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Comparative Analysis of Three Nutraceutic Plants used in the Treatment of Type 2 Diabetes in Traditional Pharmacopoeia of Cameroon

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Abstract

Many modes of action have been explored in the fight against type 2 diabetes, including the use of drugs. But these drugs, in addition to their relatively high cost, are not without side effects. As alternative to these difficulties, traditional pharmacopoeia uses several nutraceutic plants in the treatment of type 2 diabetes, including: Vernonia amygdalina, Tetrapleura tetraptera and Leptadenia lancifolia decne. The fact that those plants are used for the same treatment, means that they contain similar contents. For this reason, the goal of this study targets the comparative analyses of bioactive compounds of Vernonia amygdalina, Tetrapleura tetraptera and Leptadenia lancifolia decne that can present similarity and justify their use in the treatment of type 2 diabetes, as well as to classify them based on their phytochemical characteristics. To overcome this, the phytochemical composition of those plants was assessed by using classical methods. The results show similitude values in some phytochemical parameters. In generally, Tetrapleura tetraptera has more energetical compounds compared to Vernonia amygdalina and Leptadenia Lancifolia Decne. Mineral contents, Vernonia amygdalina showed high level of magnesium and calcium. Zinc levels are similar in Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia Lancifolia Decne. Anti-nutritionals analyses revealed that Vernonia amygdalina has high levels of tannin, oxalate and saponine. Leptadenia Lancifolia Decne presents an important level of phytate and flavonoids. Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia Lancifolia Decne have comparable value of alkaloids and total polyphenol. The presence of those bioactive compounds in these plants can explain their use in the treatment of type 2 diabetes. For this reason, more investigations are needed to assess antioxidant and antidiabetic effects in view of formulation of nutraceutic drugs.

Keywords: Comparative analyses; nutraceutic plants; treatment; type 2 diabetes.

Introduction

Type 2 diabetes is a chronic disease that occurs when a person's blood sugar level is high because their body cannot effectively use the insulin it produces. It usually affects people aged 20-79 years and accounts for about 90% of diabetes cases worldwide. Type 2 diabetes is the fifth leading cause of death worldwide, raising the alarm and classifying it as a public health problem [1]. This problem needs specific management which consists of lifestyle changes, followed by pharmacological treatment including insulin if necessary [2]. Many modes of action have been explored to fight against type 2 diabetes, including blocking the potassium-dependent ATP pump in pancreatic β-cells (Sulfonylureas-Glipizide); stimulation of Peroxisome Proliferator-Activated Receptor-γ (Thiazolidinediones-Rosiglitazone); stimulation of adenosine mono-phosphate-activated protein kinase (Biguanides-Metformin) and modulation of Glucagon Like Peptide-1 activity (Incretins-Exematide). These agents act either by stimulating insulin secretion by β-pancreatic cells (sulphonamides), or by decreasing hepatic glucose production (metformin) or else at the reduction of post prandial blood glucose by inhibiting the activity of intestinal enzymes (α-amylases and α-glucosidases) [2]. However, the drugs, in addition to their relatively high cost, are not without side effects (fatal lactic acidosis (buformin, penformin),

nausea, vomiting and diarrhoea (metformin), visual disturbances, upper respiratory infection, sinusitis and weight gain); as a result of this, many of them, in the USA/Europe, have limited use, are not marketed, have almost restricted prescribing, and are sometimes even withdrawn from the market [3]. It has been reported that only 3 out of 20 patients are able to buy prescribed drugs in hospitals and only 1 out of every 1000 patients is able to consult a specialist [4]. As a result, there is a rich tradition in the use of herbal medicines for the treatment of several ailments and plans are on the way to integrate traditional medicine in the health care system, even though the plans have not been put into action yet [5]. Cameroon however has a rich biodiversity, with ~8,620 plant species [6,7], some of which are commonly used in the treatment of several chronic diseases [8] and a range of neglected tropical diseases, including malaria, trypanosomiasis, leishmaniasis, diabetes, tuberculosis, etc. [4]. As an alternative to these difficulties Cameroonians are using nutraceutical foods. Which are ordinary foods that have components, ingredients, incorporated in them to give them a specific medicinal or physiological benefit other than a purely nutritional effect [9-11]. The economic production and availability of nutraceutical foods are highly desirable objectives to improve the health of the people of the country, especially that of the poor people [9]. Now, nutraceuticals related research for improving its quality and quantity is an important area for ongoing biotechnological investigations [12]. Moreover, the Covid 19 pandemic has proven that in Africa and especially in Cameroon, due to the strong ethnobotanical potential, it is possible to overcome many diseases such as type 2 diabetes. In the traditional pharmacopoeia, several nutraceutic plants are used in the treatment of type 2 diabetes, including: Vernonia amygdalina, Tetrapleura tetraptera and Leptadenia lancifolia decne [13]. Since nutraceuticals or functional foods can be classified based on their natural sources, pharmacological parameters or according to their chemical constitution. Hence phytochemical characterisation of these plants would help to understand their use in the treatment of type 2 diabetes since the part of each plant used is totally different. Therefore, the goal of this study is comparative assessment on phytochemical characterisation of different parts of the plants used in the treatment of type 2 diabetes.

