Original Research Article

Antioxidant and anti α-amylase activities of polar extracts of *Mitracarpus hirtus* and *Saba senegalensis* and the combinaison of their butanolic extracts.

Abstract

Diabetes mellitus is a major public health disease, and it affects all segments of the population around the world. The progression of this disease is worrying, 460 million in 2019, the number of diabetics is estimated in 2045 at 700 million worldwide. The antioxidant and antidiabetic activities of the ethyl acetate (EA) and Butan-1-ol (But-ol) fractions of *M hirtus* and *Saba senegalensis* are studied in this work. The results revealed that the fractions possessed flavonoid, tannins, terpenes et sterols and exhibit potent radical scavenging activity using DPPH as substrate. The butanolic fractions exhibited more significantly α-amylase inhibitory activities than the ethyl acetate fractions. The IC_{50} values of butanolic fractions are 0.099 and 0.41 mg/ml respectively for *M hirtus* and *S Senegalensis* versus 0.44 and 1.45mg/ml ethyl acetate fractions. The combined butanol fractions of the two plants do not lead to the expected synergetic activities. Thus, it could be concluded that due to the presence of antioxidant components in the plant, extracts have well prospective for the management of diabetes and the related condition of oxidative stress.

Keywords : Diabetes, *Mitracarpus hirtus*, *Saba senegalensis*, antioxydant, antidiabetes

1. Introduction

Diabetes mellitus is one of the most common systemic diseases in the world and it occurs when the body becomes resistant to insulin or doesn't make enough insulin. As described by the World Health Organization, diabetes mellitus of all types has exponentially grown in the past decades across the globe[1]. According to the International Diabetes Federation (IDF), in 2019, a total of 463 million people is estimated to be living with diabetes, representing 9.3% of the global adult population (20–79 years). This number is expected to increase to 578 million (10.2%) in 2030 and 700 million (10.9%) in 2045[2]. Of the people living with diabetes, half (50.1%) are unaware of their condition. In this number, higher proportions of undiag-nosed diabetes were found in low- and middle-income countries, accounting for 84.3% of all undiagnosed people with diabetes worldwide. Diabetes mellitus has become an enormous and fast developing health problem and is an increasing economic burden hampering the social and economic development of many countries. The characteristic symptoms of diabetes are pruritus, polydipsia, weight loss, polyphagia, wasting, blurred vision, polyuria, tachycardia and hypotension. Eventually, as results of these, severe complications were created in both types of diabetes mellitus such as nephropathy, retinopathy, neuropathy, dyslipidemia and cardiovascular diseases[3]. Currently available therapy for diabetes includes insulin and various oral hypoglycemic agents such as

sulfonylureas, metformin, glucosidase inhibitors, troglitazone, etc. But these are reported to produce serious adverse side effects such as liver problems, lactic acidosis and diarrhea[4]. The distressing statistics of damage that diabetes could cause, necessitate to identify and exploring new avenues for diabetes management from natural products having fewer side effects. Indeed, the use of plants in the management of several pathologies such as diabetes no longer needs to be demonstrated [5][6]. According to Saravanan et al, traditional Medicine preparations could be a potential source of novel antidiabetic compounds or phytomedicines supplements. For example, Metformin (a biguanide) is a primary line drug currently used to control diabetes mellitus in Biomedicine which was developed from galegine (a guanidine) isolated from Galega officinalis[7]. The described mechanisms of action of plants on diabetes are numerous. Among these there is on the one hand the inhibition of α-glucosidase and α-amylase activity and on the other hand the inhibition of oxidative agents. According to Banerjee et al, the α-glucosidase enzyme is responsible for the breakdown of oligo- and/or disaccharides to monosaccharides. The inhibitory action of these enzymes leads to a decrease of blood glucose level, because the monosaccharides are the form of carbohydrates which is absorbed through the mucosal border in the small intestine. Another effective method to control diabetes is to inhibit the activity of a-amylase enzyme which is responsible for the collapse of starch to more simple sugars (dextrin, maltotriose, maltose and glucose)[8]. In addition, other authors have described that the inflammation and oxidative stress encountered during diabetes are directly associated with the body's insulin resistance [9]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases. This manuscript aims to study the antioxidant and anti α-amylase activity *in vitro* of polar extracts from two plants of the Senegalese flora, traditionally used in the management of diabetes in Senegal and the combinaison of their most polar active fraction.

