ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 09, pp.22896-22900, September, 2018

ORIGINAL RESEARCH ARTICLE **OPEN ACCESS**

EVALUATION OF THE ANTIOXIDANT ACTIVITY OF ACETAL AND HEXANE EXTRACTS OF*ENTADA MANNII* (FABACEAE) *IN VITRO*

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ARTICLE INFO

Article History:
Received 22nd June, 2018
Received in revised form
17th July, 2018
Accepted 29th August, 2018
Published online 30th September, 2018

Key Words:
E.mannii, Acetal,
Hexane extracts,
Antioxidant.

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ABSTRACT

This study aimed to evaluate the antioxidant activity of the acetal and hexane extracts of Entada mannii (Fabaceae) after conducting the polyphenolics assay. E. mannii is a plant used in the traditional treatment of diabetes in the south-east of Coast Ivory. The quantitative estimate of the polyphenol content of the acetal extract gave the following results: Total Phenolic Content (19.74 ± 2), total flavonoids (0.69 ± 0.03) and total flavanols (0.33 ± 0.02). As for the hexane extract, the respective contents obtained for these same compounds are: 9, 641 ± 1 ;0.39 ± 0.01 and0.3 ± 0.01. The evaluation of the antioxidant activity of the various extracts was carried out according to two methods: the free radical scavenging by the DPPH and the measurement of the reducing power (FRAP).The results obtained indicate that the acetal extract of E. manniicontains more polyphenolic compounds than the hexane extract. The acetal extract antioxidantproperties (AEEM, IC50 = 52.30 ±2.05) are also greater than those of the hexane extract (HEEM, IC50 = 70.51±1.84). This antioxidant activity could thus represent an additional asset for the use of this plant in the traditional treatment of diabetes and some pathologies related to oxidative stress.

Citation: Doumbia Idrissa, Yeo Sounta Oumar, Dhanabal Palaniswamy, Yapi Houphouet Felix and Djaman Allico Joseph. 2018. "Evaluation of the antioxidant activity of acetal and hexane extracts ofentada mannii (fabaceae) in vitro", *International Journal of Development Research*, 8, (09), 22896-22900.

INTRODUCTION

Long regarded as molecules without special metabolic interests, secondary metabolites are attracting more and more attention. And for good reason, several molecules belonging to the group of secondary metabolites have specific pharmacological and nutritional activities that offer new perspectives in the management of cancer, metabolic diseases and various pathologies (De Beer, 2002 and Arceusz, 2013). The state of current knowledge makes it possible to better appreciate their roles through the study of their different mechanisms of action (Macheix, 1996). Several studies have made it possible to highlight the interest of polyphenolic compounds in the prevention and the treatment of numerous cancers (colon, stomach, liver, breast, prostate, lungs, skin, bladder, etc.) at all stages. Carcinogenesis (Stavric, 1992; Das, 1994 and Hayase, 1984). The mechanisms involved seem to be very varied: prevention of oxidative stress, inhibition of arachidonic acid metabolism and associated inflammatory reactions, inhibition of protein kinase C and cell proliferation, induction of apoptosis and inhibition of angiogenesis. Significant intake of polyphenol antioxidants may be correlated with a significant decrease in atherosclerosis deaths, by decreasing LDL oxidation (Frankel, 1995; Brunetton, 1999 and Bahorun, 1997). The antidiabetic action of flavonoids and polyphenols has been demonstrated by several authors (Bayle, 2018 and N'guessan, 2009). Given the multiple beneficial effects of these secondary metabolites, the search for the meticulous activity of plants hopes to discover new plant extracts with greater antioxidant powers on the one hand and

the possibility of identifying new antioxidant molecules are forcing research teams into a meticulous exploration of the activity of potentially antioxidant medicinal plants. It is in this context that we undertook to evaluate the antioxidant activity of the acetal and hexane extracts of *Entada mannii* (Fabaceae), a plant used in the traditional treatment of diabetes in southeastern of Ivory Coast.

MATERIAL AND METHODS

Plant material: The barks of *Entada mannii* (Fabaceae) from Agboville (south-east of IvoryCoast) have been identified by the National Center of Floristry at the University Felix Houphouet Boigny (Cocody-Abidjan). A specimen of the plant was deposited in the herbarium of this Center.

Preparation of the acetal extract of *Entada mannii* (Fabaceae): The harvested bark was dried at room temperature (28 ± 1 ° C) for one month out of the sun. The dried bark was ground to a fine powder. The powder (50 g) was macerated in 250 ml of ethyl acetate for 24 h at room temperature. The mixture was then filtered through the gauze and a second time on Whatman filter paper (3 MM). The evaporation of the solvent was carried out in an oven at 50 ° C. After drying, a brown powder obtained, was used to prepare the acetal extract of *Entada mannii* (AEEM).