Material and methods

Collection and processing of plant material

The leaves of Vernonia amydalina were collected from a field in the Nkolmesseng district of Yaoundé V. The fruits of Tetrapleura tetraptera were purchased at the Mfoudi market (Yaoundé Cameroon). Leptadenia lancifolia leaves and vines were collected in the Kaele area (Mayo-kani, Far-North Cameroon). The samples were then sent to the Laboratory of Food Science and Metabolism (LabSAM). They were sorted, weighed, put under a stream of water, wrung out and dried in a dehydrator at 45°C until a constant weight was obtained. The dried samples were then crushed and sieved through a 160-micron sieve and the resulting powder was packaged and labelled for phytochemical analysis.

Phytochemical characterization

Phytochemical screening of plants was carried out by El-haoud [14] methods. Water content of powders and dry matter were obtained by the AFNOR method [15]. Total ash and crude fibre content were determined by the AOAC method [16]. Total lipids were extracted with Soxhlet according to the Russian method described by Bourely [17]. The total nitrogen was determined after mineralization of the samples according to the Kjeldahl method [18], and determination according to the colorimetric technique of Devani [19]. Total sugars were extracted and determined according to the method described by Fischer [20]. The energy value of the powders was determined by the Livesey [21] method. The vitamin C content was evaluated by Harris [22] method. The minerals Zn^{2+}, Ca^{2+} and Mg^{2+}, were analyzed according to the method described by Horwitz [23]. Extraction and determination of total phenolic compounds was carried out using the Folin - Ciocalteu reagent as described by Marigo [24]. Flavonoid content was done by the described by de Vinson [25]. Total tannins were assessed by Ndhlala [26] method. Phytate content was done based on Olayeye [27] method. Oxalate content was determined by the modified titration method of Aina [28]. Saponin content was measured by Koziol [29] method.

Statistical analysis

Results were expressed as means ± standard deviation. The result obtained was the mean for three tests. All results were analyzed using a one-way analysis of variance. The data sets were expressed as mean ± standard deviation (n=3). Analysis of variance (ANOVA) was done using One-Way Analysis of Variance to test for the difference in means. Duncan's Multiple Range Test was carried out to test for the means that are significantly different ($p<0.05$) from each other, which are presented by alphabets in superscripts.

Results and Discussion

Phytochemical screening

The qualitative analyses by chemical screening of the samples studied are presented in Table 1. From the results obtained in Table 1, it can be noted that Leptadenia lancifolia contains few secondary metabolites compared to Vernonia amygdalina and Tetrapleura tetraptera. However, there is an absence of anthocyanins in both Leptadenia lancifolia and Tetrapleura tetraptera. On the other hand, a marked presence of flavonoids was tested in Leptadenia lancifolia and Tetrapleura tetraptera compared to Vernonia amygdalina. These results are like those found by Boukhezna [30] on the same plant species.

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Table 1: Results of the phytochemical screening of the plants used.

Contents/Samples	Vernonia amygdalina	Tetrapleura tetraptera	Leptadenia lancifolia
Triterpene and sterol	++	++	+
Polyphenol	++	+	+
Saponin	++	+	+
Anthocyanins	+	-	-
Tannin	++	++	+
Flavonoid	+	++	++
Alkaloid	++	+	+
Anthraquinone	++	++	+

- Negative; +: slightly positive; ++: very positive.

Triterpenes and sterols are more present in Vernonia amygdalina and Tetrapleura tetraptera, while in Leptadenia lancifolia they are moderately present. The same observation is applied to tannins and anthraquinones, which reacted in the same way to the different tests applied. The screening of polyphenols, saponins and alkaloids is more pronounced in Vernonia amygdalina, and less present in Tetrapleura tetraptera and Leptadenia lancifolia. In general, Vernonia amygdalina is richer in secondary metabolites than Tetrapleura tetraptera and Leptadenia lancifolia. This observation is in perfect agreement with the studies of Matar, Nwoba and Mlatovi [31-33].

Nutrient potential of the plants studied.

