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Phytochemical trials and evaluation of the antioxidant activity of plants used in the treatment of type 2 diabetes in the Central African Republic- Case of *Khaya anthotheca, Desmodium tortuosum and Millettia laurentii*

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ABSTRACT

Excess free radicals equivalent to oxidative stress is an important cause of many diseases that pose a serious public health problem. Type 2 diabetes is one of these diseases. In the Central African Republic, it is estimated that more than 60,000 diabetics and 75% of diabetic deaths are caused by cardiovascular complications [1]. The traditional African pharmacopoeia offers an alternative to synthetic antidiabetics. The objective of this study is the phytochemical sorting and evaluation of the antioxidant activity of three samples of plants of the Central African pharmacopoeia known for their antidiabetic activity. The extracts of the three plants are obtained after maceration in ethanol or methanol for 48 hours at room temperature (25 °C.) followed by double filtration on cotton, then on 3 mm wattman paper and evaporation of the filtrates at rotary evaporator. The powder thus obtained allowed us to carry out phytochemical and antioxidant tests. After the phytochemical sorting, the antiradical activity by the DPPH and the inhibition of the lipid peroxidation by the ferric thiocyanate method were evaluated Phytochemical sorting revealed that these plants contain sterols, polyterpenes, polyphenols, flavonoids and saponins. Those exhibiting the antioxidant and antiradical important activity are Khaya anthotheca and Desmodium tortuosum with respectively 10.4 ± 0.3; 9.5 ± 0.7, rich in polyphenols and flavonoids, whereas for *Millettia laurentii* 13.6 ± 0.5 contains more sterols and polyterpenes with ethanol as solvent but thin-layer chromatography for methanol extracts indicates that the latter also contains a fairly high content of polyphenols.

Keywords: Type 2 diabetes, Antioxidant activity, *Khaya anthotheca, Desmodium tortuosum, Millettia laurentii*.

INTRODUCTION

Approximately 18 million people die each year from cardiovascular disease, mainly related to risk factors such as diabetes and high blood pressure. Diabetes is a metabolic disorder due to insufficiency or misuse of insulin characterized by a fasting blood glucose greater than 1.26g / L verified twice [2]. This disease affects more and more people in the world and is today a real public health problem. Indeed, WHO highlights a progressive global incidence of diabetes. From 108 million in 1980, the figures rose to 135 million in 1995, 177 million in 2000 and 347 million diabetics in 2011 [3] and to 422 million in 2014 (WHO 2016). This prevalence is continuously increasing in industrialized and developing countries. Currently, according to WHO (World Health Organization), more than 80% of the world's population, especially in underdeveloped countries, use traditional treatments to meet their health and primary care needs [4]. Traditional medicine has always been used in Africa in the treatment of diabetes mellitus. The work on the study of medicinal plants known to be antidiabetic revealed the active principles responsible for the hypoglycaemic effects of some of them. While some traditional healers have begun to use modern health facilities for the diagnosis and monitoring of their patients, others base their diagnosis and therapy on purely empirical grounds, often without any proven therapeutic efficacy [5]. Traditional medicine is now regaining interest and many diabetics regularly use it. More than 1200 plant species, used in traditional medicine, have antidiabetic properties. However, for most of them scientific reports have not yet been elucidated [6]. Under normal conditions, free radicals are unstable, neutral or charged chemical species which possess an unpaired electron on their last electron layer [7]. Free radicals are physiologically in equilibrium with antioxidant compounds [8]. Under certain conditions, during inflammation or in response to certain environmental factors such as irradiation (UV or X-rays), nutritional deficiencies (in vitamins and trace elements), the production of these free radicals increases generating an imbalance in Oxidative entities called "oxidative stress". Oxidative stress is thus an imbalance in the balance of pro-oxidants / antioxidants, either by deficit in antioxidants or as a result of an overproduction of free radicals; It is implicated in many diseases as a triggering factor or

associated with complications of their evolution [10]; It is also one of the factors potentiating the appearance of multifactorial diseases such as diabetes, Alzheimer's disease, rheumatism and cardiovascular diseases [9]. To fight against free radicals, the organism has effective systems of protection: antioxidants, defined as substances capable, at relatively low concentrations, of delaying or inhibiting the oxidation of substrates [7]. Antioxidants can be enzymatic or non-enzymatic, also called natural antioxidants. In addition to substances specific to the body, food and plants can also be sources of natural antioxidants. It is therefore proposed to evaluate in this study the antioxidant activity of three plants used in the Central African pharmacopoeia against type 2 diabetes.