The first one is *Mitracarpus hirtus* L., which is a common weed belonging to the Rubiaceae family. It is widely distributed as it easily spreads in gardens, farms and fields in neotropical and tropical regions [10]. In Senegal this plant is seasonal and only grows in the wild during the rainy season. It is among other things traditionally used in the treatment of skin diseases, antibiotic and antidote for insect stings and bites. The second studied plant in this manuscript is *Saba senegalensis.* It belongs to the family of Apocynaceae, a wild plant that is a large woody liana with white latex and can reach more than 40 m high. The interest of *Saba senegalensis* in feeding and treating numerous of diseases in rural populations is widely recognized [11]. In traditional medicine, this plant is used against constipation, scleroderma, parasitic infections, headaches and food poisoning. It is also used in the treatment of urinary schistosomiasis and as an antiemetic [12].

2. Materials and methods

2.1. Materials

2.1.1. Plant materials

The leaves of *Mitracarpus hirtus* and *Saba senegalensis* used in this work were purchased from respectively « Sagne » a village in Fatick area and in « Keur Madaro » in Thiès area in Senegal. The identification was carried out at the laboratory of pharmacognosy of the

medicine, pharmacy and odontology faculty at Cheikh Anta Diop University of Dakar. The leaves were dried under sunlight and powdered well to fine powder using a Brabender mill.

2.1.2. Chemistry and biochemistry products

Chemical and biochemical products used in this work were procured from different suppliers: Methanol (Sigma Aldrich), hexane (Sigma Aldrich), DPPH (Sigma Aldrich) ; DNSA (Acros organic), Na2H2PO4 (Acros organic), NaH2PO4 (Acros organic), potassium sodium tartrate (Acros organic) ; α-amylase (mpbio) ; Ethyl acetate (Labkem), butan-1-ol (Labkem), Dichloromethane (Labkem). All solvents were freshly distilled before use.

2.2. Methods

2.2.1. Extraction of leave powders with polar solvents.

For each plant, 40g of leaves powder are macerated in 400 ml of methanol at room temperature for 48 hours. The macerated matter was filtered and the filtrate evaporated to dryness using the vacuum rotary evaporator. The crude methanol extract obtained was liquid-liquid fractionated using first ethyl acetate and butan-1-ol. The fractions from each plant are stored in refrigerator at 4 °C until use.

2.2.2. Phytochemicals screening

In this part, We looked for the presence of Flavonoids, alkaloids, saponins, tannins, antracenoids, terpenoids and steroids in the polar fractions, using the qualitative technics described by Nabil and Kazeem [13][14].

2.2.3. Determination of antioxidant activity by the DPPH radical scavenging method

Measurement of the antiradical activity of polar extracts from *Saba senegalensis* and *Mitracarpus hirtus* leaves is performed by the 2,2'-diphenyl-1picrylhydrazyle (DPPH) test in accordance with the method described in our previous study [15]. The activity of the polar extracts was evaluated by determining the IC_{50} of the samples which was compared to the IC_{50} of Ascorbic acid, used as reference sample. The percentage of trapping of the DPPH radical is calculated by the following formula.

$$\% \mathbf{I} = \frac{(\mathbf{A_c} - \mathbf{A_e}) * \mathbf{100}}{\boldsymbol{A_c}}$$

Where: **%I : i**nhibition percentage; $\mathbf{A_c}$: control absorbance ; $\mathbf{A_e}$: extracts absorbance.

It is possible to deduced from **IC_{50}** values, the efficient concentration (**EC_{50}**) and the Antiradical Power **(ARP**). Indeed, the EC_{50}, efficient concentration at 50%, is defined as the amount of antioxidant required to decrease the initial concentration of DPPH by 50%. The EC_{50}, expressed in grams of extract per mole of DPPH was calculated according to the following formula, from the IC_{50}. « EC_{50} = IC_{50} (µg / mL) / MDPPH (µmol / mL) » MDPPH = molarity of the DPPH solution. The antiradical power (**ARP**) corresponds to the inverse of the

efficient concentration « ARP = 1 / EC_{50} ». It measures the anti-radical efficient of the concerned product. The higher its value, the greater the antiradical power of the product.

