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Antioxidant Activity of the Major Soldier Salivary Glands Extracts of the Termite *Macrotermes bellicosus*

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Abstract: ***The aim of this study was to evaluate the chemical composition and the antioxidant potential of extracts (aqueous, methanol and acetic) of the major soldier salivary glands of the termite Macrotermes bellicosus. The antioxidant activities of extracts were examined using diphenylpicrylhydrazyl (DPPH) and ferric reducing power methods (FRAP). The presence of phenolics, quinones, flavonoids, terpenoids, saponins, reducing sugars, glycosides, carbohydrates was identified in the extracts. In comparison to the methanolic and acetic extracts, the aqueous extract had the highest total phenolic and flavonoid content at 584 ± 3.25 mg of GAE/g and 12.63 ± 1.88 of mg of QUE/g respectively. The results showed that the extracts presented strong activities of scavenging DPPH free radical and ferric reducing power. The IC_{50} values of extracts (aqueous, methanolic and acetic) were found 8.92 ± 0.54 µg/mL, 28.33 ± 1.35 µg/mL and 39.78 ± 2.13 µg/mL respectively for the DPPH radical scavenging activity. This higher content of total phenolics and flavonoids found in the extracts was directly associated with higher antioxidant activity. The results encourage the use of major soldier of the termite Macrotermes bellicosus for medicinal health, functional food and nutraceuticals applications, due to their antioxidant properties.***

Keywords: Antioxidant, salivary glands, termite, *Macrotermes bellicosus*

1. Introduction

In the last three decades, the search for natural bioactive compounds that can serve as antioxidant agents had increased tremendously. The reasons for these are increasing understanding of the harmful nature of reactive oxygen species (ROS) produced during oxidation processes, harmful nature of synthetic antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These compounds are hazardous to animal and human health as they can be lethal, carcinogenic, mutagenic, teratogenic, immunosuppressant, or may mimic estrogens [1]. It is well established that free radicals are very much implicated in several metabolic diseases that include heart diseases, acquired immunodeficiency syndrome, diabetes mellitus, arthritis, cancer, geing, liver disorder etc. [2], and the antioxidant therapy has gained an utmost importance in the treatment of these diseases [3]. Therefore, there is a pressing need to find and develop new antioxydant agents. In modern societies, zootherapy constitutes an important alternative among many other known therapies practiced worldwide. Wild and domestic animals and their byproducts (e.g. hooves, skin, bones, feathers and tusks) form important ingredients in the preparation of curative, protective and preventive medicine [4].

Insects had been proved to be very important sources of drugs for modern medicine since they have immunological, analgesic, anti-bacterial, diuretic, antirheumatic and anesthetic properties [5]. They contain many bioactive compounds in their hemolymph and exocrine glands that can be of interest in therapeutic. Termites are arthropods inhabiting the subterranean habitats for over several million years and form an important part of the diet of human beings around the world including tribes of western Africa. It's also a rich source of food for various other vertebrates and plants. Besides termite used by the south Indian tribes for treating asthma and as food to enhance lactation in women, revealed a high percentage of protein followed by lipids and carbohydrates [6]. Studies dietary supplementation of termites treated separately with pesticides like acephate and endosulfan, significantly decreased the toxic effect of pesticides and increased litter production more than those given the pesticides alone both in the generations, implying that the components of termite may be inducing the activities of detoxifying enzymes present in Swiss albino mice [7]. High nutritive value of the termite coupled with its probable antitoxic role [8].

The aim of this study was to evaluate the chemical composition and the antioxidant potential of extracts of the major soldier salivary glands of the termite *Macrotermes bellicosus* from Côte d'Ivoire.

2. Materials and Methods

2.1 Biological Material

Materials used in the present study were the major soldier salivary glands of the termite *Macrotermes bellicosus*. The salivary glands were isolated from major soldier of the termite *Macrotermes bellicosus* collected in the termitary surrounding the University Felix Houphouet Boigny (Abidjan, Côte-d'Ivoire).

