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Research Article

DPPH Free Radical Scavenging Activity of Two Extracts from *Agelanthus dodoneifolius* (Loranthaceae) Leaves

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ABSTRACT
Agelanthus dodoneifolius (Loranthaceae) commonly named African mistletoe is traditionally used in Burkina Faso as a decoction for the treatment of asthma, stomach-ache and cardiovascular diseases. The current study was designed to assess the DPPH free radical scavenging effect and the flavonoid and phenolic contents of the decoction and maceration extracts from *Agelanthus dodoneifolius* leaves. Moreover, the phytochemical analysis was also carried out. The decoction and maceration were prepared in water. Phytochemical analysis was realized according to the standard protocols. The scavenging activity was determined using the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The flavonoid and phenolic contents were assessed using the Neu and Folin-Ciocalteu reagents, respectively. The phytochemical investigation showed the presence of flavonoid, tannins, steroids, triterpenes, carotenoids, anthocyanins and sugars in *Agelanthus dodoneifolius* dried and powdered leaves. Both decoction and maceration exhibited a significant dose-dependent radical scavenging activity with EC_{50} values of 8.20 ± 0.25 and 13.20 ± 0.37 µg/mL, respectively. This significant radical scavenging effect may be attributed to their richness in flavonoid and phenolic compounds. This study enhances the ability of *Agelanthus dodoneifolius* leaves as excellent natural source for antiradical scavenging. Therefore, the results may be taken account for the development of an herbal medicine to treat diseases related to an oxidative damage.

Keywords: *Agelanthus dodoneifolius*, decoction, maceration, DPPH, antiradical activity, phenolic compounds.

INTRODUCTION

The use of medicinal plants for the treatment of various diseases is as old as mankind[1]. Nowadays, people have demonstrated a significant enthusiasm towards traditional and complementary medicine that several countries included this kind of medicine in their health programs[2]. Indeed, the traditional and complementary medicine constitutes sometimes the only and most reliable source of care for many millions of people as it is more accessible, affordable and culturally acceptable and trusted[2]. Medicinal plants are more and more gaining success since their compounds are generally better tolerated and have less side effects when compared to the non-natural or synthetic drugs[3]. The study of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) is still growing, evidenced with a tremendous literature[4]. Free radicals are formed as byproducts of normal biological process and can intervene positively or negatively in the body[5]. Some of their positive actions concern energy production, regulation of cell growth and phagocytosis, while their harmful effects are related to the fact that they seriously damage important biomolecules such as nucleic acids, proteins, lipids and cell membranes[5-6]. The noxious effects of free radicals are balanced with endogenous and exogenous antioxidants that act by preventing the onset of oxidative stress which is involved in the development of important diseases such as cancer, neurodegenerative disorders and aging[7]. The plant-derived antioxidants such as polyphenolic compounds are still preferred to the synthetic antioxidants which are suspected to promote negative health effects[8]. Moreover, as stated by some epidemiological studies, a long-term consumption of diet rich in plant polyphenols contributes to fight oxidative stress and thereby preventing the onset of serious chronic diseases[9]. *Agelanthus dodoneifolius* (Loranthaceae), also named African mistletoe is an ubiquitous and evergreen shrub that is largely distributed throughout Africa[10,11]. *Agelanthus dodoneifolius* is used to treat various diseases such as cardiovascular diseases, asthma, wound and cancer[12,13]. The traditional uses of *Agelanthus dodoneifolius* involve mainly decoction and local application. The current study was undertaken to assess the DPPH free radical scavenging effect of both decoction and maceration extracts obtained from the leaves of *Agelanthus dodoneifolius.*

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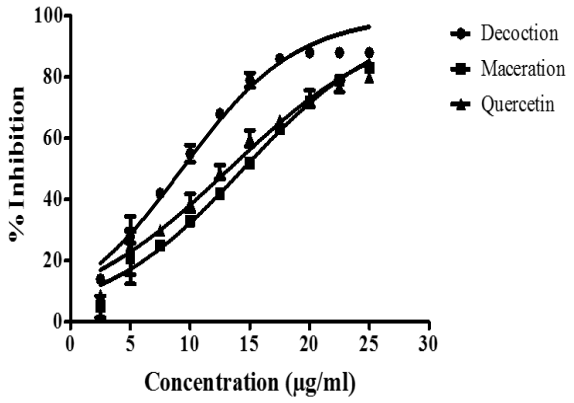

Figure 1: DPPH radical scavenging activity of Agelanthus dodoneifolius extracts and quercetin. *Values are presented as mean ± standard deviation of duplicate experiments.*

Table 1: EC_{50} (µg/ml) and ARP values of *Agelanthus dodoneifolius* leaves aqueous extracts

Sample	EC_{50} (µg/mL)	ARP
Quercetin	11.81± 0.32[a]	0.08
Maceration	13.20 ± 0.37[c;a]	0.08
Decoction	8.28 ± 0.25[b;a]	0.12

Values are mean ± standard deviation of two independent experiments. Within a column; means not sharing the same letter (a; b; c) are significantly different (p < 0.05; unpaired t-test with Welch's correction). Quercetin was used as standard.

