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POTENTIAL ANTIOXIDANT EFFECT OF A PREPARATION OF TWO PLANTS (*DISTEMONANTHUS BENTHAMIANUS* AND *DISSOTIS ROTUDIFOLIA*)

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ABSTRACT

Background: Plants are sources of natural antioxidants and some of their compounds have important antioxidant properties and health benefits. *Distemonanthus benthamianus* and *Dissotis rotundifolia* are used in traditional medicine for the treatment of many diseases. **Objective**: To evaluate the in vitro and in vivo antioxidant potential of extracts from a preparation of a mixture of *D. benthamianus* stem bark and *D. rotundifolia* leaves in diabetic rats. **Methodology**: Total phenols and flavonoids in the extracts were determined using spectrophotometric methods. In vitro antioxidant activity was determined by DPPH method and ABTS+ assay. Rats (25) were used for the evaluation of the in vivo antioxidant activity. These animals were divided into 04 batches (05) made diabetic and one control batch (05). After 21 days of treatment with the different extracts, these animals were sacrificed and the markers (SOD, Catalase, MDA and TBars content) were measured. **Results:** We note a strong presence of phenolic compounds and flavonoids in the hydroethanolic extract compared to the aqueous extract. This extract also showed a strong antioxidant activity in vitro (IC50 = 115.38 ug/ml and % =51.46 mg TE/g) in contrast to the aqueous extract (IC50 = 167.74 ug/ml and % =37.21 mg TE/g). Furthermore, the in vivo activity showed a better regulation of stress markers with the hydroethanolic extract. Conclusion: The extracts seem to have a promising antioxidant potential which can be used as a complementary treatment to various oxidative stress related diseases including diabetes.

KEYWORDS: Antioxidants, plants, phenolic, flavonoids, oxidative damage.

INTRODUCTION

Diabetes is a chronic metabolic disease characterised by hyperglycaemia resulting from a defect in insulin secretion and/or action.[1] This condition increases the risk of much co-morbidity and mortalities including CVD[2] and this relationship are even stronger combining several risk factors.[3] Furthermore, it is commonly accepted that oxidative stress is involved in most degenerative diseases such as diabetes.[4] This oxidative stress during diabetes includes an imbalance in the redox balance from increased reactive oxygen species (ROS) and decreased antioxidant defence system.[5] Due to the involvement of free radicals in the etiology of various pathologies including diabetes[6], studies on antioxidants aimed at compensating for a deficit in the natural anti-free radical protection system are needed.[7;8] The study of natural antioxidants in medicinal plants in relation to their therapeutic properties has attracted much interest. Scientific studies have reported biological properties of plants due to their richness in bioactive compounds.[9;10;11] *Distemonanthus benthamianus*, (Fabaceae) and *Dissotis rotundifolia* (Melastomaceae) are two plants recognised in folk medicine for the treatment of many diseases. Their richness in polyphenolic compounds, flavonoids, gall tannins, saponins, quinones, alkaloids can be used for the prevention of many diseases.[12;13]

The present study aimed to evaluate the effect of a preparation of *D. benthamianus* stem bark and *D. rotundifolia* leaves on markers of oxidative stress in diabetic rats.

1. MATERIALS AND METHODS

1.1 Plant material

The plant material consisted of *D. benthamianus* stem bark and *D. rotundifolia* leaves collected in the Issia and Dabou regions (Côte d'Ivoire). These plants were identified by the National Centre of Floristics of Côte d'Ivoire where a sample of each plant is kept.

The animal material consisted of albino rats of the Wistar strain weighing between 185 and 220 g supplied by the physiology laboratory of the Félix Houphouët-Boigny University in Abidjan.