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2.2 Preparation of extracts

Ten (10) g of the major soldier salivary glands of the termite *Macrotermes bellicosus* were homogenized with 8 mL of sterile water and sonicated as previously described [9]. The homogenate was centrifuged (HERMECE Z 300K) at 12000 rpm for 30 min. The supernatant collected constituted the aqueous crude extract of the salivary glands. The methanolic and acetic extract was prepared by homogenized 10 g of salivary glands in 8 mL of a methanol or ethyl acetate solution. After centrifugation, the supernatant collected were evaporated (50°C) to dryness under reduced pressure using a Rotavapor and appropiate amount of the residue was used for the antioxidant assays.

2.3 Chemical screening

The extracts of the salivary glands were subjected to preliminary chemical testing to detect for the presence of different chemical groups of compounds. The extracts of the salivary glands were screened for the presence of different classes of secondary metabolites including alkaloids, flavonoids, phenols, saponins, tannins, quinones, carbohydrates, glycosides, fixed oils and fats and terpenoids using previously described methods [10].

2.4 Determination of total phenolic content

The total phenolics were estimated the extracts using Folin-Ciocalteu (FC) reagent method [11], employing gallic acid as standard. Briefly, 0.2 mL of extracts (1.25 g/mL) was mixed thoroughly with 0.8 mL of sodium carbonate (75 mg/mL) reagent and incubated for 5 min at room temperature. Then 1 mL of 10 % Folin-Ciocalteu (FC) was added and allowed to stand for a further 90 min at room temperature in the dark. The absorbance was measured at 765 nm using UV-Visible spectrophotometer (Spectronic Genesys 5), and the results were expressed as mg of gallic acid equivalent (GAE)/g of extract.

2.5 Determination of total flavonoid content

Total flavonoid content of the extracts of the salivary glands was determined using the aluminium chloride colorimetric method as described [12]. One (1) mL of extract was mixed with 1 mL of 2 % $AlCl_3$ solution dissolved in methanol. The sample was incubated for 15 min at room temperature. The absorbance was determined using UV-Visible spectrophotometer (Spectronic Genesys 5) at 430 nm. The same procedure was repeated for the standard solution of quercetin and the calibration line was obtained. Based on the measured absorbance, the concentration of flavonoids was read (mg/mL) on the calibration line. Then, the content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of QUE/g of extract).

2.6 Antioxidant assay

DDPH free radical scavenging activity

Quantitative measurement of radical scavenging properties of the extracts was carried out according to the method [13]. Briefly, 3 mM solution of 2,2-diphenyl-1-picryl-hydrazyl (DPPH, Sigma-Aldrich) in ethanol was prepared and 1 mL of this solution was added to 2 mL of the extracts at various concentrations (0–50 µg/mL) and ascorbic acid was used as a positive control. After incubating the mixture for 30 min in the dark, the discoloration was measured at 517 nm. The capacity to scavenge the DPPH radical was calculated and expressed as percent inhibition using the following equation:

I, % = (Absorbance of control – Absorbance of test/ Absorbance of control) × 100

The IC_{50} values (concentration of sample required to scavenge 50 % of free radicals) were calculated from the regression equation prepared from the different concentrations of extracts.

Ferric reducing/antioxidant power activity (FRAP)

Ferric reducing/antioxidant power (FRAP) was determined following the method reported [14]. Extracts (methanol and aqueous) of salivary glands at various concentrations (0–50 µg/mL) was mixed with 5 mL of 200 mM phosphate buffer (pH 6.6) and 5 mL of potassium ferricyanide (1%) and were incubated at 50°C for 20 min. To the incubated mixtures 5 mL of 10 % trichloroacetic acid was added and the tubes were centrifuged at 3.000 rpm for 10 min. Further, five milliliters of the upper layer of the solution was mixed with 5 mL distilled water and 0.5 mL of 0.1 % ferric chloride and absorbance of the reaction mixtures was measured at 700 nm (UV-VIS spectrophotometer).

2.7 Statistical analysis

All experiments were carried out in triplicates. The significant differences between the antioxidant activities of the extracts were determined by one-way analysis of variance and means were compared by Duncan's multiple range tests. The level of significance was set at $p < 0.05$. Excel 2007 and Statistica version 7.1 were used.

