fruit-mesocarp fraction (EFF), aqueous stem-bark fraction (ASF), and ethyl acetate stem-bark fraction (ESF) were obtained and used in the study.

Total phenolics and total flavonoids content of solvent-fractions derived from ethanolic extract of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp is shown in Table 1. It showed varying levels of total phenolics and flavonoids. The result revealed that the aqueous fruit-mesocarp fraction has the highest amount of total phenolics (0.77±0.0011 mg g^{-1}GAE) while ethyl acetate stem-bark fraction has the less total phenolics content (0.06±0.0076 mg g^{-1}GAE) but highest in the amount of total flavonoids (0.11±0.0001 mg g^{-1}QE) while aqueous fruit-mesocarp fraction is the less in total flavonoids (0.02±0.0004 mg g^{-1} QE). However, phenolics was high in the fractions compared to flavonoids.

B. ACUTE TOXICITY STUDY

From the experiment performed as per the OECD Guidelines 425 for up-and-down acute toxicity test, the result reveals that solvent fractions of ethanolic extracts of *Balanites aegyptiaca* parts were safe up to a dose of 4000 mg/kg body wt. Observation made 4 hours and later 24 hours after administration shows; no treatment-related mortality at all the tested doses, no significant changes in behavior such as apathy, hyperactivity, morbidity, etc. recorded in the treated animals. The solvent fractions derived from ethanolic extracts of *Balanites aegyptiaca* parts was safe up to a dose of 4000 mg/kg body wt and therefore, the LD_{50} value for oral toxicity is considered to be greater than 4000 mg/kg body wt.

	Ethanolic Extract-Fractions					
	Aqueous Leave	Ethyl acetate Leave	Aqueous Fruit-mesocarp	Ethyl acetate Fruit-mesocarp	Aqueous Stem-bark	Ethyl acetate Stem-bark
Fractions	68.80	31.1	84.55	13.18	87.0	12.2
Yield		4			8	6
(g/100g)						
Phenolics (mg g^{-1}GAE)	0.66± 0.0625^{bc}	0.56± 0.002^{b}	0.77± 0.016^{bcd}	0.07± 0.0199^{a}	0.52± 0.0416^{b}	0.06 ± 0.0076^{a}
Flavonoids (mg g^{-1}QE)	0.04± 0.0001^{bc}	0.02± 0.0004^{a}	0.03± 0.0007^{b}	0.05± 0.0003^{bcd}	0.04± 0.0001^{bc}	0.11± 0.0001^{bcde}

Values are Mean ± Std of triplicate determinants with different superscript in the same row are significantly different ($P \leq 0.05$)

Table 1: Total phenolics and flavonoids content of solvent-fractions of ethanolic extracts of Balanites aegyptiaca leaves, stem-bark and fruit-mesocarp

C. HYPOGLYCEMIC EFFECT OF EXTRACT OF BALANITES AEGYPTIACA ON NORMOGLYCEMIC RATS

Test for hypoglycemic effects of oral administration of solvent fractions of ethanolic extracts of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp on normoglycemic rats are presented in Figure 1. All the fractions of the ethanolic extracts of *Balanites aegyptiaca* leaves, stem-bark, and fruit-mesocarp produce significant reduction of fasting blood glucose level in time dependent manner. Precisely, the reduction in fasting blood glucose was more pronounced by the metformin, aqueous leaves fraction (ALF) and aqueous stem-bark fraction (ASF) when compared to the other solvent-fractions.

D. EFFECT OF EXTRACTS OF BALANITES AEGPYTIACA ON GLUCOSE LOADED IN NORMOGLYCEMIC RATS

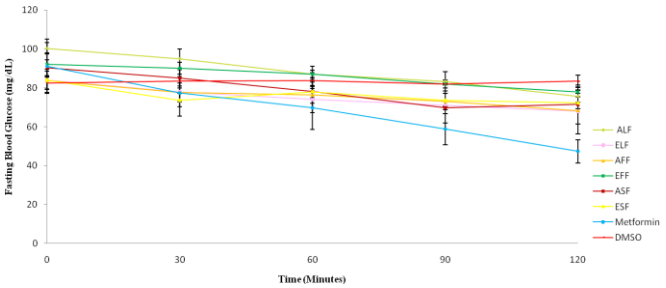
Effects of solvent-fractions of ethanolic extracts of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp on glucose loaded normoglycemic rats are presented in Fig 2. Rats treated with the aqueous fractions of the plant parts displayed most significant reduction. To be precise, the suppression in postprandial blood glucose level was more pronounced by the metformin and aqueous fruit fraction (AFF) compared to the others.