1.2. Methods

1.2.1. Preparation of the Extracts

The aqueous extract was prepared by decoction, according to[14], with adaptations. Leaves and bark were shed dried and then powdered. The powdered plant material (20 g) was mixed with distilled water (400 ml) and then boiled for 15 min, left to cool for 5 min, and filtered through Whatman grade 1 filter paper, using a pressurized suction filtration system. Upon concentration *in vacuo* at 40°C, the filtrate was defatted with n-hexane (1:1 v/v). The resulting aqueous extract was freeze-dried, sealed, and kept in a refrigerator for further use. A fresh stock solution was prepared for the experiment whenever required.
The method of[15] was used for the preparation of the hydroethanolic extract,

1.2.2. Phytochemical Characterization of the Extracts

The phenolic compounds and flavonoids in hydroethanolic and aqueous extract were identified by UHPLC-DAD-ESI-MS^n analysis, performed on a Ultimate 3000 (Dionex Co., Sunnyvale, CA, United States) apparatus equipped with an ultimate 3000 Diode Array Detector (Dionex Co.), and coupled to a mass spectrometer, according to the method previously described by.[16]

The quantification of total polyphenols was done according to the linear regression equation of the Gallic acid calibration curve: Y= 0.0104x + 0.0204; R2= 0.998.
The total flavonoid contents in each extract are calculated using the calibration range regression equation: Y= 0.001x + 0.056; R2=0.9550.

1.2.3. In vitro antioxidant activity

- Diphenyl-1-picrylhydrazyl Radical (DPPH) Scavenging Activity

The effect of the extract against DPPH radical was determined following the method adopted by **[17]**. In this assay, the sample (25 µL) solution (or methanol, in the case of blank) and 0.6 mM DPPH solution (250 µL) were mixed in a 96-well microplate. Quercetin was used as a positive control. After shaking the microplates, the mixture was incubated in dark for 30 min and subsequently measured on a plate reader (Spectra Max 190) at a wavelength of 515 nm. The results were calculated as inhibition percentage (%I).

- Evaluation of the antiradical activity

It was evaluated by the ABTS+- cation radical discoloration test according to the method used by.[18] Quercetin was used as standard and the final result was expressed as micromoles of quercetin equivalent per gram of dry matter.

1.2.4. Evaluation of antioxidant activity in vivo

Rats (25) of the Wistar strain were divided into 05 batches of 05 rats each (01 control batch and 04 experimental batches). They were housed in standard polypropylene cages and maintained under standard laboratory conditions (12 h light-dark cycles, temperature 20 ± 2 °C). They were fed a standard pelleted rat diet and had access to water during the ten-day acclimatization period. Diabetes was induced in fasted experimental rats by intraperitoneal (IP) injection of alloxan monohydrate (98%) at a dose of 150 mg/ kg. After diabetes induction, the animals were treated as described by[19] for 21 days. Thus, the control lot receives physiological water daily by mouth. Batches 2 and 3 received aqueous extract (200 and 400 mg/kg) and batches 3 and 4 received hydroethanolic extract at the same dose daily. At the end of the 21 days of treatment, the animals were anaesthetized by intra-abdominal injection of chloral hydrate and then sacrificed and the kidneys were collected for the determination of the various markers of oxidative stress.

- Preparation of homogenates and determination of oxidative stress marker parameters

Tissues (kidneys) were homogenized in 200 Mm phosphate buffer (pH 7.8) for the determination of superoxide dismutase (SOD) activity according to the method described by.[20] Catalase (CAT) activity was determined by the method of [21] using 0.12 Mm phosphate buffer (pH 7.2). Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were determined by the biochemical method according to.[22]

2. RESULTS

2.1. Phytochemical Characterization of the Extracts

Table I shows the different concentrations of polyphenols and flavonoids in the extracts.

- Polyphenol content of the extracts

As regards the total polyphenol content, the results showed that the phenolic compound content of the extracts is significantly affected by the extraction solvent. The high content was obtained with the hydroethanolic extract (87.21 ± 0.96 mg GAE/g extract) compared to the aqueous extract (30.77 ± 0.57 mg GAE/g extract).