MATERIALS AND METHODS

Reagents and products

Products and reagents used for the various analyzes are provided by Sigma-Aldrich–Fluka (Saint Quentin France).

Preparation of the ethanoic extracts of plants

According to the method described by Bidie *et al*. [20] 5 g of ground product of the various organs of plants used were mixed with 150 ml of absolute ethanol. The mixture obtained is stirred for at least 72 hours using a magnetic stirrer of the Heidolph MR Hei Tec type. The mixture is then filtered on WATTMAN paper. The filtrate is evaporated at reduced pressure and at 40 ° C. using a Heidolph HB Digital Laborota 4000 rotary evaporator. The powder obtained was used for the various tests. I Phytochemical Screening: The different groups of compounds (sterols, polyterpenes, alkaloids, tannins, polyphenols, flavonoids, quinones and saponins) contained in the extracts have been demonstrated according to the methods described by Ronchetti and Russo [11], Hegnauer [12-13], Bekro *et al*. [14].

Phytochimical screening

Sterols and polyterpenes

To demonstrate the sterols and polyterpenes we used the LIEBERMANN reagent:

> 5 ml of each extract evaporated to dryness + 1 ml of acetic anhydride 0.5 ml of sulfuric acid in triturate.

Polyphenols

By reaction with ferric chloride (FeCl 3) 2 ml of each solution + one to two drops of alcoholic solution of 2% ferric chloride (Jeulin Evreux).

Flavonoids

By the so-called cyanidine reaction: 2 ml of each dry extract + 5 ml of hydrochloric alcohol (10%) diluted twice + 2 or 3 chips of Magnesium + 3 drops of isoamyl alcohol (Fisher scientific Elancourt).

Tannins

The catechic tannins by the STIASNY reagent (30% Formol, concentrated HCl: 1 / 0.5) 5 ml of each solution evaporated to dryness + 15 ml of the STIASNY reagent; Mixture maintained in a water bath at 80 ° C. for 30 min. Gallic tannins / Addition of 3 drops of 2% FeCl 3 to the filtrate of the previous solution collected and saturated with sodium acetate (Fischer scientific Elancourt).

Free or combined quinone substances

By the BORNTRAEGEN reagent: 2 ml of each extract evaporated to dryness. The residue is triturated in 5 ml of 1/5 HCl. The triturate is taken to the Marie bath for 30 min and then extracted with 20 ml of chloroform (Sigma Aldrich). Then the diluted NH3 2 times added to the chloroform solution.

Alkaloids

By reagent of DRAGENDORFF and MAYER: 6 ml of each solution were evaporated to dryness and then taken up in 6 ml of alcohol at 60 ° C. 2 drops of the DRAGENDORFF reagent or 2 drops of the MAYER reagent added to the alcoholic solution.

Saponins

10 ml of each of each of the aqueous extracts are stirred for 15 seconds and then allowed to stand for 15 min.

Total phenol contents

The total phenol contents of the plants were determined by the Folin-ciocalteu method [16]: A volume of 0.5 mL of each plant extract (0.1 g / mL) or of gallic acid (0.1 Mg / mL) is mixed with 5 mL of Folin-ciocalteu reagent diluted 1/10 with distilled water and 4 mL of sodium carbonate (1 M). Gallic acid is the reference antioxidant. After 15 minutes incubation at room temperature (25 ° C.), the absorbance is read at the spectrophotometer at 765 nm. The standard curve is obtained under the same conditions as previously using a range of concentrations (0-250 mg / L) of gallic acid solution prepared in methanol. The total phenol contents of the plant extracts are determined graphically and expressed in terms of gallic acid equivalent (mg / g dry matter).

Thin Film and CCM of Polyphenols

Extraction of polyphenols

To extract the polyphenols from the different samples by maceration, we based ourselves on the protocol described by Romani *et al*. [17]: 10 to 30 g of the powder of each sample are macerated at ambient temperature for 48 hours (twice) with 100 ml of methanol and then filtered on Watmann filter paper and stored at 4 ° C. until use.

Thin-layer chromatography

On silica chromatographic plate was placed one drop of each aqueous and methanolic sample on the starting line of the elution. The eluant is formed of n-hexane and ethyl acetate At concentrations 10/0, 3/7 and 5/5.