E. EFFECT OF EXTRACT OF BALANITES AEGPYTIACA ON GLUCOSE TOLERANCE TEST IN STRPTOZOTOCIN-INDUCED DIABETIC RATS

Blood glucose level of streptozotocin diabetic control and treated rats with solvent-fractions of ethanolic extracts of *Balanites aegyptiaca* leaves, fruit-mesocarp and stem-bark at different time periods (0, 30, 60, 90 and 120 min) after oral administration of glucose (3 g/kg) is shown in Fig 3. In the diabetic control, increase in blood glucose level was observed after 30 min and remained high over the next 120 min. Metformin, Aqueous (ALF) and ethyl acetate (ELF) fractions

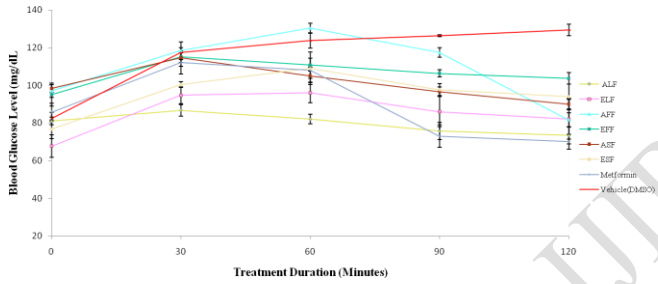
ISSN: 2394-4404

of the leaves ethanolic extracts lowered the blood glucose level at 60 min and remained low over the next 120 min. While ethyl acetate fruit-mesocarp (EFF) and stem-bark (ESF) fractions lower the blood glucose at 90 min through 120 min. However, aqueous fruit-mesocarp (AFF) and stem-bark (ASF) fractions showed fluctuation in blood glucose level throughout the tested periods.



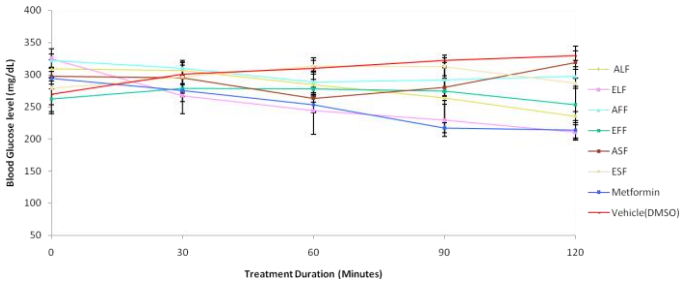
ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit fraction, EFF = Ethyl acetate Fruit fraction, ASF = Aqueous Stem-bark fraction, DMSO = Dimethylsulfoxide (vehicle)

Figure 1: Hypoglycemic Effect of Oral Administration of Solvent-fractions of Ethanolic Extracts of Balanites aegyptiaca Leaves, Stem-Bark and Fruit-Mesocarp on Normoglycemic Rats



ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit fraction, EFF = Ethyl acetate fruit fraction, ASF = Aqueous Stem-bark fraction, DMSO = Dimethylsulfoxide (vehicle)

Figure 2: Effect of Oral Administration of Solvent-fractions of Ethanolic Extracts of Balanites aegyptiaca Leaves, Stem-Bark and Fruit-Mesocarp on Glucose Loaded in Normoglycemic Rats



ALF = Aqueous leaves fraction, ELF = Ethyl acetate leaves fraction, AFF = Aqueous Fruit fraction, EFF = Ethyl acetate fruit fraction, ASF = Aqueous Stem-bark fraction, DMSO = Dimethylsulfoxide (vehicle)

Figure 3: Effect of Oral Administration of Solvent-Fractions of Ethanolic Extract of Balanites aegyptiaca Leaves, Stem-Bark and Fruit-Mesocarp on Postprandial Blood Glucose Level in Streptozotocin-induced Diabetic Rats

IV. DISCUSSION

Plant parts have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being. The medicinal value of plants lies in some chemical substances known as phytochemicals that have a definite physiological action on the human body. Among the most important of these bioactive constituents of plants are flavanoids and phenolics (Hill, 1952; Atawodi *et al*., 2011; Egan *et al*,. 2014). Quantification of solvent fractions of *Balanites aegyptiaca* leaves, stem-bark and fruit-mesocarp ethanolic extracts revealed the presence and varying amount of phenolics and flavonoids contents that confers to the usefulness of the plant in disease intervention.

Results indicated that phenolics compounds are high in the plant solvent fractions. It was stated that water enhances interaction of hydroxyl and or carboxylic groups, hence promote the dissolution of phenolics in the solvents (Mota, *et al*., 2008). On the other hand, flavonoids have low solubility in water and this suggest its low quantity in the fractions. Solvent fractions of the ethanolic extracts of *Balanites aegyptiaca* leveas, stem-bark and fruit mesocarp exhibited no toxic effect when given orally at concentration up to 4000 mg/kg body weight. Normalcy and insignificant changes in wellness parameters observed reveals the safety of the plant extracts.

The ultimate targets of antidiabetic therapy are; blood glucose homeostatic regulation and amelioration of metabolic derangement. In this study, *in vivo* hypoglycemic effects of solvent fractions of *Balanites aegyptiaca* were observed. The plant solvent-fractions caused a reduction in blood glucose levels in a time dependent manner. The hypoglycemic activity of the plant was further substantiated by its improved glucose tolerance in the normalglycemic/diabetic treated rats suggesting that the plant solvent fractions contain compounds that have the capacity to correct impaired glucose tolerance in diabetes, hence exhibit antidiabetic effect. Khathi *et al* (2013) have reported similar event by *Syzigium aromaticum* extract in streptozotocin-induced diabetic rats following an oral glucose tolerance test.