MATERIALS AND METHODS

Plant material collection**:** Leaves of *Agelanthus dodoneifolius* were harvested from *Vitellaria paradoxa* (Sapotaceae) in the area of Ouagadougou. Samples were authenticated and a voucher specimens (01 & 02) were deposited in the herbarium of the Plant Ecology and Biology Laboratory, University of Ouagadougou, Burkina Faso.Reagents and chemicals**:** 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu phenol reagent, 2-aminoethyl diphenylborinate, quercetin, gallic acid and sodium carbonate were purchased from Sigma (St-Louis, USA). All other chemicals and solvents were of analytical grade. Preparation of extracts**:** The aqueous extracts were obtained with the shade dried leaves of *Agelanthus dodoneifolius* that were powdered using a grinder machine. Maceration was prepared by dissolving 200 g of powdered leaves into 1 L of distilled water. The extract was allowed to shake for 24 h. The decoction was obtained by dissolving 100 g of leaves powder in 1 L of distilled water and then brought it to boil for 15 min. All the extracts were then filtered and freeze dried (Christ Alpha, 1-2 LD). The yield of each extract was obtained from the formula: $Yield\ (\%) = (m_f / m_i) \times 100$

m*f*: final mass of extract obtained after the lyophilization

m*i*: mass of initial dry powder used

Phytochemical screening**:** The phytochemical screening was adapted from qualitative standard methods described by[14]. The detection of steroids, triterpenes, anthocyanins, flavonoids, tannins and sugars was carried out with the dried powdered leaves of *Agelanthus dodoneifolius*. DPPH radical scavenging assay**:** The principle of this assay is based on the reduction of DPPH, a free stable radical by an antioxidant according to the following reaction[15].

$DPPH^{\bullet} + A\mathbf{H} \rightarrow DPPH\mathbf{H} + A^{\bullet}$

During the reaction, alcoholic solution of DPPH turns from deep violet color to light yellow color.

The DPPH free radical assay was carried out in a 96-well microplate using the method previously described[16]. with slight modifications. Briefly, 100 µL of various concentrations of extract in methanol (from 5 to 25 µg/mL) were added to 100 µL of 0.01% methanolic DPPH solution. The plate was incubated for 30 min in the dark at ambient temperature and the absorbance was recorded at 540 nm using a spectrophotometer (Bio-Rad, Belgium). Quercetin at different concentrations (5-25 µg/mL) was used as standard. The DPPH radical scavenging activity (%) was calculated as follows: $DPPH\ scavenging\ activity\ (\%) = [(Ac - As) / Ac] \times 100$

Where Ac was the absorbance of control [DPPH + Methanol without sample] and As was the absorbance of sample [DPPH + Sample (extract/standard)]. In order to assess the antiradical potency, the EC_{50} (defined as the concentration of substrate that causes 50% reduction of the DPPH color) and the antioxidant radical power (ARP) were determined. The ARP is calculated as the inverse of EC_{50} value[17].

$ARP = 1/EC_{50}$

Determination of flavonoid content**:** The flavonoid content of the aqueous extracts of *Agelanthus dodoneifolius* leaves was adapted to a 96-well plate using the Neu's reagent (2-aminoethyl diphenyl borinate) according to the method of[18]. with minor modifications. The extracts (0.5%) were diluted 50% with methanol (80%) and then, 2 mL of the resulting solution was mixed with 100 µL of Neu'reagent (1% in methanol). The absorbance of the mixture was measured at 405 nm using a multiwell plate reader (Bio-Rad, Belgium). The extract absorptions were compared to that of a standard methanolic solution of quercetin (0.05 mg/mL) treated with the same reagent and under the same conditions. The flavonoid content was calculated as follows: $F\% = [(0.05 \times Ae) / (Aq \times Ce)] \times 100$

Where Ae was the absorption of aqueous extract, Aq was the absorption of quercetin and Ce was the extract concentration (Ce = 2.5 mg/mL under our experimental conditions). Determination of total phenolic content**:** The amount of total phenolic compounds in the aqueous extracts of *Agelanthus dodoneifolius* leaves was determined using Folin-Ciocalteu reagent according to the method described by[19] with some modifications. Briefly, 25 µL of Folin-Ciocalteu's reagent (50%, v/v) was mixed with 10 µL of aqueous plant extract (1

