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Chemical Composition of *Newbouldia Laevis* (P. Bauv) Used in the Treatment of Diabetes Mellitus in Nigeria

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Abstract

Diabetis mellitus (DM) is a chronic metabolic disease that affects both Humans and animals. The prevalence of DM is on the increase especially in the developing countries due to sedentary lifestyle and nutrition habits. Most people in these countries and elsewhere that suffers from chronic diseases take some form of herbal drugs either alone or in combination with orthodox medicine. These herbal drugs act based on their phytochemical composition. In this work, we examine the composition of one of the plants *Newbouldia laevis,* used in the treatment of diabetes in Africa generally in folk medicine. The result reveals the presence of alkaloids, Saponins, flavonoids, glycosides, tannins and cyanide (21.73±0.36, 15.99±0.04, 40.78±0.27, 12.13±0.01, 0.088±0.06 and 1.03±0.02)% respectively. It also contain both heavy metals which are implicated in diabetes such as Lead (29.80±2.84 mg/100g) and Mercury (12.62±0.02 PPM) as well as trace elements such as selenium (11.51±0.5) mg/100g and chromium (8.31±0.03) PPM which are beneficial in the treatment of diabetes. The plants also contain Vitamins A, B_1, B_2, B_3, C and E in that order (1190.3±0.41 iu/l, 5.50±0.05 mg/100g, 0.95±0.05 mg/100g, 1.93±0.07 mg/100g, and 1.92±0.02% and 5.81±0.03 mg/kg respectively in reasonable quantities. The proximate analysis indicated the presence of carbohydrate, protein, lipids, fibre, ash and moisture in various percentages. It is therefore observed that the pharmacological activities of the extract may principally be due to its phytochemical, elemental and Vitamin compositions. It is important to be careful of the heavy metals and cyanide presences which may pose some health challenges in prolong usage.

Keywords: *Newbouldia laevis;* Phytochemicals; Vitamins; Elements; Proximate; Diabetes

Introduction

The universal role of plants in the treatment of diseases is exemplified by their employment in all the major branches of medicine irrespective of the underlying philosophical premise throughout history and continue to serve as the basis for many pharmaceuticals today [1]. How and when such medicinal plants were first used, is in many cases lost in prehistory [2]. In view of the above facts, the World Health Organization (WHO) has recognized the role of herbal or traditional medicine in primary health care, especially in developing countries and has encouraged member nations to develop national policies for proper identification, sustainable exploitation, scientific development and appropriate utilization of herbal medicines appropriate for their situations [3]. Diabetes is defined as a pathophysiologic condition in which there is excessive glucose in the blood due to disturbance in homeostasis of carbohydrate, protein and lipid metabolism regulated by the hormone insulin [4]. Diabetes mellitus has been reported in several animals including dogs and cats, sheep, cattle horses, pigs and virtually all laboratory animals like guinea pigs, hamsters, mice, rats and non-human primates and the incidence seems to be on the increase [5-11]. The high prevalence rate of diabetes and its attendant high cost on healthcare have necessitated search for cheaper, effective and readily available alternatives in plants. *Newbouldia laevis* (P. Beauv) Seeman ex Bureau also known as "Border" or "Boundary tree" is one of such plants. It belongs to the family Bignoniaceae and the genus Newbouldia [12]. It has various ethnic names in Nigeria such as "Aduruku" or 'Bareshi" in Hausa, "Ogirisi" in Igbo, "Akoko" in Yoruba, and"Kontor" in Tiv languages. In Ivory Coast, the Fula-Fulfulde calls it "Sokunde" and it is known as "Sesemasa" by the Akan-Asante in Ghana [12-15]. It is widely used in traditional medicine in Nigeria and other west African countries to treat various diseases [13,16-18]. The effects of these plants are based on their phytochemical constituents. Plants contain an array of phytochemicals that are proven to be pharmacologically active and have been used in the management of

many diseases of man and animals [2]. The aim of this work was to assess the phytochemical composition of the plant *Newbouldia laevis* with a view to understanding the basis for its folkloric uses in the treatment of diabetes and other diseases as well as to assess the safety level based on its composition.