Biological activity

Methanolic extracts were obtained after maceration of the individual samples in absolute methanol for 24 h, followed by filtration of the supernatant on Wattman paper; The filtrates represent the stock solutions from which the various ¾, ½ and ¼ dilutions are carried out [23].

Antioxidant by DPPH

The antioxidant test was carried out using the DPPH method based on that of Blois in 1958. 50 µL of each methanolic solution of the extracts at different concentrations (from 0.0125 to 5 mg / ml) are added to 1.95 ml of the DPPH methanol solution (0.025 g / l). At the same time, a negative control is prepared by mixing 50 µL of methanol with 1.95 ml of the DPPH methanol solution. The reading of the absorbance is made against a blank prepared for each Concentration at 517 nm at the Jenway spectrophotometer after 30 min incubation in the dark and at ambient temperature. The positive control is represented by a solution of a standard antioxidant, the quercetin complex whose absorbance was measured under the same conditions as the samples and for each concentration the test is repeated 2 times. The results were expressed as percent inhibition (%):

$$\% \text{ inhibition} = 100 \times (A_{blank} - A_{sample}) / A_{blank}$$

The antioxidant activity of the extract is expressed as IC_{50}, which defines the concentration of the extract that reduces by 50% the free radical (DPPH)

Antioxidant activity by inhibition of lipid peroxidation and by the ferric thiocyanate (FTC) method.

The antioxidant activity of plant extracts is measured by inhibiting the peroxidation of linoleic acid using the ferric thiocyanate method according to the method described by Takao *et al*., [15] The reaction mixture containing 0.4 ml of extracts (0.1 g / ml) or complex Quercetin, 0.4 ml of linoleic acid (2.52% in absolute ethanol) and 0.8 ml of Phosphate buffer (pH 7.4) in a water bath for 1 hour at 40 ° C. 0.1 mL of this solution + 5 mL of 70% ethanol and 0.1 mL of ammonium thiocyanate (30%). After 3 minutes, 2 drops of FeCl 2 prepared in 3.5% HCl (20 mM) are added to the reaction medium. A white test consists of distilled water. The absorbance of the red coloration resulting from the solution is read twice a day at 500 nm on the spectrophotometer until the absorbance of the negative control reaches its maximum. The percentage inhibition of lipid peroxidation.

$$\% \text{ inhibition} = 100 \times (A_{blank} - A_{sample}) / A_{blank}$$

RESULTS AND DISCUSSION

The histogram below shows the results of the extraction yields of the different parts of the plants subjected to the study. The best yield is obtained with K. anthotheca with 41.6%, followed by M. laurentii and D. tortuosum with 8.8% and 7% respectively.

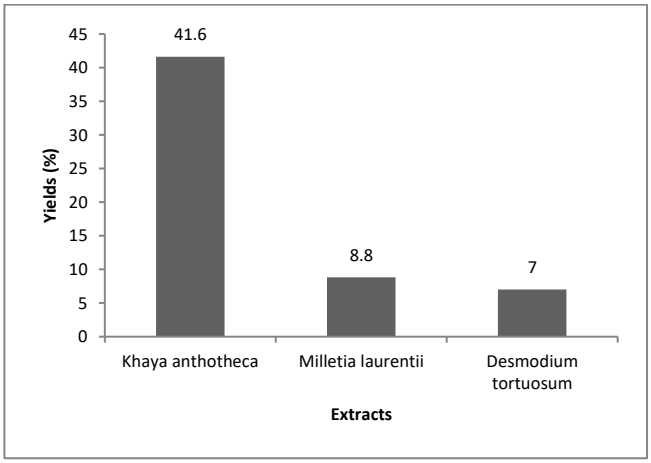


Figure 1: Yields of ethanoic extracts

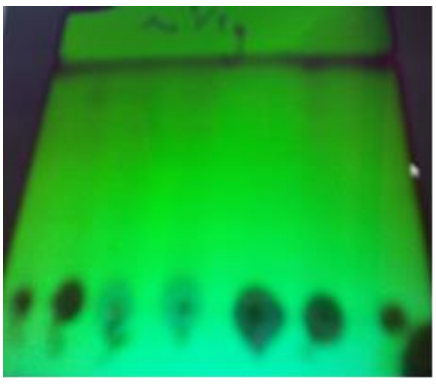
Table 1: Phytochemical screening

According to the results, *K. anthotheca* and *D. Tortuosum* contain polyphenols and tannins in addition to Saponins also present in *M. laurentii.* The search for total phenols shows that *K. anthotheca* contains > 103mgEAG / g in contrast to *M. laurentii*, which contains very little 26.41 ± 4.94 mgEAG / g, whereas *D. tortuosum* has an average of total phenols = 70.91 ± 3.07 mgEAG / g.