Preparation of hexanic extract of Entada *mannii* (Fabaceae): The dry bark powder (50 g) obtained above was macerated in 250 ml of hexane for 24 hours at room temperature. The mixture was then filtered through the gauze and a second time on Whatman filter paper (3 MM). The evaporation of the solvent was carried out in an oven at 40 ° C. After drying, we obtain a brown powder used to prepare the hexane extract of *Entanda mannii* (HEEM).

Experimental protocol

Determination of polyphenols: The polyphenolic compounds targeted in this study are: total phenols, total flavonoids, total flavanols and total proanthocyanidins.

Determination of Total Phenolic Contents (TPC): Total phenol content will be determined in plant extracts by the Folin-Ciocalteu method (Mc Donald, 2001). To 0.5 ml of each plant extract (0.1 g / mL) are added respectively 5 mL of the Folin-ciocalteu reagent diluted 1/10 with distilled water and 4 mL of sodium carbonate (1M). After 15 minutes of incubation at room temperature, the optical density is measured spectrophotometrically at 765 nm. Gallic acid prepared in a solvent mixture of methanol / water (50 :50, v / v) is used as a standard at concentrations ranging from 0 to 250 mg / mL. The total phenol content of the total plant extracts is expressed in terms of equivalents of gallic acid per g of solids (mg EAG / g extract).

Determination of total flavonoids

The aluminum chloride colorimetric method will be used to determine the flavonoid content in plant extracts (Chang, 2002). To 0.5 mL of the extract (0.1 g / mL) are successively added 1.5 mL of methanol; 0.1 mL of aluminum chloride 10% (w / v); 0.1 mL of potassium acetate (1M) and 2.8 mL of distilled water. After 30 minutes of incubation at room temperature, the optical density is measured spectrophotometrically at 415 nm. A methanol solution of quercetin is used as a standard at concentrations ranging from 0 to 100 µg / mL. The total flavonoid content of the total plant extracts is expressed in terms of equivalents of quercetin per g of solids (mg EQ / g extract).

Determination of total flavanols

The total flavonol content of the total plant extracts was determined according to the method described by [14]. To 2 mL of extract (0.1 mg / mL), 2 mL of an ethanolic solution of aluminum chloride 2% (w / v) and 3 mL of sodium acetate (50 g / l) are added. After 2h 30 min of incubation at 20 ° C, the absorbance is read spectrophotometer at 440 nm. Quercetin is used as standard at concentrations ranging from 0 to 100 µg / mL. The total flavonol content of the total plant extracts is expressed in terms of equivalents of quercetin per g of solids (mg EQ / g extract).

Evaluation of antioxidant activity

Measurement of antiradical activity; trapping of free radicals by DPPH

The evaluation of the antiradical activity was done according to the method of Sanchez-Moreno C. *et al.,* 1998[15, 16] slightly adapted. By double successive dilutions from a stock solution (0.1 mg / mL), we prepared a range of concentrations of extracts varying from 1.56 to 100 µg / mL. A volume of 50 µl of each extract is taken and then added to the methanolic solution of DPPH (diphenyl picryl-hydrazyl) at 0.025 g / l. After incubating for 30 minutes at room temperature and in the dark, the absorbance is read on a spectrophotometer at 515 nm against the sample blank (medium consisting of 50 µl of methanol with 1.95 mL of the methanolic solution of DPPH). Ascorbic acid (0.1 mg / mL), prepared under the same conditions, is used as a standard. For each concentration range of extracts as well as for vitamin C, the test is repeated 3 times.

The results were expressed as percent inhibition (I%).

$I\% = [(Abs_{control} - Abs_{test}) / Abs\ control] \times 100$

$Abs_{control}$: is the absorbance of the DPPH solution without plant extract

Abs_{sample} : is the absorbance of the reaction medium containing the DPPH and the plant extract or vitamin.

The values of the plant extract concentrations which result in 50% inhibition of the DPPH (IC 50) radicals are determined graphically by linear regression and then compared to that of vitamin C.

Ferric Reducing Antioxidant Power (FRAP): The reducing power of the plant extracts is determined according to the method described by Benzie*et al.,* 1996, and Oyaizu, M., 1986**[**17, 18]. This method measures the ability of the extracts to reduce the ferric ion (Fe3 +) to ferrous ion (Fe2 +).One milliliter of the extract at different concentrations (from 0.007 to 2.5 mg / mL) is mixed with 2.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a solution of potassium ferricyanide K3Fe (CN) 6 to 1%. After 30 minutes of incubation in a water bath at 50 ° C and then addition of 2.5 mL of 10% trichloroacetic acid, the reaction medium is centrifuged at 3000 rpm for 10 minutes.