Materials and Methods

One kilogram (1 kg) of the leaves of *Newbouldia laevis* (NLE) were collected and identified by Mr. Terry Waya with a specimen number UAM/FHM/205 deposited at the Forestry hebarium at the University of Agriculture, Makurdi. The leaves were grinded and extracted by cold maceration using 80% aquous methanol. Rotary evaporator (Rotavapor-R-215) was used to concentrate and dry the extract *in vacuo* and the percentage yield was calculated.

A comprehensive qualitative phytochemical analysis of the plant extract was carried out. The methods of Harbourne, (1991) and Evans (2009) were used [2,19]. Two (2) grammes of *Newbouldia laevis* leaf extract was dissolved in 40 ml of distilled water to obtain a concentration of 50 mg/ml and was used to test for starch, carbohydrates, alkaloids, saponins, flavonoids, polyuronoids, tannins and sterols/terpenes. A quantitative analysis of some of the phytochemicals was also carried out as described by the Association of Organic and analytical Chemists [20]. The phytochemicals assessed include alkaloids, flavonoids, saponins, hydrocyanide (HCN) reducing sugars and tannins.

Elemental/mineral Analyses

The mineral and elemental analyses of the NLE were carried out using atomic absorption spectrophotometer (AAS) (Schimadzu – Japan). The aim was to identify heavy metals which are sometimes involved in toxicities and are implicated in diabetes as well as some pharmacologically important trace elements that improve the function of β-cells and are beneficial in the management of diabetes [2,21]. The method of the Association of Organic and Analytical Chemists was adopted. The following elements were assessed quantitatively [20]. Aluminium (Al), Lead (Pb), Mercury (Hg), Cadmium (Cd), Zinc (Zn), Copper (Cu), Calcium (Ca), Chromium (Cr), Iron (Fe), Magnesium (Mg), Sodium (Na), Potassium (K) and Selenium (Se).

Vitamin Analysis

Vitamin composition of NLE was carried out with a view to assessing particularly the antioxidant vitamins such as vitamins A, C, E and the B groups. The AOAC (2006) guidelines for vitamin analysis were adapted: Vitamin A was estimated by colorimetric method [18]. This involved reacting the extract with Antimony trichloride that converts the vitamin into a coloured compound whose absorbance was measured and compared against a standard. Vitamin C was estimated by titrimetric method. The extract was titrated against the dye DPIP (2, 6-dichlorophenolindophenol). Vitamin C reduced this dye from blue to a colorless form. The dye was standardized against a solution of vitamin C of known concentration, and then the extract was titrated and assayed. The B group and vitamin E were estimated by fluorimetric method. This method is used in assaying vitamins with natural fluorescence such as riboflavin by measuring the degree of fluorescence of the extract and comparing it against a set of standards. Vitamins that do not possess natural fluorescence were converted to fluorescent derivatives and their concentration determined.

Proximate Analysis

The proximate analysis of NLE was carried out by Kjeldahl's method.The analysis consists of moisture, ash, fibre, fats/oil, protein and carbohydrate. This was done in line with the AOAC (2006) guidelines [18].

Moisture

A clean crucible was dried to a constant weight in an air oven at 105 °c, cooled in a dessicator and weighed (W1). Two (2) g of finely ground sample of NLE was accurately weighed into the previously labelled crucible and weighed (W2). The crucible containing the sample was dried in an oven to a constant weight (W3). The percentage moisture content was calculated thus:

$$\% \text{ Moisture content} = (W2-W3)/(W2-W1) \times 100$$

Ash

A porcelain crucible was dried in an oven at 100 °c for 10 minutes, cooled in a dessicator and weighed (W1). Two (2) g of the finely ground sample was placed into the previously weighed porcelain crucible and reweighed (W2). It was first ignited and then transferred into a furnace, which was then set at 550 °C. The sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed cooled in the dessicator and weighed (W3). The percentage ash content was calculated as:

$$\% \text{ Ash Content} = (W3-W1)/(W2-W1) \times 100$$

Crude Lipid

A clean, dried 500 ml round bottom flask, containing few anti-bumping grnules was weighed (W1) and 300ml of petroleum ether (40-60 °C) for the extraction was poured into the flask fitted with soxhlet extraction unit. The extractor thimble containing twenty grams (20 g) was fixed into the soxhlet extraction unit. The round bottom flask and a condenser were connected to the soxhlet

extractor, and cold water circulation was put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for six hrs. The solvent was recovered and the oil was dried in the oven at 70 °C for one hour. The round bottom flask and oil was cooled and then weighed (W2).
The lipid content was calculated thus:

% crude lipid content =(W2-W1)/(Wt of sammple) ×100

Crude Fibre

Two grammes (2 g) of finely ground NLE was weighed out into a round bottom flask, 100 ml of 0.25M sulphuric acid solution was added and the mixture boiled under reflux for 30 mins. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. It was transferred into a flask and 100 ml of hot 0.31M sodium hydroxide solution was added and the mixture boiled again under reflux for 30 mins and quickly filtered under suction. The soluble residue was washed with boiling water until it was base free. It was dried to constant weight in the oven at 100 °C, cooled in a dessicator and weighed (C1).

The weighed sample (C1) was the incinerated in a muttlefurnce at 550 °C for 2 hours, cooled in the dessicator and reweighed (C2).

Calculation
The loss in weight on incineration = C1 – C2. The calculation was carried out thus:

% crude fibre = = (C1-C2)/(Weight of original sample) ×100

Protein

The technique involves digestion of the compound with sulphuric acid in which each nitrogen atom in the original molecule produces one molecule of ammonia and the ammonia is then collected in boric acid and titrated with 0.1M HCl. The crude protein is calculated thus:

% N2 = (14 ×M ×V1 × Tv ×100)/(Wt of sample (mg)×Va)

% crude protein = % nitrogen (N2) x 6.25

Where M = actual molarity of acid

Tv = titre volume of HCl used

V1 = total volume of diluted digest

Va = aliquot volume distilled

Carbohydrade

The total carbohydrate content was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100 thus:

% Total carbohydrate = 100 - % moisture + % ash + % fat + % protein + % fibre.

Result

A complete phytochemical, elemental/mineral, Vitamin and proximate analysis of NLE are presented in Tables 1-4. All the results were read in triplicate.

Phytochemical composition of NLE

The qualitative and quantitative phytochemical composition of NLE is presented in Tables 1A and B respectively. The major phytochemicals found in NLE include: flavonoids, alkaloids, saponins, glycosides/reducing sugars, sterols/terpenes and even cyanides but no starch.

S/No	Tests	Inference
1	Starch	-
2	Carbohydrate:	+
3	Alkaloids	++
4	Saponins	++
5	Flavonoids	++

S/No	Tests	Inference
6	Glycosides/ reducing sugar	+
7	Tannins	+
8	Sterols/terpenes	+
9	Polyuronoids	+

Table 1A: Qualitative assessment of Phytochemicals present in NLE
Key: - Absence; + Present moderately; ++ Present aboundantly

S/No	Phytochemical	Unit	Mean ± SEM
1	Flavonoids	%	40.78±0.27
2	Alkaloids	%	21.73±0.36
3	Saponins	%	15.99±0.04
4	Glycosides/ Reducing sugars	%	12.13±0.01
5	Tannins	%	0.088±0.06
6	Cyanides	%	1.03±0.02

Table 1B: Quantitative assessment of phytochemicals present in NLE

Elemental/Mineral Composition of NLE

The elemental/mineral composition of NLE is presented in Table 2. The table showed that NLE contains some of the heavy metals like lead, cadmium and mercury but not aluminium. It also contains some trace elements such as zinc, calcium, selenium, potassium, chromium etc. in reasonable quantities.

S/No	Element	Unit	Mean ± SEM
1	Lead	Mg/100g	29.80±2.84
2	Mercury	PPM	12.62±0.02
3	Cadmium	PPM	9.44±0.5
4	Copper	Mg/100g	23.16±0.07
5	Calcium	Mg/100g	0.493±0.007
6	Chromium	PPM	8.31±0.03
7	Zinc	Mg/100g	1250.3±0.05
8	Iron	Mg/100g	6.34±0.44
9	Magnesium	Mg/100g	0.89±0.01
10	Sodium	PPM	107.3±2.7
11	Potassium	PPM	421±1.5
12	Selenium	%	11.51±0.5
13	Aluminium	%	0.00±0.0

Table 2: Elemental/mineral composition (heavy metals and trace elements) of NLE

Vitamin Analysis of NLE

The vitamin composition of NLE is presented in Table 3. The result showed that NLE contains 1190.3±0.41 iu/l of vitamin A, 1.92±0.02% of vitamin C and 5.81±0.03 mg/100g of vitamin E. For the B group of vitamins, NLE contains 5.50±0.05 mg/100g of vitamin B1, 0.95±0.05 mg/100g vitamin B2 and 1.93±0.07 mg/100g vitamin B3.