3. Results and Discussion

3.1 Results

Chemical analysis

The extracts of major soldier salivary glands of the termite *Macrotermes bellicosus* were subjected to a preliminary chemical screening for various constituents. The results revealed the presence of phenolics, terpenoids, saponins, flavonoids, quinones, glycosides, carbohydrates and reducing sugars in the extracts. But, alkaloids, tannins, fixed oils and fats were not detected in these extracts (Table 1). Total phenolics levels were expressed as mg gallic acid equivalent/g of salivary glands and were found to be highest in these extracts. In comparison to the methanolic and acetic extract, the aqueous extract had the highest total phenolic and flavonoid content at 584 ± 3.25 mg of GAE/g and 12.63 ± 1.88 of mg of quercetin equivalent/g respectively (Table 2). The concentrations of total phenols and flavonoids levels in methanol extract were 217.33 ± 1.24 mg of gallic acid equivalent/g of salivary glands and 7.66 ± 1.15 mg of quercetin equivalent/g of salivary glands respectively.

Table 1: Chemical evaluation of extracts

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(+) indicate presence, (-) indicate absent

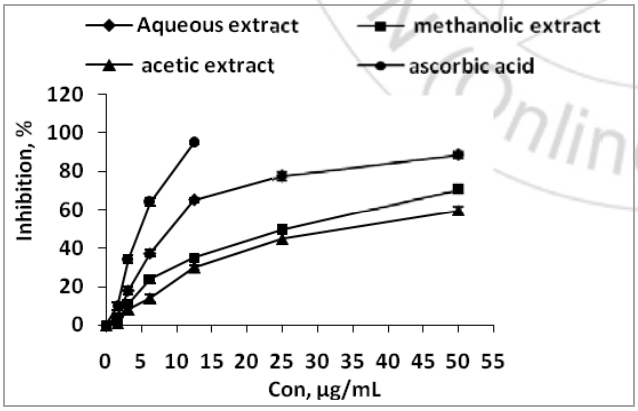
Chemical analysis	*Aqueous extract*	*Methanolic extract*	*Acetic extract*
Phenolics	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Quinones	+	+	+
Tannins	_	_	_
Alkaloids	_	_	_
Carbohydrates	+	+	+
Glycosides	+	+	+
Saponins	+	_	_
Fixed oils and fats	_	_	_

Table 2: Total phenolic and total flavonoid content of the extracts

Salivary glands extracts	*Total phenols (mg of GAE/g)*	*Total flavonoids (mg of QUE/g)*
Aqueous extract	584 ± 3.25	12.63 ± 1.88
Methanolic extract	217.33 ± 1.24	7.66 ± 1.15
Acetic extract	147.66 ± 2.56	0.33 ± 0.28

Scavenging effect on DPPH radicals

The anti-radical activity of the extracts was evaluated by the DPPH method. The percentage of DPPH radicals scavenging was expressed in terms of the concentration of extract (Graph 1). The antioxidant activity of the extracts is expressed as IC_{50}. This parameter defines the effective concentration of the extract that causes the loss of 50 % of the activity of DPPH (color). The IC_{50} values of aqueous, methanolic and acetic extracts were found to be 8.92 ± 0.54 µg/mL, 28.33 ± 1.35 µg/mL and 39.78 ± 0.13 µg/mL , respectively (Table 3). The results indicate that the aqueous extract processed potent DPPH scavenging activities than methanolic and acetic extracts. However, the scavenging effects of the aqueous extract tested on the DPPH radical were lower compared to that of vitamin C taken as a reference substance.



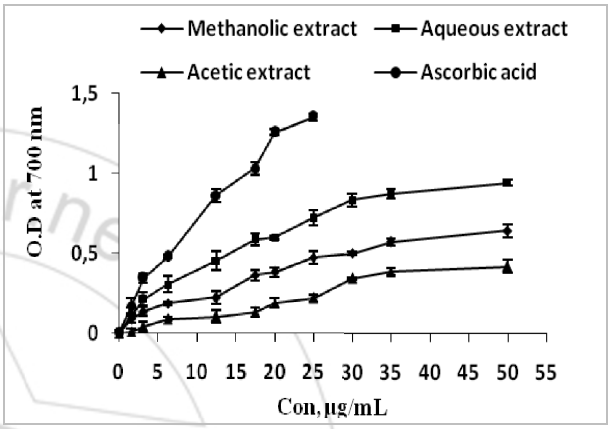
Graph 1: Free radical scavenging activity of the extracts and ascorbic acid was used as a positive control