2.2.4. Anti-α-amylase activities of polar extracts

The anti α-amylase activity of polar extracts was carried out using a modified procedure of Al Waleed et al [13]. A total of 250 µL of the polar fractions (1.25 – 10 mg/ml) was placed in a tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/ml) was added. The content of the tubes was pre-incubated at 25°C for 10 mins, after hitch 250 µL of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals. The reaction mixtures were incubated at 25°C for 10 min. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent and further incubated in boiling water for 5 min and cooled to room temperature. The content of each test tube was diluted with 5 ml distilled water and the absorbance measured at 620 nm using Elisa plate reader (Thermo scientific multiskan fc). A control was prepared using the same procedure except that the fraction was replaced with distilled water. The α-amylase inhibitory activity was calculated as in the following equation.

$$\text{Inhibition (\%)} = \frac{[\text{Abs}_{620}\,(\text{control}) - \text{Abs}_{620}\,(\text{extract})] * 100}{\text{Abs}_{620}\,(\text{control})}$$

The IC_{50} values were determined from plots of percent of inhibition versus concentration of polar fractions and were calculated by the equation of linear regression. All tests were performed in triplicate.

3. Results

3.1. Phytochemical composition of the studied polar fractions

Table 1: Compounds present in the polar extracts of *M hirtus* and *S senegalensis*

	M hirtus		*S senegalensis*	
	EA	**But-ol**	**EA**	**But-ol**
Flavonoids	+	+	+	+
Alcaloids	+	+	-	-
Saponins	-	-	+	-
Tannins	+	+	+	+
Antracenoids	-	-	**nd**	**nd**
Sterols and erpenes	+	+	+	+

EA = Ethyl acetate; But-ol =Butane-1-ol; + = Presence; - absence; nd = non determined

3.2. Antioxidant activities of the studied fractions

The IC_{50} values were determined from plots of percent inhibition versus concentration of polar fractions and were calculated by the equation of linear regression. Ascorbic acid was used as the reference. All tests were performed in triplicate.

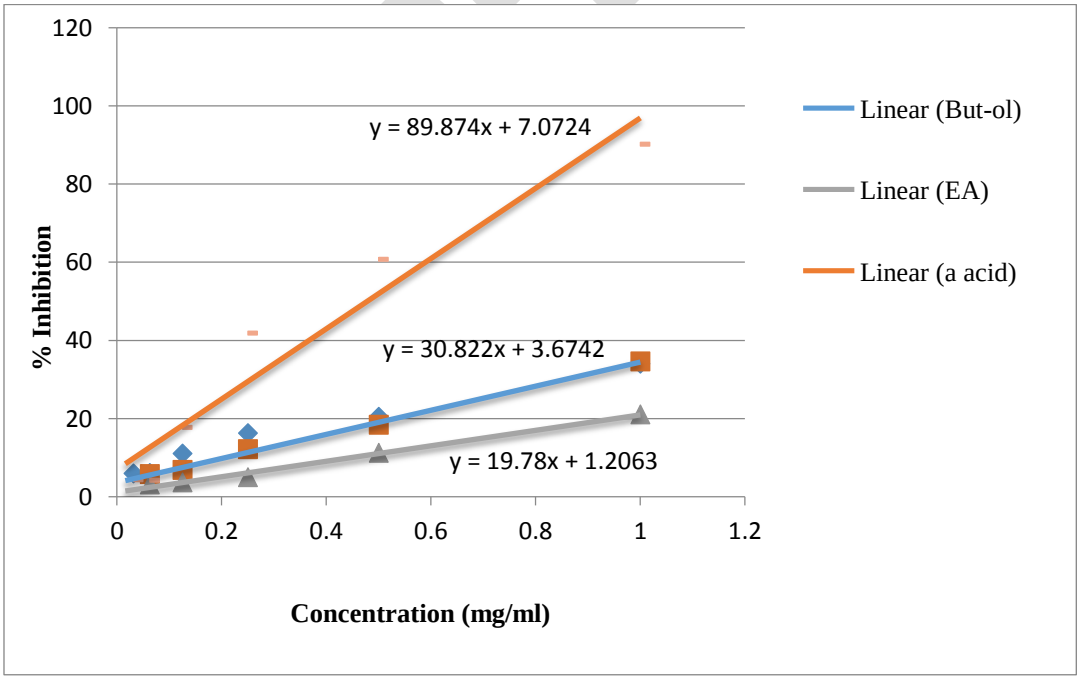

Figure 1 Linear regression equations which permit to calculate the IC_{50} of the antioxidant activities of EA and But-ol fractions *M hirtus*