Extracts	Sterols and polyterpenes	Polyphenols	Flavonoïds	Tanins	Quinones	Alcaloïdes	Saponins	Total Phenols
K. anthotheca	-	+	±	±	-	-	+	**103±1.1**
M. laurentii	+	+	-	-	-	-	+	**26.43±4.9**
D. tortuosum	-	+	-	+	-	-	+	**70.43±3**

Chromatographic profiles of methanol and aqueous extracts

At the 3/7 concentration of the eluant, the chromatographic plate subjected to NH3 vapors reveals more clear spots of similar polyphenols for the methanolic extracts of *K. anthropheca* and *M. laurentii*. According to the relationship between fluorescence under UV and the structure of flavonoids [18], the polyphenols shown by thin layer chromatography, the fluorescent light blue color corresponds to the free 5 - OH - free flavones or to the 5 - OH - free flavones Free with 3-substituted -OH. Quantitatively, methanolic extracts of *K. anthotheca* show a high concentration followed by those of *M. laurentii* and to a lesser extent *D. tortuosum*.

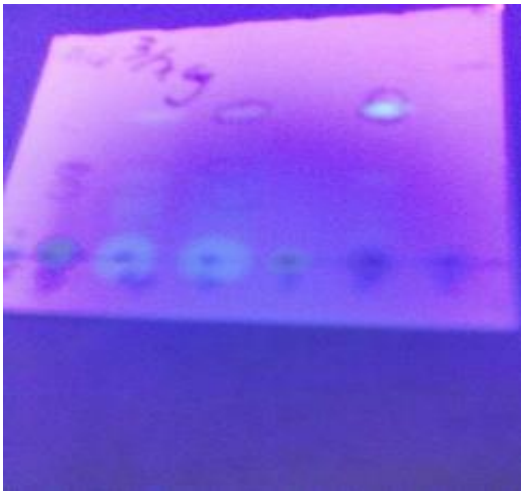


DA- DM- MM- MM- MA -KM - KA

At 254nm

DA- DM- MM- MM- MA -KM-KA

At 365nm



DA-DM- MM- MM- MA -KM -KA

At 365 nm + Vapor NH_3

DA- DM- MM- MM-MA-KM-KA

A 254 nm+Vapor NH_3

MA= Aqueous extract of *M. laurentii*
MM = Methanolic extract of *M. laurentii*
DA = Aqueous extract of *D. tortuosum*
DM = Methanolic extract of *D. tortuosum*
KM = Methanolic extract of *K. anthotheca*
KA = Aqueous extract of *K. anthotheca*

Anti-radical activity by DPPH

The antiradical activity detected with DPPH indicates an IC_{50} reached at concentrations of extracts ≤ 10mg / L for *K. anthotheca* and *D. tortuosum* but > 10mg / L for *M. laurentii* whereas that of complex quercetin, Reference sample has an IC_{50} of 6.3 mg / L.

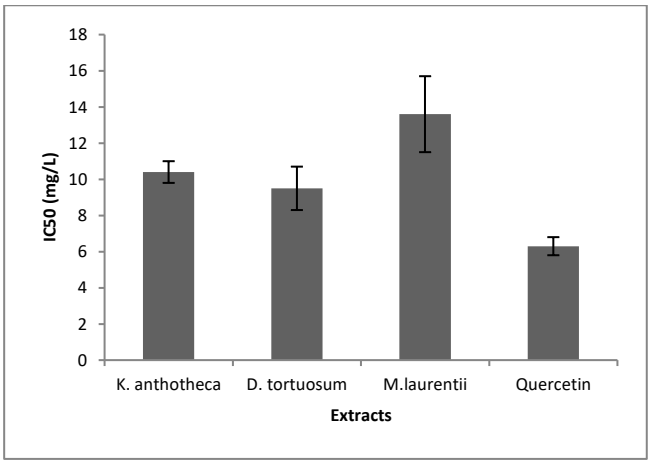
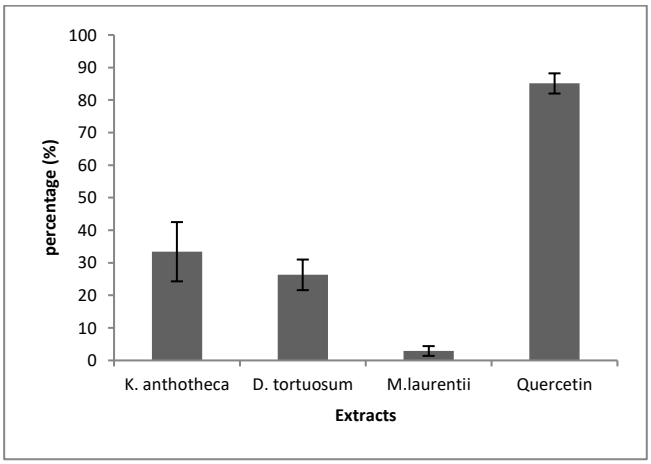