- Flavonoid content of the extracts

Concerning the flavonoids content of the two extracts, the results obtained showed no significant difference. Nevertheless, the concentration of total flavonoids in the hydroethanolic extract was higher (23.72 ± 0.17 mg EQ/g extract) compared to the aqueous extract (10.63 ± 0.12 mg EQ /g extract).

Table I: Content of total polyphenols and flavonoids in the extracts.

Metabolites	Aqueous extract	Hydroethanolic extract
Total polyphenol content (in mg GAE /g extract)	30.77±0.19	87.22 ± 0.38
Total flavonoids content (in mg EQ /g extract)	10.63 ± 0.12	23.72 ± 0.17

GAE/g: gallic acid equivalent per gram; QE/g: quercetin equivalent per gram

2.2. Evaluation of the antioxidant activity of extracts in vitro

The antioxidant potential of the extracts was determined on the basis of their free radical scavenging activity, i.e. against DPPH and on their ability to reduce the ABTS. As shown in Table 3 and Figures (1, 2 and 3).

The free radical scavenging effects by the DPPH test showed a better activity of the hydroethanolic extract (IC50= 115.38 µg/ml) compared to the aqueous extract (IC50= 167.74 µg/ml). It should be noted that the antioxidant effects of the extracts were relatively lower compared to that of gallic acid (IC50= 25.12 µg/ml).

In terms of the reducing activity of the ABTS radical, the hydroethanolic extract showed a better antioxidant power (51.46 ± 0.24 mg TEAC/g) with respect to the ABTS radical compared to the aqueous extract (37.21 ± 3.73 mg TE/g).

Table II: Inhibitory concentration 50 (IC50) of extracts and gallic acid.

Parameter	Gallic acide	hydroethanolic Extract	Aqueous extract
Level inhibition (ug/ml)	25.12	115.38	167.74

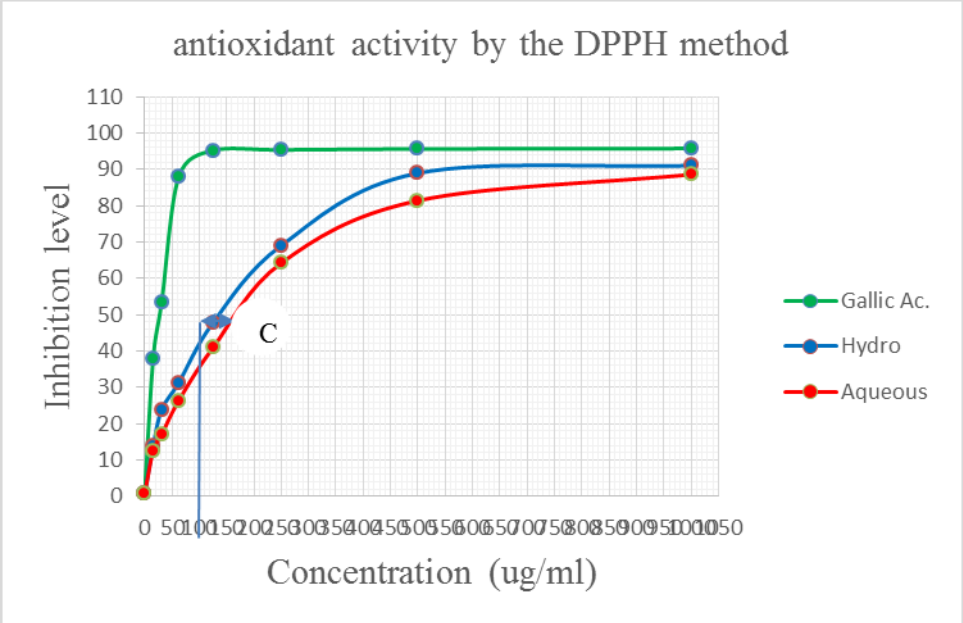

Figure 1: Inhibition concentration curves for extracts.