Table 1. Total phenols, total flavonoids and total flavanols contents of *Entada manni* extracts

Plant organ	Extracts	Total Phenolic contents (mg GAE / g of extract)	Flavonoids (mg QE / g of extract)	Flavonols (mg QE / g of extract)
E. mannii (Bark)	Ethyl acetate	19.74 ± 2	0.69 ± 0.03	0.33 ± 0.02
	Hexane	9. 641 ± 1	0.39 ± 0.01	0.3 ± 0.01

The values are the averages of 3 repetitions ± standard deviation.

To an aliquot of the supernatant (2.5 mL) is then added 2.5 mL of distilled water and 0.5 mL of 0.1% iron III chloride. After another 10 minutes of incubation at room temperature, the absorbance is measured spectrophotometrically at 700 nm against a blank. Ascorbic acid at different concentrations is used in comparison with plant extracts. An increase in the absorbance of the samples indicates an increase in the reducing power of the extracts tested.

RESULTS AND DISCUSSION

Content of polyphenolic compounds: The results of the determination of the various polyphenol compounds are summarized in Table 1. These results are the average of the 3 determinations carried out. Total phenol content has been reported as mg gallic acid / g dry plant material. In contrast, total flavonoid and total flavanol contents were reported in mg equivalent of quercetin / g dry plant material. The results reveal that the acetal extract concentrates the polyphenolic compounds better than the hexane extract. Moreover, these results indicate that total flavonoids and total flavanols represent respectively 3.49% and 5.94% ofTotal Phenolic contents in the acetal extract. Hexanic extract represents 4.04 %and 3.11 % respectively.

Evaluation of antioxidant activity

Measurement of antiradical activity; trapping of free radicals by DPPH: The antioxidant activity of the acetal and hexane extracts of *Entada mannii* and the standard antioxidant (ascorbic acid) with respect to the DPPH radical was evaluated using a spectrophotometer SHIMADZU Spectrophotometer (UV-1700 PharmaSpec; UV-Vis) following the reduction of this radical which is accompanied by its passage from the violet color (DPPH •) to the yellow color (DPPH-H) measurable at 515nm. This reduction capacity is determined by a decrease in the absorbance induced by antiradical substances. Figure 1 shows the percent inhibition of DPPH according to different concentrations of ascorbic acid. Figures 2 and 3show respectively the % inhibition of DPPH according to different concentrations of the acetal and hexane extracts. The different concentrations of IC50 determined from figures 1, 2 and 3 are reported in Table 2. The results obtained indicate that the acetal and hexane extracts of *E.mannii* are endowed with an antioxidant power (AEEM/IC50 = 52.30± 2.05; HEEM/IC50 = 70.51± 1.84µg /mL). This antioxidant activity, which is certainly lower than that of the reference antioxidant represented by ascorbic acid (IC50 = 45.716 ± 1.185), remains nonetheless important, especially with regard to the acetal extract (IC50 = 52.30± 2.05). The important antioxidant activity of the acetal extract would be dependent on the presence of polyphenolic compounds or phenolic compounds. Indeed, the correlations between antioxidant activity and a high content of polyphenolic compounds such as: gallic acid, caffeic acid, rosmarinic acid, salvianolic acid and phenolic acids present in several plants such as *Salvia officinalis* L., *S. virgata,S. nemorosa,S. officinalis, S. bulleyana,S. campanulata S. albicaulis* and *S. castanea* have been established by several authors (Bougandoura, 2013; Apak, 2007; Yinrong, 2001; Kamatou, 2010; Jeshvaghania, 2015 and Li, 2013).

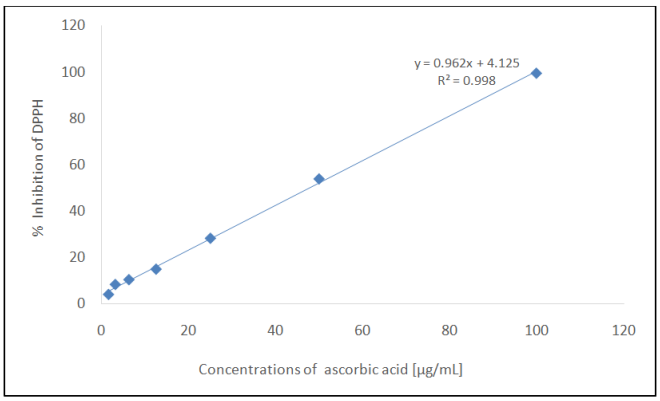

Figure 1. % inhibition of DPPH according to different concentrations of ascorbic acid