Table 2 presents the nutritional potential of the plants studied. The analysis of the water contents of the studied plants (Vernonia amygdalina, Tetrapleura tetraptera, Leptadenia lancifolia) reveals that the water contents vary from 6.55±0.40 g/100g DM (Vernonia amygdalina) to 7.84±0.14 g/100g DM (Tetrapleura tetraptera). It is observed that the values of Vernonia amygdalina and Leptadenia lancifolia (6.40±0.84 g/100g DM) do not show any significant difference (p<5%). However, the values of Tetrapleura tetraptera are significantly different (p<5%) from those contained in Vernonia amygdalina and Leptadenia lancifolia. These different values are lower than 79.92 (%); 40.25±0.18 (%) and 80.5 (%) found by Usunobun, N'zebo and Lapo [34-36] respectively on the same plants. Table 2 shows that the protein contents of the plants used are 0.49% (Vernonia amygdalina), 0.27% (Tetrapleura tetraptera) and 0.29% (Leptadenia lancifolia) respectively. The values obtained in the different samples show a significant difference at the 5% threshold. These values are lower than those obtained by Usunobun [34] (19.23%); N'zebo [35] (7.01%) and Lapo [36] (5%) respectively on the same plants.

Table 2: Content of some nutritional elements of the studied plants (g/100g DM)

Contents/Samples	Vernonia amygdalina,	Tetrapleura tetraptera	Leptadenia lancifolia
Water content	6.547±0.398a	7.838±0.143b	6.397±0.843a
Total Protein	0.490±0.003d	0.267±0.000b	0.294±0.003c
Total Sugar	19.239±0.218b	30.195±0.376d	22.742±0.296c
Fat	2.521±0.377b	2.061±0.016b	4.022±1.05c
Fibre	10.203±0.263b	30.997±0.146d	29.330±0.080c
Vitamin C	0.043±0.0001b	0.005±0.0001a	0,042±0.0009b
ΔE	101.6±0.38	140.38±0.64	128.3±0.84

Values are expressed as mean ± standard deviation on the mean (percentage variation of the mean); Values assigned different letters on the same line are significantly different (p<0.05).

The total sugar contents of Vernonia amygdalina, Tetrapleura tetraptera, Leptadenia lancifolia are respectively 19.24±0.22%; 30.19±0.38% and 22.74±0.30%. Duncan's test shows that there is a significant difference (p<5%) between the total sugar contents of the tested samples. These values are all lower than the contents obtained by Usunobun [34] (68.35%) and N'zebo [35] (63.73±0.5%), but higher than 11.3% determined by Lapo [36] respectively on the same samples. The lipid contents of Vernonia amygdalina, Tetrapleura tetraptera and Leptadenia lancifolia samples presented in Table 2 are 2.52±0.38; 2.06±0.02 and 4.02±1.06 respectively. According to Duncan's test, there is no significant difference (p<5%) between the lipid contents

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of Vernonia amygdalina and Tetrapleura tetraptera. However, a significant difference is revealed between the lipid content of Leptadenia lancifolia and those of Vernonia amygdalina and Tetrapleura tetraptera. The lipid contents assessed in Vernonia amygdalina and Tetrapleura tetraptera were lower than those obtained by Usunobun [34] (4.70%); N'zebo [35] (3.40±0.18%) respectively. In contrast, the lipid content of Leptadenia lancifolia was higher than that found by Lapo [36] (0.13%) on the same samples. Evaluation of the fibre content of Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia lancifolia gave the values 10.20±0.26%; 31.0±0.15% and 29.33±0.08%. There was a significant difference (p<5%) between the fibre contents of the samples analysed. The values obtained with Vernonia amygdalina, Tetrapleura tetraptera are higher than those determined by Usunobun [34] (9.75%) and N'zebo [35] (11.78%) respectively on the same plants.

The vitamin C content of the different samples ranged from 0.01% (Tetrapleura tetraptera) to 0.04% (Vernonia amygdalina and Leptadenia lancifolia). Duncan's test showed a significant difference (p<5%) between the vitamin C content of Tetrapleura tetraptera, and that of Vernonia amygdalina and Leptadenia lancifolia. The latter two showed no significant difference (p<5%) between them. These vitamin C contents determined in Vernonia amygdalina and Leptadenia lancifolia are within the recommended range for daily intake of ascorbic acid (20-40mg/day) in adolescents. In addition, it should be noted that the vitamin C contents of the plants studied are lower than those found by Usunobun [34] (0.23%) and N'zebo [35] (0.42±0.01%) but higher than those found by Lapo [36] (0.0076%) on these same plants.