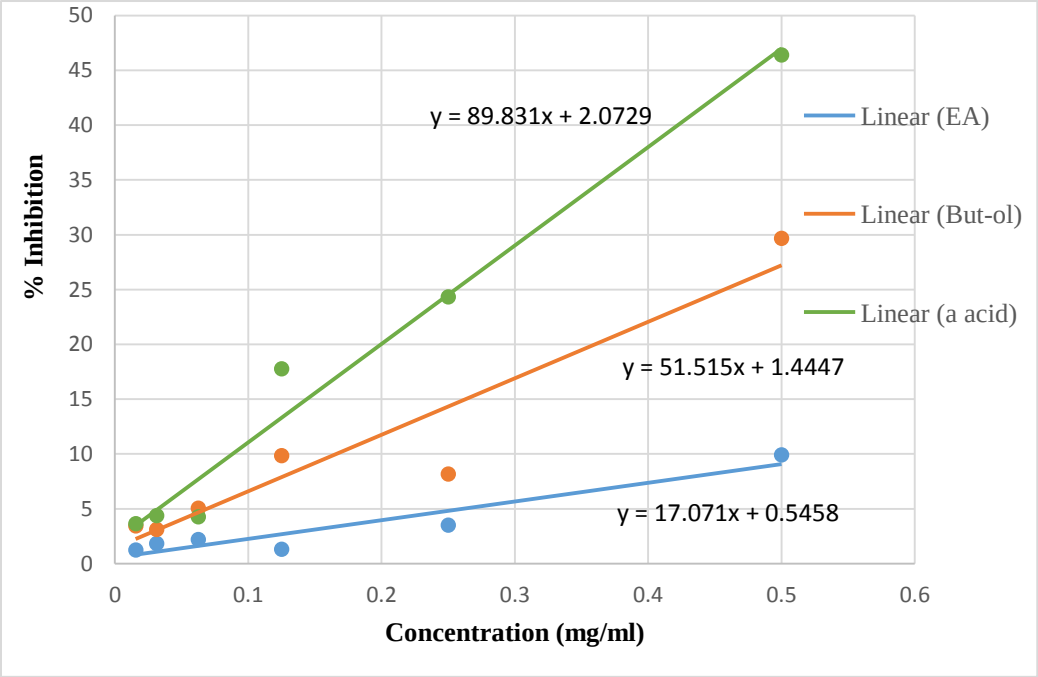

Figure 2 Linear regression equations which permit to calculate the IC_{50} of the antioxidant activities of EA and But-ol fractions of *S senegalensis*