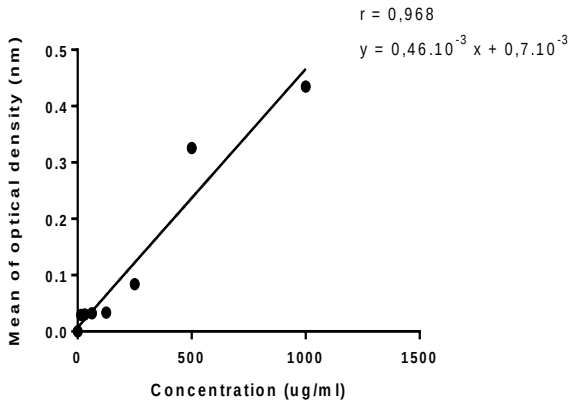

Figure 2: Calibration curve of Trolox optical density versus concentration.

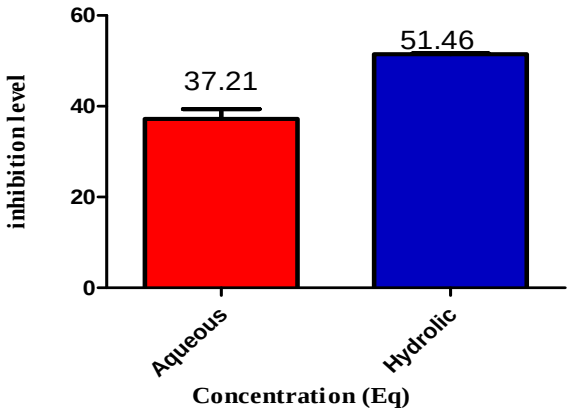

Figure 3: Evaluation of the reducing effect of the different extracts on the ABTS°+ radical.

2.3. Evaluation of the effects of the extracts on oxidative stress markers

The effects of the different extracts on the markers of oxidative stress (SOD; MDA, TBar content and Catalase) measured in the different kidneys of normal and experimental rats are reported in the different **figures** (**4, 5, 6, 7**).

Induction of diabetes in rats resulted in a significant ($p<0.05$) reduction in SOD levels and TBars concentrations followed by a significant ($p<0.05$) increase in tissue (kidney) MDA levels.

For MDA, there was an increase in kidney levels in diabetic rats (104.1 ± 5.91 nmol) compared to normal control rats (61.80 ± 0.95 nmol). During the 21 days of treatment, the hydroethanolic extract at the dose of 400 mg/kg showed a better effect (53.24 ± 4.38 nmol) compared to the aqueous extract at the same dose (67.23 ± 2.52 nmol).

In terms of SOD levels, a reduction in activity (11.11 ± 1.28 U/g tissues) was observed in diabetic rats compared to control rats (22, 23± 0.18 U/g tissue respectively). The treatment effect was better observed with the hydroethanolic extract (26.05 ± 0.53 U/g tissue) at the dose of 400 mg/kg compared to the aqueous extract at the same dose (12.68 ± 0.46 U/g tissues).

In terms of catalase enzyme activity (CAT), the results showed a reduction in renal CAT activity (0.11 ± 0.01 U/mg tissue) in diabetic rats compared to normal control rats (0.34 ± 0.03). Treatment with the different extracts showed a significant increase in enzyme activity in the rats compared to diabetic rats. This effect of the extracts is best seen with the hydroethanolic extract at the dose of 400 mg/kg (0.65 ± 0.03 U/mg tissue) compared to the aqueous extract at the same dose (0.30 ± 0.01 U/mg tissue).

With regard to thiobarbituric acid (TBars) levels, induction of diabetes in experimental rats' results in reduced levels (22.23 ± 1.72 mmol/mg tissue) compared to the normal control group (78.38 ± 0.36 mmol/mg tissue). After 21 days of treatment, the results showed an increase in TBars levels in the rats. The best effect was observed with the hydroethanolic extract at the dose of 400 mg/kg (82.12 ± 1.25 mmol/mg tissues) compared to the values obtained with the aqueous extract (61.03 ± 3.39 mmol/mg tissue).

In addition, dysregulation of various markers of oxidative stress was observed in untreated diabetic rats compared to those treated at the end of the experiment.