The energy values of the different plants are respectively 101.6±0.38 kcal/100g (Vernonia amygdalina), 140.38±0.64 kcal/100g (Tetrapleura tetraptera) and 128.3±0.84 kcal/100g (Leptadenia lancifolia). The energy values obtained with the different plants show a significant difference (p<5%) between them. These energy values are lower than the 446.67 kcal/100g obtained by Focfack [37] on a compound formulated from Zingiber officinale rhizomes, Moringa oleifera Lam leaves, Stevia rebaudiana Bertoni leaves, Sodium guava fruits and Hibiscus sabdariffa flowers. The comparative analysis of nutritional compounds shows that Tetrapleura tetraptera is the most energetic plant compared to Vernonia amygdalina and Leptadenia Lancifolia Decne.

Mineral and ash content

Table 3 shows the ash and mineral contents of the plants studied. Analysis of total ash of Vernonia amygdalina, Tetrapleura tetraptera and Leptadenia lancifolia reveals values of 10.270±0.919%; 2.323±0.123% and 4.277±0.135% respectively. These values are significantly different (p< 5%) between them. The ash content of Vernonia amygdalina and Leptadenia lancifolia are close to the contents found by Usunobun [34] (10.22%); Lapo [36] (4.35%) and Musa [38] (2-4%) respectively. It is also noted that the value estimated with Tetrapleura tetraptera is lower than that found by N'zebo [35] (5.38±0.18%). Table 3 reveals that the magnesium contents of Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia lancifolia are 0.532±0.007; 0.203±0.042 and 0.340±0.002% respectively. Duncan's test shows a significant difference (p<5%) between the different magnesium contents obtained. The magnesium contents of Vernonia amygdalina, Tetrapleura tetraptera, are higher than those found by Usunobun [34] (0.088%) and N'zebo [35] (0.141%) respectively on these two plants. The calcium contents presented in Table 3 reveal a significant difference (P<5%) between Vernonia amygdalina (0.355±0.005%) and Tetrapleura tetraptera (0.158%). The same observations are noted with the values obtained in Leptadenia lancifolia (0.248±0.002%). These values are lower than those recommended daily, which are between 500mg-1200mg [39].

Table 3: Ash and mineral contents of the plants studied (g/100g).

Contents/Samples	Vernonia amygdalina,	Tetrapleura tetraptera	Leptadenia lancifolia
Total ash	10.270±0.919c	2.323±0.123a	4.277±0.135b
Mg2+	0.532±0.007c	0.203±0.042a	0.340±0.002b
Ca2+	0.355±0.005c	0.158±0.00a	0.248±0,002b
Zn2+	0.002±0.000a	0.008±0.006a	0.001±0.000a

Values are expressed as mean ± standard deviation on the mean (percentage change of the mean); Values assigned different letters on the same line are significantly different (p<0.05).

The evaluation of the zinc content shows that the values of the different samples are 0.002%; 0.008±0.006% and 0.001±% respectively. Duncan's differential analysis shows no significant difference (p<5%) between these different values. The Zinc contents of Vernonia amygdalina, and Tetrapleura tetraptera are approximately equivalent to the recommended values of 3 and 8 mg/day respectively for children, men, and women [40]. But the values obtained with Leptadenia lancifolia is lower. A comparative study of the minerals in the plants Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia Lancifolia Decne shows that Vernonia amygdalina has higher magnesium and calcium contents. At least it is noted that Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia Lancifolia Decne have comparable zinc contents.

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Secondary metabolites and anti-nutrients

Table 4 shows the secondary metabolite and anti-nutrient contents. From the results in Table 4, the tannin contents of the plants studied are 7.0±0.001mg leu eq/100g; 0.4±0.001mg leu eq/100g; 0.013±0.000 mg leu eq/100g for Vernonia amygdalina, Tetrapleura tetraptera and Leptadenia lancifolia respectively. The analysis of variance shows that there is a significant difference (p<5%) between the values obtained. These tannin contents are below the 10% dry matter value which could cause a reduction in voluntary intake by goats [41].

Table 4: Secondary metabolite and anti-nutrient content of plants.