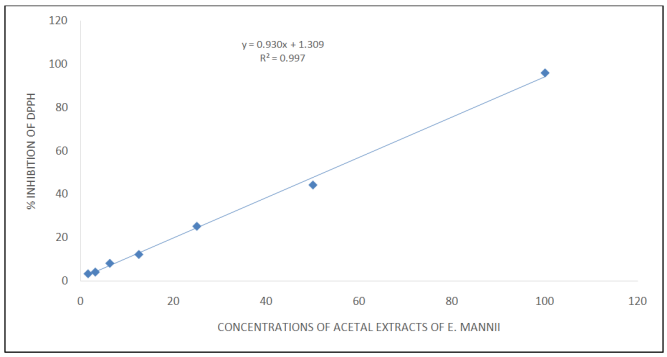

Figure 2. % inhibition of DPPH according to different concentrations of acetal extracts of*E. mannii.*

Ferric Reducing Antioxidant Power (FRAP): The iron reduction test is a simple, fast and reproducible test. It is a test developed by Benzie *et al.,* 1996, and can be applied in plants as well as plasmas and in organic and aqueous extracts (Li, 2008). The presence of reducing agents in plant extracts causes the Fe3 + / ferricyanide complex to be reduced to the ferrous form. Therefore, Fe2 + can be evaluated by measuring and monitoring the increase in the density of the blue color in the reaction medium at 700 nm (Siddhuraju, 2007). On the other hand, it is worth noting that the reducing power of acetal and hexane extracts is dose-dependent. In other words, this power increases proportionally to the concentration. At the concentration of 2.5 mg / ml, the reducing power of the acetal extract of *Entada mannii* is much higher (OD = 0.754)

compared to the hexane extract (OD = 0.631), but much lower than that of the ascorbic acid (Figure 4). The reducing power of the extracts would therefore be related to the presence of polyphenolic compounds.

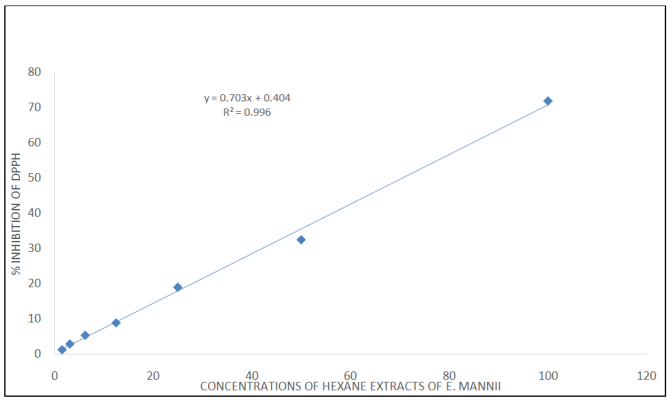

Figure 3. % inhibition of DPPH according to different concentrations of hexane extracts of *E. mannii*

Table 2. Antioxidant test result expressing the effective concentration 50% in µg / mL

		Entadamannii	
	Ascorbic acid (µg /mL)	Acetal extract (AEEA) (µg /mL)	Hexane extract (HEEA) (µg /mL)
% IC50 (515 nm)	45.716 ± 1.185	52.30 ± 2.05	70.51 ± 1.84

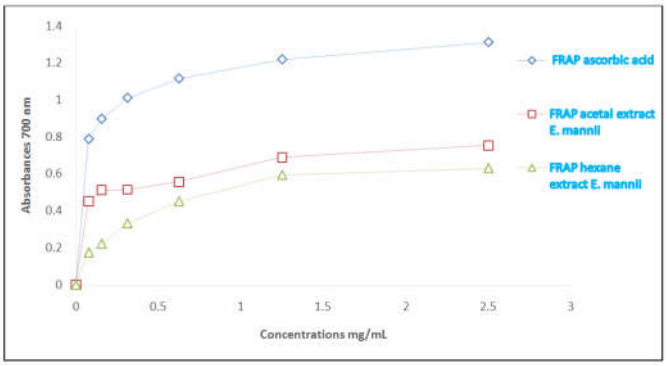

Figure 4. Reducing power of acetatic and hexane extracts of E m*annii* and ascorbic acid

In conclusion the determination of phenolic compounds in the acetal and hexane extracts of *Entanda mannii* has shown that the acetal extract concentrates the polyphenolic compounds better than the hexane extract. This high concentration of polyphenolic compounds in the acetal extract gives it greater antioxidant activity than the hexane extract. But, this activity remains lower than that of ascorbic acid. However, since these are crude extracts consisting of several crude compounds, it is therefore not excluded that they contain compounds which, once purified, can have a much more improved antioxidant activity. Further research to identify, isolate and purify these constituents will therefore be necessary.

Acknowledgment

Our thanks to the FICCI and the Government of India for granting the CV Raman Fellowship to Dr. DOUMBIA Idrissa.

The authors also thank the teams of the Department of Pharmacognosy and Phytopharmacy at JSS College of Pharmacy of Ooty in India for their valuable assistance.

Conflict of Interest: The authors claim that there is no conflict of interest.

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