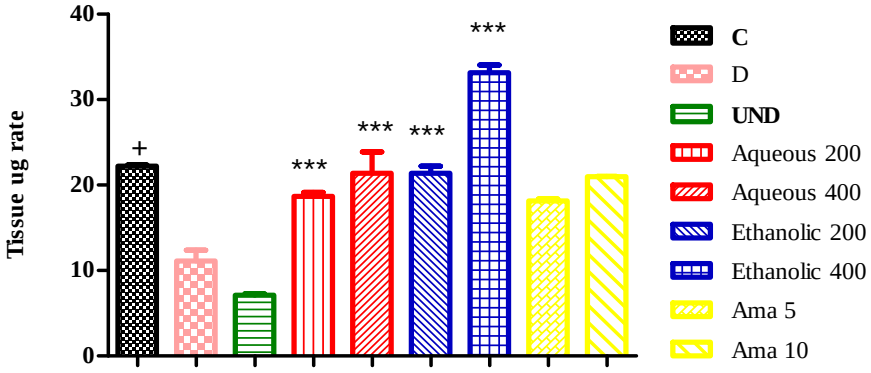

Figure 4: Variation in SOD levels after treatment.

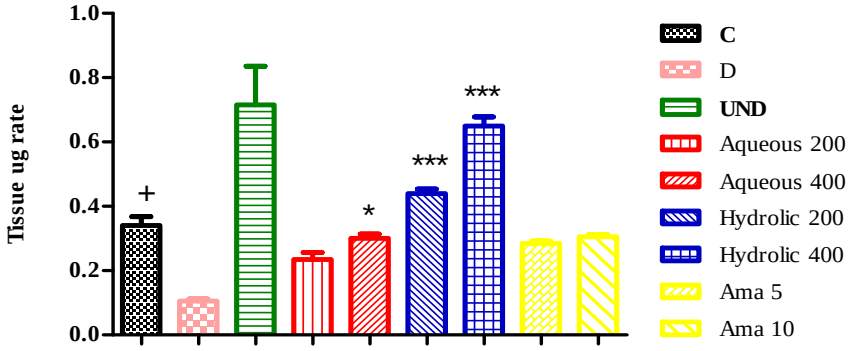

Figure 5: Variation in SOD levels after treatment.

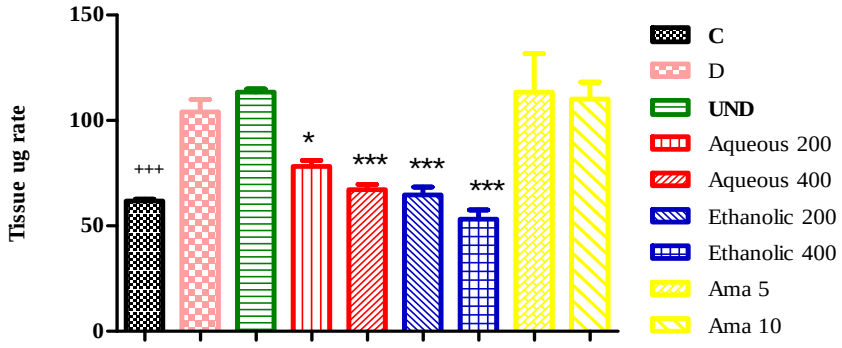

Figure 4: Variation in MDA levels after treatment.

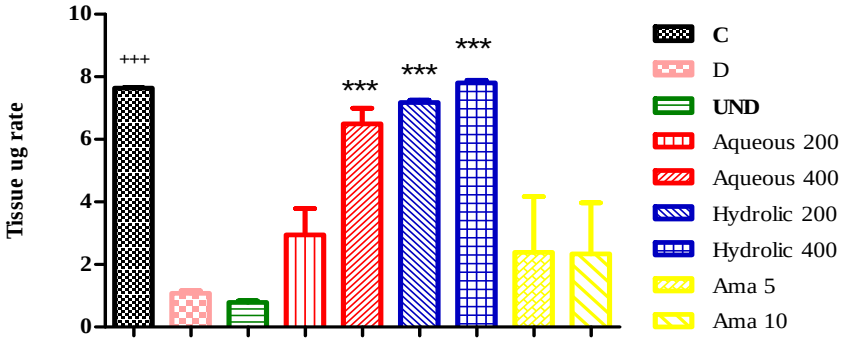

Figure 7: Variation in TBars levels after treatment.