Figure 1: Results of antioxidant activity of ethanoic extracts

Lipid peroxidation by the FTC method



The inhibition percentage (%) of lipid peroxidation important for *K. anthotheca* = 33.43 ± 9.17 and *D. tortuosum* = 26.32 ± 29.17 is very low for *M. laurentii* = 2.99 ± 1.5. It should be noted that in another study, *K. ahthotheca* had a low antioxidant activity with a higher IC50 = 176.40 ± / ml [24]; this would be another variety of *K. anthotheca*. Inhibitory activity of lipid peroxidation.

DISCUSSION

The samples studied all contain antioxidant substances mainly polyphenols of the poyterpenes and tenants known for their therapeutic effect in the control of many diseases including fatty diabetes. Negative reactions contrary to what is asserted in the literature, such as the presence of quinones for *K. anthotheca* [19], are due either to a low solubility of the samples in ethanol or to a low concentration of the extracts. The antioxidant and antiradical activity detected is relatively high for *K. anthotheca* followed by *D. tortuosum* and to a lesser extent *M. laurentii*. This activity is strongly linked to the presence of antioxidant substances, namely polyphenols,

polyterpenes and, in addition, tannins and saponins, but for *M. laurentii*, the presence of antioxidant substances was only demonstrated in the methanol extract by Chromatography on a thin layer and a high-performance liquid, which explains why its anti-radical activities with DPPH and antioxidant by the method of ammonium ferrothiocyanate with the ethanoic extract proved to be weak. The numerous spots obtained in this case could be explained by the capacity of methanol to inhibit the action of polyphenol oxidase which causes the oxidation of polyphenols in plant tissues [21].

The mechanisms contributing to the generation of oxidative stress in diabetes mellitus may include free oxygen species (ROS), non-enzymatic glycosylation, glucose auto-oxidation, modified glutathione metabolism (GSH), Changes in antioxidant enzymes and production of lipid peroxide. The free radicals that may be involved in diabetes, the antioxidant substances highlighted in this study, could play an important role in the trapping of these free radicals justifying the antidiabetic activity of *K. anthotheca*, *M. laurentii* and *D. tortuosum*. By releasing a hydrogen atom from their hydroxyl group or by their chelating properties of the metal ions [22]; Long-term consumption of catechins may be beneficial for diet-induced obesity and type II diabetes and may reduce the risk of coronary artery disease in synergy with rutin.

CONCLUSION

At the end of this study, we can say that the three samples studied *K. anthotheca* and *D. tortuosum* and *M. laurentii* which contain mainly polyphenols, polyterpenes and flavonoids, metabolites responsible for antioxidant and antiradical activity and secondarily saponins and tannins have certain antioxidant activity. The secondary metabolites identified in this study and at the concentrations used are, among others, flavones, rutin and catechin which may play an important role in the antidiabetic activity of the plants studied. Nevertheless, and at the doses used, this activity remains clearly lower than that of the reference molecule, in this case quercetin. The solvent used and the concentration of the solutes are thus very important to demonstrate the presence of secondary metabolites determining the pharmacological properties of plants used in traditional medicine.

Conflict of Interest

None declared.

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None declared.