Table 2: Total flavonoid and total phenolic contents of *Agelanthus dodoneifolius* leaves aqueous extracts

Sample	Flavonoid content (g QE/100 g extract)	Total phenolic (g GAE/100 g extract)
Maceration	1.40 ± 0.03^{b}	18.55 ± 0.21^{a}
Decoction	1.48 ± 0.01^{a}	18.33 ± 0.45^{a}

Values are mean ± standard deviation of two independent experiments. Within a column; means not sharing the same letter (a; b; c) are significantly different ($p < 0.05$; unpaired t-test with Welch's correction).

mg/mL). The plate was allowed to incubate 5 min at room temperature,
after which 25 µL of 20% (w/v) sodium carbonate was distributed in the wells and then distilled water was added in order to reach a volume of 200 µL per well. The plate was then incubated for 30 min at room temperature and the absorbance was read at 540 nm with a microplate reader (Bio-Rad, Belgium) against a blank (the reagent was replaced by water). Gallic acid was used as standard and the phenolic content, expressed as gram of Gallic Acid Equivalents (GAE) per gram of lyophilized extract (g GAE/g of extract), was obtained with the calibration curve of gallic acid ($y = 0.0302x – 0.0128$, $R^2 = 0.996$).

Statistical analysis

Assays were realized in duplicate and the results are expressed as mean ± standard deviation (SD). The plotted of DPPH radical scavenging activity graphs and the EC_{50} values were obtained using the GraphPad Prism® (version 5.0) software. An unpaired *t*-test with Welch's correction was used for statistical comparisons and a *p*-value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

The yields of maceration and decoction extracts were practically the same with respective values of 18.34 and 17%. This result suggests that the decoction extract may contain compounds that are thermoresistant. The qualitative phytochemical screening revealed the presence of phytochemicals such as steroids, triterpenes, carotenoids, flavonoids, anthocyanins, tannins and sugars in the dried and powdered leaves of *Agelanthus dodoneifolius*. Their presence may explain some of the pharmacological activities of *Agelanthus dodoneifolius*[13, 20-22]. There are various *in vitro* chemical models to evaluate the radical scavenging activity of a pure compound or plant extract. In the present study, the radical scavenging effect was assessed according to a modified method of[16]. using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. DPPH• assay is routinely employed in laboratories for determining the free radical scavenging potential of purified phenolic compounds and natural plant extracts since the assay is rapid, easy and inexpensive[17,23-24]. Fig.1 presents the DPPH radical scavenging activity of quercetin compared to that of both maceration and decoction extracts. The DPPH assay measures the ability of a compound to act as free radical scavenger or hydrogen donor[25]. Our results demonstrated that the scavenging effect was dose-dependent for quercetin and the aqueous extracts of *Agelanthus dodoneifolius*. The efficient concentration (EC_{50}) and the antioxidant radical power (ARP) are some of parameters that are used to express the antioxidant potency[25-26]. Thus, it's reported that an antioxidant is more potent when the EC_{50} and the ARP values are respectively lower and larger[17,25]. Therefore, considering the results reported in Table 1, the antiradical capacity can be ranked in the following order: decoction > quercetin > maceration. Phenolic compounds such as phenolic acids, flavonoids and tannins are widely distributed in the plant kingdom and therefore represent the most important secondary metabolites of the plants[27]. These metabolites, generally involved in protecting plants from UV light and pathogens aggression, are known as radical free scavengers[9,27]. Hence, the antiradical scavenging capacity of a plant may be attributed to its content in polyphenols. Table 2 expressed the flavonoid and phenolic contents of the maceration and decoction extracts from the leaves of *Agelanthus dodoneifolius*. The flavonoid content of the decoction extract was slightly higher than that of the maceration extract while there was no statistically significant difference in their phenolic content. The phenolic content reported in this study was lower than that of our previous report[22] since, here, a modified Folin-Ciocalteu colorimetric assay method was used. Therefore, the results may have been underestimated. Based on these results, it can be argued that the best potent antiradical effect of the decoction may be attributed to its flavonoid content.

CONCLUSION

In the present study, we reported the DPPH free radical scavenging effect as well as the total flavonoid and phenolic contents of decoction and maceration extracts of *Agelanthus dodoneifolius* leaves. The extracts, especially the decoction, exhibited a significant DPPH radical scavenging activity that can be related to their polyphenolic content. Therefore, *Agelanthus dodoneifolius* leaves are excellent natural source for antioxidants and the plant may be used for the development of an herbal drug in the treatment of various diseases such as asthma and cardiovascular diseases. Taken together, the results give a valid justification to the use of *Agelanthus dodoneifolius* as a decoction and, hence confirm that oral route and decoction are the most preferred method of herbal administration and preparation.

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