V. CONCLUSION

This study shows that *Balanites aegyptiaca* leaves, fruit-mesocarp and stem-bark with profound investigation exhibit hypoglycemic activity. The activity might be attributable to chemical compounds like phenolics and flavonoids which were presence in the extracts. The leaves and fruit-mesocarp of the plant contain the highest content of the compounds hence exhibit better antihyperglycemic effects.

ACKNOWLEDGEMENT

I wish to appreciate the effort of my able supervisors (Prof. KM Anigo, Prof IA. Umar, and Dr (Mrs) JO Alegbejo) and, the technical staff of the Department of Biochemistry, ABU Zaria, Nigeria.

IJIRAS

CONFLICT OF INTEREST

No conflicts of interest exist in regard to this manuscript.

REFERENCES

[1] Atawodi, SE., Atawodi, JC., Pfundstein, B., Spiegelhelder, B., Bartsch, H., Dwen, R (2011). Assessment of the polyphenol components and *in vitro* antioxidant properties of *Syzygium aromaticum (L). Merr & perry. EJEA Ch*, 10(3):1970-8.

[2] Bruce, R.D (1985). An up-and-down procedure for acute toxicity testing. *Fundamental Applied Toxicology*, 5: 151-152.

[3] Daya LC and Vaghasiya HU (2011). A review on Balanites aegyptiaca Del (desert date): phytochemical constituents,traditional uses, and pharmacological activity. *Pharmacogen rev*. 5(9): 55-62.

[4] Duncan, B. D. (1957). Multiple range test for correlated and heteroscedastic means. Biometrics 13: Pp 359–364.

[5] Govorko D, Logendra S, Wang Y, Esposito D, Komarnytsky S, David R, et al (2007). Polyphenolic compounds from *Artemisia dracunculus L*. inhibit PEPCK gene expression and gluconeogenesis in an H4IIE hepatoma cell line. *Am J Physiol Endocrinol Metab* 293: E1503–E1510.

[6] Hall JB, and Waljer DH (1991). In: Daya LC and Vaghasiya HU (2011). A review on Balanites aegyptiaca Del (desert date): phytochemical constituents,traditional uses, and pharmacological activity. *Pharmacogen rev*. 5(9): 55-62.

[7] Hall JB (1992). Ecology of a key African multipurpose tree species Balanites aegyptiaca Del. The state of knowledge. *Forest Ecol Manag*., 50: 1-30.

[8] Jung MY, Jeon BS, Bock JY (2002). Free, esterified and insoluble bound phenolic acids in white and red ginsengs (Panax ginsengC.A. Meyer). *Food Chem*,79:105–111.

[9] Khathi, A.; Serumula, M.R.; Myburg, R.B.; Van Heerden, F.R.; Musabayane, C.T (2013). Effects of *Syzygium aromaticum*-derived triterpenes on postprandial blood glucose in streptozotocin-induced diabetic rats following carbohydrate challenge. *Plos ONE*, 8, e81632.

[10] Meda, A., Lamien, CE., Romito, M., Millogo, J., Nacoulma, OG (1999). Determination of the total phenolic, flavonoid and praline contents in Burkina Fason honey, as well as their radical scavenging activity. *Food Chemistry*, 91:571-7.

[11] Mota, FL., Queimada AJ, Pinho SP, Macedo EA(2008). Aqueous solubility of some natural phenolic compounds. Indust Eng Chem Res, 47:518-5189.

[12] Newmann DJ and Cragg CM (2007). Natural product as source of new drug over last 25 years. *J Nat Prod*., 70:461-477.

[13] OECD Guidelines for testing chemicals 425. Acute oral toxicity; up-and-down procedure. *Organization of Economic Company and development* (*OECD)*, 1998a. Paris.

[14] Salwa AM, El Hadidi MN. Flavonoids of *Balanites aegyptiaca* (Balanitaceae) from Egypt. *Plant Syst Evol.* 1988;160:153–158.

[15] Sarker SD, Bartholomew B, Nash RJ. Alkaloids from *Balanite aegyptiaca*. *Fitoterapia*. 2000;71:328–330.

[16] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. (2011). Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *Afr J Tradit Complement Altern Med*., 8(1): 1–10.

[17] Singleton, V.L., Orthofor, R., and Lamuel-Ranevtos, R.M (2002). Analysis of total phenol and other oxidation substrates and antioxidants by means of Folin-ciocalteauls reagent.*Met. Enzymology*, 299:152-157.

[18] Tain, C.(2013). Green-tea polyphenols reduced fat deposits in high fat-fed rats via erkil 2-PPARY-adiponectin pathway (Abstract). *Plos One*, 8 (1): 53796.

[19] Wang Y, Xiang L, Wang C, Tang C, He X (2013). Antidiabetic and antioxidant effects and phytochemicals of Mulberry fruit (Morus alba L) polyphenol enhanced extract. *Plos One*, 8(7): e71144.

IJIRAS