3. DISCUSSION

Numerous studies have shown that the increased level of free radicals in the human body is involved in the pathogenesis of diabetes.[23;4] Thus, scavenging these reactive oxygen and nitrogen species is considered an effective measure to reduce oxidative stress.[24] Some bioactive compounds with antioxidant properties could play an important role in the prevention and treatment of diabetes.[25]

In the present study, the results showed the richness of the extracts in polyphenols and flavonoids. Our results are in agreement with those of.[26] Indeed the latter, after quantitative phytochemical analysis of the root and leaves of *Dissotis rotundifolia*, showed the presence of flavonoids and polyphenols. In the same order[27], after phytochemical screening of the bark of *Distemonanthus benthamianus*, showed the presence of flavonoids and phenolic compounds in the aqueous extract. This richness of the extracts in these different compounds was better observed in the hydroethanolic extract compared to the aqueous extract. This could probably be attributed to the different solubility of these compounds in the extracts.[28]

Antioxidant activity is manifested by various actions, such as hydrogen transfer, chelation of transition metals, and inhibition of oxidative enzymes or enzymatic detoxification of ROS. The IC50 values revealed that the extracts have a dose-dependent antiradical activity. Thus, of the two extracts, the hydroethanolic extract exhibited antioxidant activity (DPPH) and a strong capacity to reduce the ABTS radical compared to the aqueous extracts. These results corroborate those obtained by[29;30] who reported a significant correlation between polyphenol content and scavenger effect of plant extracts. Similar results on *Hertia cheirifolia* extracts were reported by.[31] Indeed, phenolic compounds and more particularly flavonoids are mainly responsible for the scavenger effect of free radicals.[32] The difference in activity noted between the two extracts could be attributed to the fact that organic solvent extracts are richer in polar, medium and low polarity secondary metabolites.[33]

For markers of oxidative stress, it should be noted that the induction of diabetes by alloxan in animals through chemicals selectively destroys pancreatic β-cells.[34] This results in the dysregulation of these parameters (CAT, SOD, MDA, TBars content). Note that these antioxidant enzymes are considered the first line of cellular defense against oxidative damage. Treatment of diabetic rats showed regulation of these enzymes. These results show that the extracts could prevent alloxan-induced alteration, playing an important role in preventing complications induced by lipid peroxidation [35]. Thus, the hydroethanolic extract showed a better performance compared to the aqueous extract. This remarkable

activity of this extract could be related to its richness in flavonoid phenolic compounds which could be responsible for the antioxidant activity.[36] Indeed, flavonoids are powerful antioxidants and are known to modulate the activity of different enzymes due to their interaction with various biomolecules. They protect and regenerate damaged ß-cells in alloxan-diabetic rats.[37] Our results are similar to those of[37; 32] who reported that phenolic compounds and especially flavonoids are mainly responsible for the scavenger effect of free radicals. This could demonstrate the electron donating property of this extract and consequently its ability to neutralize free radicals.

CONCLUSION

Medicinal plants continue to be the ideal source of secondary metabolites, which explains their increased exploitation in the pharmaceutical industry. Polyphenols are the most interesting and studied plant compounds nowadays. The present study showed a high richness of phenolic compounds and flavonoids in the preparation of both plants (*Distemonanthus benthamianus* and *Dissotis rotudifolia***)** and a remarkable free radical scavenging activity. Thus, this preparation could strengthen the antioxidant enzyme defense system and play a preventive role in the development of diabetes by improving markers of oxidative stress.

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