REFERENCES

1. Apema R, Mozouloua D, Abeye J, Salamate FML. Les plantes médicinales utilisées dans le traitement du diabète par les tradipraticiens à Bangui, 2011.
2. Afssaps-Has. Traitement Médicamenteux Du Diabète De Type 2-Recommandation de Bonne Pratique, 2006.
3. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants, *Lancet*, 2011; 378(9785):31–40.
4. Farnsworth NR, Akerele O, Bingel AS, *et al*. Medicinal plants in therapy. Bull World Health Organ, 1985; 63:(9):65–81.
5. Famuyiwa OO. The efficacity of traditional medicine in the management of diabetes mellitus in southwestern Nigeria, Afr J Med Sci. 1993; (22):7-31.
6. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents.
7. Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radical Research, 1999; 31(4):261-272pp.
8. Romani A, Pinelli P, Cantini C, Cimato A, Heimler D. Characterization of Violetto di Toscana, a typical Italian variety of artichoke (Cynara scolymus L.). J. Food Chem), 2006; 95:221-225.
9. Favier F. Le stress oxydant. L'actualité chimique, Novembre-décembre, 2003; 108-115pp.
10. Sohal *et al*,. Role of oxidative stress and protein oxidation in the aging process. Free Radical Biology and Medicine, 2002; 33(1):37-44.
11. Ronchetti F, Russo G. A new alkaloid from Rauvolfia. Phytochem. 1971; 10:1385-1388pp.
12. Hegnauer R, Chemotaxonomie der Pflanzen, Bikhäuser Verlag, Basel, Suttgart. 1973; 6:761.
13. Wagner H, Bladt S. Plant Drug Analysis:A thin Layer Chromatography Atlas (2nd ed.). Springer, Berlin, 2001; pp. 349-364.
14. Békro YA, Békro JAM, Boua BB, Tra BFH, Ehilé EE. Etude ethnobatanique et screning phytochimique de *Caesalpinia benthamiana* (Baill.) (Caesalpiniaceae). Rev. Sci. Nat. 2007; 4:217-225.
15. Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K. A simple screening method for antioxidant and isolation of several antioxidants produced by marin bacteria from fish and shellfish. *Biosc Biotechnol Biochem*, 1994; 58:1780-1783.
16. Mc Donald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts, Food Chem, 2001; 73:73-84.
17. Romani A, Pinelli P, Cantini C, Cimato A, Heimler D. Characterization of Violetto di Toscana, a typical Italian variety of artichoke (*Cynara scolymus* L.). J. Food Chem. 2006; 95:221-225.
18. Lahouel M. Interaction flavonoïdes-mithochrondrie et role de la propolis dans la prevention de l'apopose induite par certains medicaments anticancéreaux, Thése de doctorat de l'université Mentouri de Constantine, 2005.
19. Sung-Eun Leea, Mi-Ran Kimb, Jeong-Han Kimb, Gary R. Takeokac, Tae-Wan Kimd, Byeoung Soo Parke. Antimalarial activity of anthothecol derived from Khaya anthotheca (Meliaceae), 2007.
20. Bidié AP, Koffi E, N'guessan JD, Djaman AJ, Guédé-Guina F. Influence of *Mitragyna ciliata* (MYTA) on the microsomalactivity of ATPase Na+/K+ dependent extract on a rabbit (heart) Afr J Trad CAM. 2008; 5:294-301.
21. Yao HW, Li J, Chen JQ, Xu SY. Inhibitory effect of leflunomide on hepatic fibrosis induced by CCl4 in rats, ActaPharmacologica Sinica, 2004; 25(7):915-920.
22. Pastre J, Pastre O, Pastre C. Intérêt de la supplémentation en antioxydants dans l'alimentation, Thèse de Vétérinaire, Université de Toulouse, 2005.
23. Parejo I, Codina C, Petrakis C, Kefalas P. Evaluation of scavenging activity assessed by Co (II)/EDTA-induced luminal chemilunes-cence and DPPH (2,2-diphényl- 1-pycryl hydrazyl) free radical assay. J Pharmacol Toxicol Method, 2000; 44:507-512.

24. Suleiman MM, Bagla V, Naidoo V, Eloff JN. Evaluation of selected South African plant species for antioxidant, antiplatelet, and cytotoxic activity.
25. Kuntie V, Pejie N, Ivkovie B, Vugie Z, Ilie K, Mieie S, Vukojevie V. Isocratic R-PHPLC method for rutin determination in solid oral dosage forms. Journal of pharmaceutical and biomedical analysis. 2007; 43:718-721.

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