S/No	Element	Unit	Mean ± SEM
1	Vitamin A	iμ/L	1190.3±0.407
2	Vitamin B1	Mg/100g	5.50±0.05
3	Vitamin B2	Mg/100g	0.95±0.05
4	Vitamin B3	Mg/100g	1.93±0.07
5	Vitamin C	%	1.92±0.02
6	Vitamin E	Mg/kg	5.81±0.03

Table 3: Vitamin composition of NLE

Proximate Analysis of NLE

The proximate analysis of NLE is presented in Table 4. It showed that NLE contains 77.97±0.02% Moisture, 5.20±0.01% Ash, 0.07±0.01% Fibre, 9.2±0.20% Lipids 6.32±0.01% Protein and 1.21±0.18% Carbohydrate.

S/No	Element	Unit	Mean ± SEM
1	Moisture	%	77.97±0.02
2	Ash	%	5.20±0.01
3	Fibre	%	0.07±0.01
4	Fat/Oil	%	9.2±0.20
5	Protein	%	6.32±0.01
6	Carbohydrate	%	1.21±0.18

Table 4: Proximate Analysis of NLE

Discussion

Phytochemicals which are mostly secondary metabolites of plants boisynthesis are primarily used by the plants for various purposes including defence and protection [22,23]. Many of these phytochemicals have been reported to have various pharmacological effects including hypoglycaemic activities. They includes flavinoids, tannins, saponins, alkaloids and glycosides [2,23]. In this study, it can be seen that NLE contains most of these constituents (Table 1A). A quantitative assessment of these phytochemicals also showed that NLE contains 40.78±0.27% flavonoids, 21.73±0.36% alkaloids, 15.99±0.04% saponins, 12.13±0.01% glycosides/reducing sugars, 0.088±0.06% tannins and even 1.03±0.02 cynanide (HCN) (Table 1B). Flavonoids are known with various pharmacological effects including antihyperglycaemic, anti-inflammatory and antioxidant activities [2,23]. Therefore the high level of flavonoids seen in NLE can be responsible for all or some of these pharmacological activities in NLE. Alkaloids apart from being used by the plants primarily for defence purposes also have various pharmacological activities. Some of which include antidiabetic, anti-inflammatory, analgesic, antidiarrhoeas etc [2,23]. A lot of them are used as bitters to stimulate the digestive system and are very popular in traditional medicine in the present times. Saponins have various pharmacological activities including bacterial and antiparasitic effects in addition to its hemolytic effect [22]. Tannins are used traditionally to precipitate proteins as well as antidiarrhoeas. They also help in wound healing processes [23]. These phytochemicals are also known to possess other properties like free radical scavenging effects and rejuvenating potentials. These effects are beneficial in chronic diseases management including diabetes and cancers [24]. Therefore, the hypoglycaemic effect of NLE may not be unconnected to the presence of these phytochemicals. Other workers like Ogunlana and Ogunlana, 2008; Tanko et al., 2008 and Kolawole and Akanji, 2013, have reported the presence of these phytochemical and the antidiabetic effect of *Newbouldia laevis.*

Elemental and mineral analysis revealed two major classes of elements that are important in diabetes and other disease processesw. They include the heavy metals like lead, mercury and cadmium but not aluminum. The second group of trace elements is potassium, zinc, calcium, chromium, selenium, manganese and iron. The presence of heavy metals and in large quantities especially leads and mercury, poses a serious health challenge in the use of plants for treatment of diseases. This is because some fatalities have been reported from their uses [25]. The heavy metals are implicated in diabetes. Heavy metals like lead, mercury, arsenic, cadmium and aluminum plays a role in the actual destruction of β-cells through stimulating an autoimmune reaction to the β-cells after binding to these cells in the pancreas. Mercury and lead in addition attach themselves to highly vulnerable junctions of proteins that they find great capacity to provoke morphological changes in the body [26]. The trace elements are believed to have beneficial effects in the treatment of diabetes and other diseases [2]. In the present study NLE contained more of the trace elements in reasonable quantities (Table 2).These trace elements may also contribute to the overall anti diabetic effect of NLE.