Table 3: IC_{50} values of salivary glands extracts

Salivary glands extracts	*IC50 values, µg/mL*
Aqueous extract	*8.92 ± 0.54*
Methanolic extract	*28.33 ± 1.35*
Acetic extract	*39.78 ± 2.13*
Ascorbic acid	*4.85 ± 0.03*

Ferric reducing/antioxidant power activity (FRAP)

The reducing capacity of compounds or extract may serve as a significant indicator of their potential antioxidant activities. The presence of a reductant, such as the antioxidant substances in extract, causes the reduction of Fe^{3+} ferricyanide complex to the ferrous form, Fe^{2+} increasing the absorbance of the reaction medium at 700 nm. The reduction capabilities of the aqueous, methanolic and acetic extracts of salivary glands are indicated as in Graph 2. The result has shown that the aqueous extract have a good affinity and reducing power of Fe^{3+} ions. In comparison with the methanolic and acetic extract, the aqueous extract had better reducing power at a concentration of 50µg/mL. However, the power and ability of the aqueous extract to reduce Fe^{3+} ions is significantly lower than ascorbic acid.



Graph 2: Ferric reducing power of the extracts of salivary glands

3.2 Discussion

The aim of this study was to evaluate the chemical composition and the antioxidant potential of extracts of major soldier salivary glands of the termite *Macrotermes bellicosus* from Côte d'Ivoire. Chemical screening results revealed the presence of polyphenols, terpenoids, saponins, quinones, flavonoids, glycosides, carbohydrates and reducing sugars in both extracts. These results were in accordance to the results previously obtained in 15 species of termite soldiers [15]. In this study the water was the best solvent for extraction of phenolic and flavonoids compounds of the salivary glands compared to methanol and ethyl acetate. This would probably due to make that these compounds have more affinity in water. So salivary glands termites have water tanks [16], therefore these compounds would be more stable in water. The total phenolic compound level in these extracts was higher as compared to that observed in many medicinal plants and secretions of certain arthropods. The phenolic compound content of propolis bees *Api melifera* was various between 2.93 and 8.76 mg/mL [17]. The higher amount of total phenolic and flavonoid content of aqueous extract suggests that it possesses high antioxidant activity. DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants [18]. These results indicate that the extracts have a significant and noticeable effect on scavenging free radicals by DPPH method and this can be directly associated to the high phenolic constituents present in the extract. There, this activity could be due to an interaction of both the phenolic

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compounds and some other characterized molecules such as terpenoids or glycosides. Thus the anti-radical power against DPPH of extracts tested is similar as that found in propolis of several bees *Apis melifera* (IC_{50} = 0.007-0.069 mg/mL) [17]. These extracts also possess ferric reducing power. The degree of chelating ferrous ions by extract is lower than ascorbic acid. The ferric reducing power of extract may be attributed to the phenolic and flavonoid contents of the extracts. The ability to reduce Fe (III) may be attributed to the hydrogen donation from phenolic compound, which is related to the presence of a reducing agent [19]. The phenolics compound of major soldier salivary glands of the termite *Macrotermes bellicosus* could come from the insect's own secretions or from an accumulation of products from lignocellulose compounds degradation consummed by the insect. These results are important especially to their action on the DPPH scavenging and ferric reducing power. Indeed, these extract could be incorporated in some ointments to fight effectively against several metabolic diseases.

4. Conclusion

This study demonstrated the potent antioxidant activity of the extracts of major soldier salivary glands of the termite *Macrotermes bellicosus*. Further, it was observed that there was a strong association between the higher antioxidant activities with that of higher total phenolic and flavonoid content in these extracts. The results of this study reveal that the major soldier salivary glands of the termite *Macrotermes bellicosus* could be considered as a natural antioxidant source that can be used for medicinal health.

Future work will be interesting to determine the toxicologycal profile and to learn the chemical composition and better understand the mechanism of action of the antioxidants present in the extract for development as drug for therapeutic application.

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