3.2. Anti α-amylase activity of the studied polar extracts of *M hirtus* and *S senegalensis*

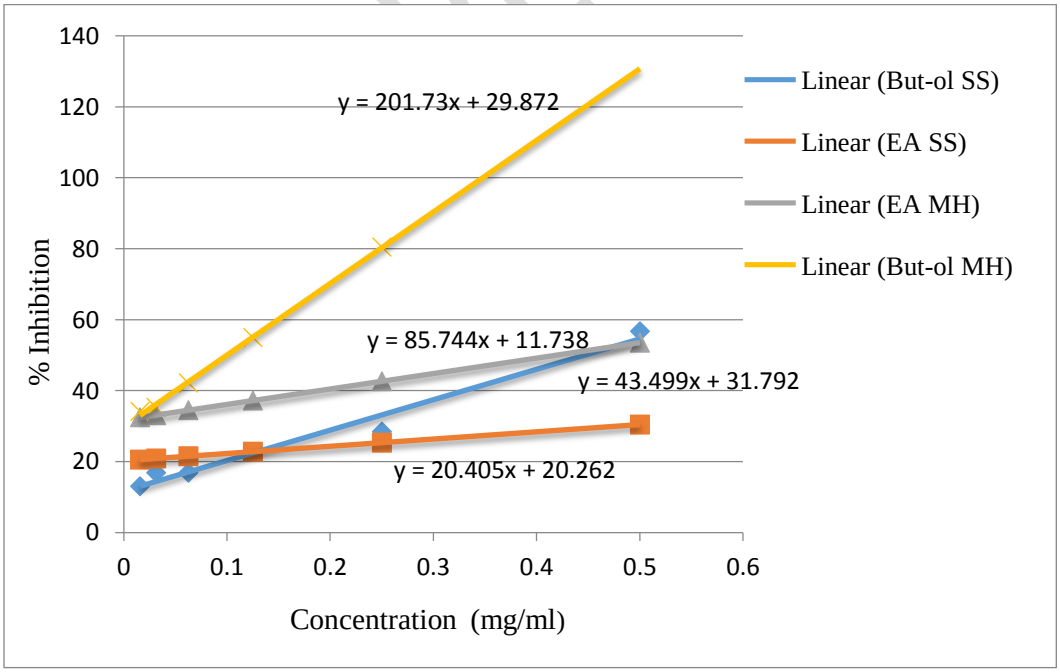

Figure 3. Linear regression equations which permit to calculate the IC_{50} of the anti α-amylase activities of EA and But-ol fractions of M *hirtus* and *S senegalensis*

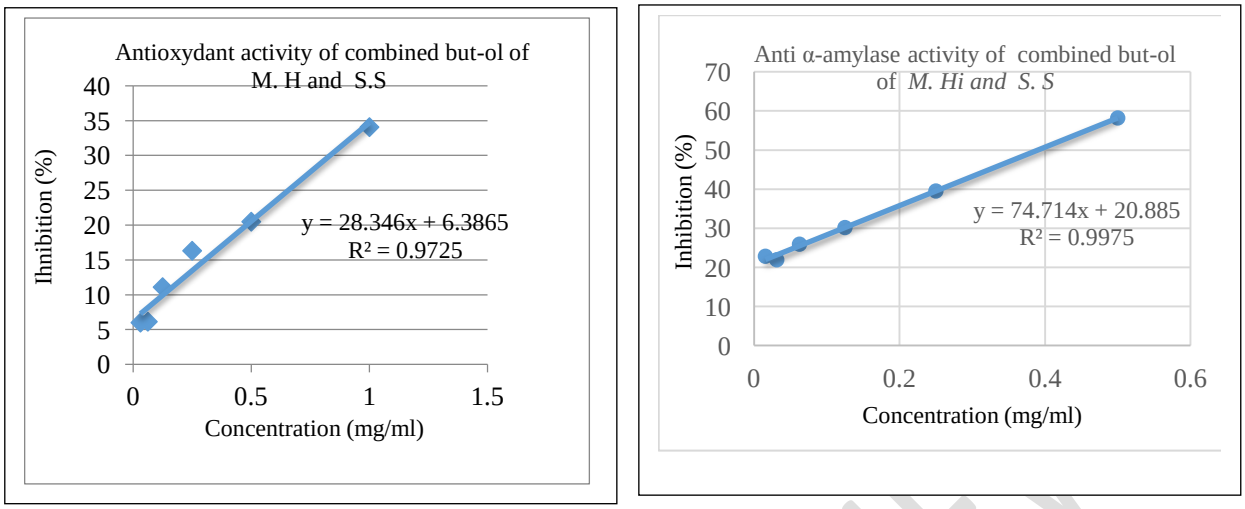

Figure 4. Linear regression equations of antioxidant (left) and anti α-amylase (rigth) activities of the but-ol combinaison of *M hirtus* and *S senegalensis* which permit to calculate the IC_{50}.

Table 2 : Summary of the antioxidant and anti α-amylase activities of the studied fractions

Studied parameters	*M. hirtus*		*S. senegalensis*		A acid	Comb
	EA	But-ol	EA	But-ol		
IC_{50} ao	2.46	1.50	2.89	0.94	0.47	1.53
ARP	0.016	0.026	0.014	0.042	0.087	0.026
EC	61.50	37.50	72.25	23.50	11.50	38.25
IC_{50} aa	0.44	0.099	1.45	0.41		0.38

Comb = combined but-ol of *M hirtus* and *S senegalensis;* **IC_{50} aa** = anti alpha amylase; **IC_{50} ao** = antioxydant