Antioxidants are secondary constituents or metabolites found naturally in the body and in some plant parts such as fruits and vegetables. They inhibit or prevent oxidation of susceptible substrate [27,28]. Common antioxidants include vitamins A, C and E, and some compounds like carotenoids (lutin, β-carotene) and flavonoids found in plants [29]. These plant-based dietary antioxidants play an important role in the maintenance of human/animal health because endogenous antioxidants provide insufficient protection against the constant and unavoidable challenges of reactive Oxygen species (ROS) [30]. There are lots of evidences that ROS is increased in both diabetes and other chronic diseases, manifested by increase in oxygen free radicals such as superoxide (SO_2) hydrogen peroxide (H_2O_2) and hydroxide (OH---) radicals and a deficiency in antioxidant defense mechanisms [31-33]. Numerous plants have been mentioned to possess this antioxidant properties including NLE [15,17]. In this study NLE had shown to possess a significant quantity of the above mentioned vitamins including members of the vitamin B group (Table 3). These are expected to contribute to the antioxidant properties of the NLE [18]. This also agrees with Montenon, *et al.*, (2004) and Hana *et al.,* (2006). It is believed that if sufficient antioxidants are made available in the body they can help in prevention and management of chronic degenerative diseases [27,28]. Proximate analysis is conventionally used to assess the food value of feed subtances [20]. The proximate analysis of NLE also showed it to contain protein, lipids, ash in reasonable quantities as well as carbohydrate, fibre and lot of moisture. (Table 4). The presence of carbohydrate was earlier observed in the phytochemicals, though

not much has been confirmed qu`antitatively in the proximate analysis. The presence of lipids in NLE was also reported earlier. Two out of ten fractions obtained from NLE using a combination of column and thin-layer chromatography were oily at room temperature [18]. The proximate analysis also showed that NLE contain some proteins.

Conclusion

The phytochemical, elemental/mineral, vitamins and proximate analysis of NLE has amply demonstrated that it contain some phytochemicals, minerals and vitamins in reasonable proportions that are capable of managing diabetes and other diseases as used in folk medicine in Nigeria and such folkloric uses have pharmacological basis. It is however important to employ caution in its long term use because of the high level of some heavy metals such as lead and mercury. The presence of cyanide in it also is of toxicological concerns.

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References

1. Wagner H, Ulrich-MG (2009) Synergy research in approaching a new generation of phytopharmaceuticals. Phytomedicine 16: 97-110.

2. Evans WC (2009) Trease and Evans Pharmacognosy (16th edn). Saunders Elsevier: 436-44.

3. WHO (2005) National policy on traditional medicine and regulation of herbal medicines. Report of WHO global survey: 168.

4. Rakesh, B, Sanjay J, Deep O, Armit J, Girraj ST, et al. (2010) Antidiabeticactivity of aqueous root extract of Inchnocarpusfruitescens in STZ and Nicotinamide-induced Type 2 diabetes in rats. Indian J Pharmacol 40: 19-22.

5. Lang CM, Mungar BL (1976) Diabetes mellitus in the Guinea pig. Diabetes 25: 434-43.

6. Kaneko T (1997) Troglitazone (CS-045): A new antidiabetic agent. Horm.Metab Res 29: 203-13.

7. Tajima M, Yuasa M, Kawanabe M, Taniyama H, Tamato O, et al. (1999) Possible causes of diabetes mellitus in cattle infected with Bovine Viral Diarrhoea Virus. J Vet Med 46: 207-15.

8. Clark Z (2003) Diabetes mellitus in a 6-month old Charolias heifer calf. Can Vet J 44: 921-2.

9. Catchpole B, Ristic JM, Fleeman LM, Davison LJ (2005) Canine diabetes mellitus: Can old dogs teach us new tricks. Diabetologica 48: 1948-56.