Contents/Samples	Vernonia amygdalina	Tetrapleura tetraptera	Leptadenia lancifolia
Tannin (mg leu/100g)	7.00±0.01c	4.01±0.001b	0.013±0.001a
Oxalate (mg/100g)	0.9±0.002c	0.08±0.002b	0.05±0.001a
Phytate (mg eq AP/100g)	8.50±0.03b	6.2±0,02a	11.51±0.051c
Saponin (mg/100g)	3.1±0.01c	0.97±0.002b	0.42±0.004a
Alkaloid (mg/100g)	1.01±0.00a	1.01±0.000a	1.02±0.000a
Total polyphenols (mg eq AG /100g)	2.00±0.00a	2.00±0.000a	2.00±0.000a
Flavonoid (mg eq EQ/100g)	119.0±0.000a	340.0±0.001b	478.01±0.004c

GA: gallic acid; EQ: quercetin; PA: phytic acid; leu: leucocyanidin. The values are expressed as mean ± standard deviation on the mean (percentage of variation of the mean); Values with different letters on the same line are significantly different (p<0.05).

It is also noted that the oxalate contents obtained are lower than the quantity of oxalate tolerated by the body, which is 200 to 500 mg [42]. These values are 0.9±0.002 mg/100g; 0.08±0.002 mg/100g; 0.05±0.001 mg/100g respectively for Vernonia Amygdalina, Tetrapleura Tetraptera and Leptadenia Lancifolia. Duncan's test shows that there is a significant difference (p<5%) between these different values.

These same observations are also highlighted in the analysis of phytate content. The Duncan test therefore shows a significant difference (p<5%) between these different values. These values are distributed as follows: Vernonia amygdalina (8.5±0.03mg eq AP/100g Ms), Tetrapleura tetraptera (6.20±0.02 mg eq AP/100g) and Leptadenia lancifolia (11.51±0.51 mg eq AP/100g). These Phytate contents are well below the maximum acceptable dose for humans which is 250 to 500 mg/100g [43]. Saponin contents ranged from 3.1±0.01mg/100g; 0.97±0.002 mg/100g; 0.42±0.004 mg/100g for Vernonia amygdalina, Tetrapleura tetraptera and Leptadenia lancifolia respectively. The analysis of variance shows that there is a significant difference (p<5%) between these values obtained. All these saponin levels are below the LD50 of this compound which is 200 mg/kg [44]. The alkaloid contents hardly vary, the values obtained are therefore 1.001±0.010mg/100g; 1.001±0.01mg/100g; 1.001±0.000 g/100g respectively for Vernonia amygdalina, Tetrapleura tetraptera and Leptadenia lancifolia. Duncan's test shows a significant difference (p<5%) between these different values. These alkaloid levels are far below the dose corresponding to most alkaloids which is 34-300 mg/kg in humans [1].

The results in Table 4 show that the polyphenol contents of the plants studied are 2±0.00 mg eq GA/100g; 2±0.00mg eq GA/100g; 2±0.00mg eq GA/100g respectively for Vernonia amygdalina, Tetrapleura tetraptera and Leptadenia lancifolia. Statistically, there is a significant difference(P<5%) between these values. The total polyphenol contents of Vernonia amygdalina, Tetrapleura tetraptera are lower than those found by Usunobun [34] (9.75 mg/100g) and N'zebo [35] (2407.10±8.36 mg/100g) respectively. The flavonoid contents of the plants studied were 119±0.00mg eq EQ/100g (Vernonia amygdalina); 340±0.001mg eq EQ/100g (Tetrapleura tetraptera); 478±0.004 mg eq EQ/100g (Leptadenia lancifolia). Duncan's test shows a significant difference (p<5%) between these different values. The flavonoid contents of different samples are lower than the recommended daily flavonoid dose of 897 mg/g [45]. The analysis of anti-nutritional compounds shows that Vernonia amygdalina has higher levels of tannin, oxalate and saponin while Leptadenia Lancifolia Decne has high levels of phytate and flavonoids. At least it is noted that Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia Lancifolia Decne have comparable levels of total alkaloids and polyphenols. It is perhaps these two compounds that give them their anti-diabetic properties.

Conclusion

The comparative study of the anti-diabetic plants (Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia Lancifolia Decne) revealed that those plants contained similar phytochemical contents with some little difference. In terms of Nutritional

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contents, Tetrapleura tetraptera is the most energetically plant compared to Vernonia amygdalina and Leptadenia Lancifolia Decne. Regarding the mineral contents, Vernonia amygdalina has higher magnesium and calcium contents. At least it is noted that Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia Lancifolia Decne have comparable zinc contents. Concerning antinutritional contents, Vernonia amygdalina has higher levels of tannin, oxalate and saponin while Leptadenia Lancifolia Decne has high levels of phytate and flavonoids. At least it is noted that Vernonia amygdalina, Tetrapleura tetraptera, and Leptadenia Lancifolia Decne have comparable levels of total alkaloids and polyphenols. It is perhaps these two compounds that give them their anti-diabetic properties. For more information, antioxidant and anti-diabetic studies of these plants would be needed to develop a nutraceutical formulation.

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