4. Discussion

In the phytochemical characterization of the studied plants (**table 1)**, we found almost the same chemical groups sought in the polar extracts of the two plants. Indeed, the flavonoids the tannins; sterols and terpenes are present in all studied fractions. While alkaloids are only found in polar extracts of *Mirtracarpus hirtus*. Sarr et al in their study also did not find

alcaloids in *Saba senegalensis* leaves [16] . However Nassirou et al described the presence as a traces of alkaloid in the leaves of *Saba senegalensis* using the two methods of alkaloids determination [17]. Showing that the leaves of this plant are poor in alkaloids. The Saponins absent in *Mitracarpus hirtus* are only found in the ethyl acetate extract of *Saba senegalensis*. The antracenoid compounds ares not found in the polar extracts of *Mitracarpus hirtus.*

The polar fractions of the two studied plants exhibit inhibitory activity on the DPPH radical. In both cases the butanolic fractions were found to be more active than those of ethyl acetate. The IC_{50} values are respectively 1.5 and 0.9 mg / ml for *M hirtus* and *S senegalensis* against 2.49 and 2.89 mg / ml for the ethyl acetate fractions (**table 2**). While ascorbic acid used as reference to an IC_{50} of 0.47 mg / ml. In their studies on the fruits of *S senegalensis*, Sarr et al [11] showed a high content of vitamin C, this could partly explain the greater activity of the Butanolic fraction of this plant. The activity obtained in the ethyl acetate fraction of *S senegalensis* leaves is greater than that obtained by Belemlilga et al who found an IC_{50} of 12.24 ug / ml for the same extract of *S senegalensis* [18]. The antiradical activity of combination of the two butanolic fractions (1.53 mg / ml) did not lead to an expected synergistic effect of the fractions. This activity is less important than that of the two fractions tested separately. We can imagine that in one of these fractions, compounds could inhibit the activity of other active molecules. Very few studies have been done on *M hirtus*, the only one found so far and which was published very recently [19] attributes the antioxidant activity of this plant to the presence of polar compounds such as rosmarinic acid, rutin and coumarin derivatives. In diabetes mellitus, it was described that the pain experienced is strongly related to the production of reactive oxygen species (ROS), oxidative stress and inflammatory factors. Antioxidants are one of the most important biological molecules that protect the body against the dangers of endogenous and exogenous oxidants [20]. The use of these plants in the management of diabetes could help to prevent the dangerous consequences of oxidants in diabetes disease.

The percentage inhibition at the concentrations 1.25 to 10 mg/mL of polar fractions from the studied plants on a-amylase showed a concentration dependent on reduction in percentage inhibition. The calculated IC_{50} showed that anti α-amylase activities of the butanolic fractions are more actives than those of ethyl acetate from the two plants. They are respectively 0.09 and 0.41 for *M. hirtus* and *S. senegalensis* while the ethyl acetate fractions are 0.44 and 1.41 mg/ml in the same order. The combination of the two butanolic fractions of these two plants exhibits an IC_{50} of 0.38 mg / ml. Here also the desired synergistic effect is not observed in relation to the combined fractions. The α-amylase and α-glucosidase are key carbohydrate hydrolyzing enzymes responsible for breaking α-1-4 bonds in disaccharides and polysaccharides, liberating glucose. The glucose surge observed a few minutes after ingestion contributes to hyperglycemia, the hallmark of diabetes mellitus [21]. The antidiabetic effect of *the studied polar fractions* of *M. hirtus* and *S. senegalensis* might attribute to its inhibitory effect against α-amylase that retarding the digestion of carbohydrate to delay the postprandial rise in blood glucose.

5. Conclusion

In this manuscript, we investigated the antioxidant and anti-α-amylase activity *in vitro* of polar fractions of *Mitracarpus hirtus* and Saba senegalensis. The results showed that the ethyl acetate and butanol extracts from the leaves of these two plants exhibit valuable activities. However, the butanol fractions remain significantly more active than those of ethyl acetate. The combination of the butanolic fractions of these two plants did not give the expected synergistic effect. The active compounds such as flavonoids, tannins, alkaloids terpens and steroids contained in these fractions would play an important role in the management of diabetes and oxidative stress. However, it will be necessary in future steps to isolate and characterize the active compounds responsible for the activities observed.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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