10. Durham AE, Huges KJ, Cottle HJ, Rendle DI, Boston RC (2009) Type 2 diabetes mellitus with Pancreatic β cell dysfunction in 3 horses confirmed with minimal model analysis. Equine Vet J 9: 924-9.

11. Khan MC (2010) Diabetes mellitus In: The merck's Veterinary Manual (10th edn): 439-40.

12. Keay RWJ (1989) Trees of Nigeria.Clarendon Press Oxford 432-6.

13. Burkil HM (1994) the useful plants of West Tropical Africa. Families A-D. Royal Botanical Gardens. Kew.Vol 4.

14. Nigeria Natural Medicine Development Agency (NNMDA) (2006) Medicinal Plant of South-West Zone Vol.1 NNMDA Publication: 26.

15. Ogunlana OI, Ogunlana OO (2008) In vitro assessment of antioxidant activity of Newbouldia Laevis J Med Plant Res 2: 176-9.

16. Tanko Y, Okasha MA, Saleh MIA, Mohammed A, Yerima M, et al. (2008) Antidiabetic effects of the ethanolic flower-extracts of Newbouldialaevis on blood glucose level in Streptozotocin- induced diabetic Wistar rats. Medwell Research J Med Sci 2: 62-5.

17. Kolawole OT, Akanji MA (2013) Anti-inflammatory and Antioxidant activities of Ethanolic extract of leaves of Leaves of Newbouldialaevis in Diabetic Rats. SCRO Annual Res J 1: 82-8.

18. Bosha JA (2015) Pharmacological effects and anti-diabetic constituents of the methanol leaf extract of Newbouldialaevis (P.Beauv) A PhD Thesis submitted to the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka: 171-3.

19. Harbourne JB (1991) phytochemical methods. A guide to modern techniques of plant analysis (2nd edn). Chapman and Hall, London: 222-36.

20. Association of Official Analytical Chemist (AOAC) 2006. The Official publication.Vol 10

21. Surcus M (2008) Alloxan that makes flour white and clean (bleach) can cause diabetes. Alloxannews Accessed on 23 June 2013.

22. Wagner H, Bladt S, Zgainski EM (1984) Plant Drug Analysis: A Thin Layer Chromatography Atlas. Translated by Th A Scott. Springer-Verlag Berlin: 125-78.

23. Bruneton J (1994) Pharmacognosy, Phytochemistry, Medicinal plants (2nd edn). Lavoisier Publishing, New York: 1-880.

24. Hana M, Zdenek S, Pavel D, Jitrenka JI Ondrej C. (2006). Absence of breast feeding is associated with the risk of Type 1 diabetes: A case-control study in a population with rapidly increasing incidence. European Journal of Pediatrics 165: 114-9.

25. Park M, Choi H, Kim J, Lee H, Ku S (2010) Twenty eight days repeated oral dose toxicity test of aqueous extracts of Mahwangyounpaeteng, a polyherbal formula. Food Chem Toxicity 48: 2477-82.

26. Zimmet P (2007) ABC health and wellbeing. International Diabetes Institute.

27. Parezo I, Viladomat F, Bastida J, Rosas-Romero A, Fieriage N, et al. (2002). Comparison between the free radical scaverging activity and antioxidant activity of six distilled and non-distilled Mediterranean herbs and aromatic plants. J. Agric Food Chem 50: 6882-90.

28. Sadighara P (2009) Tool for oxidant agents screening test. Austr J Basic Appl Sc 10: 2070-3.

29. Hayer MG (2000) Dietary Vitamin E improves immune function in cats In: Recent Advances in Canine and Feline Nutrition WB Saunders: 120-7.

30. Fridorich I (1998) Oxygen toxicity, a radical explanation.The J Experi Biol 201 : 1203-9.

31. Johansan JS, Harris AK, Richly DJ, Ergul A (2005) Oxidative stress and the use antioxidants in diabetes: Linking basic science to clinical practice. Cardiovascular Diabetology 4: 5-9.

32. Montenon J, Knekt P, Javein R, Reunanen A (2004) Dietary antioxidant intake and risk of Type 2 diabetes.Diabetic Care 27: 362-7.

33. Schneider E (2004) Encyclopedia of Medicinal Plants. Pamplona-Rogers Editorial Safeliz Spain: 19.