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Research Article

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Antioxidant activity and phytochemical study of leaf extract of *Prosopis africana (*Guill & Perr Taub) an anti-tumor plant used traditionally

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ABSTRACT

A therapy with natural antioxidants to support the endogenous antioxidant defense system could best protect more effectively to oxydative stress and cancer. This work aims to extract and characterize the major components of Prosopis africana first, to evaluate the antioxidant activity of compounds. The method applied to measure the antioxidant activity was the free radical scavenging by using DPPH• and ABTS$^{•+}$. Ethanolic and aqueous extracts inhibited the absorbance of DPPH and ABTS radical depending on the different concentrations of each solution. The calculated IC_{50} are 0.97; 2.28; 2.56 and 20.83µg/ml for the quercetin, the ascorbic acid, the ethanol and aqueous extracts respectively. The phytochemical revealed that extracts contained alkaloids salts, saponins, tannins and flavonoids. Prosopis africana can be considered as an antioxidant plant.

Key words: Antioxidant, phytochemical, ABTS$^{•+}$, DPPH•

INTRODUCTION

Recorded history from ancient Egypt, Assyria, China and India shows that the use of plants for medicinal purposes is the most ancient approach to healing. There is a worldwide green revolution which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs [1]. Medicinal plants have been a rich source of useful drugs, some of which have acted as lead compounds for further development of synthetic and semi-synthetic compounds. Initial screening of plants for probable antimicrobial actions typically begins with crude aqueous or alcoholic extract of plant followed by a range of separation and identified technologies. The analysis of plant extracts and isolated compounds for biological activity discovered that secondary metabolites may be useful as a resource for new active drug agents [2] In Nigeria, *Prosopis africana* is used to treat body pains, anxiety and toothache [3]. It contains chemically diverse compounds such as: alkaloids, terpenoids, flavonoids and glycosides which are of interest for their biological activities [4].

Reactive oxygen species, in turn, are significantly present in biological processes of energy production and phagocytosis. The main superoxide anions are (O_2^-), hydroxyl radical (OH^-), nitric oxide (NO), hydrogen peroxide (H_2O_2), and lipid radical (L^-). Among these, hydroxyl radical is more reactive in the induction of lesions in cellular molecules whilst hydrogen peroxide is sufficiently able to cross the nuclear membrane and cause damage to the DNA molecule. Thus, effective and safe antioxidants acquired sustainably from the biodiversity can diminish the threat of free radicals and reactive oxygen species damage over lifetime [5,6,7]. Polyphenols are among the most common antioxidants [8]. A therapy with natural antioxidants to support the endogenous antioxidant defense system could best protect more effectively to oxidative stress; hence an important therapeutic challenge [9,10,11]. So it seems important to study the antioxidant activity of extracts of medicinal plants as *Prosopis africana*. This work aims to extract and characterize the major components of this plant first, to evaluate the antioxidant activity of compounds by the methods of ABTS$^{•+}$ and DPPH• other hand.

EXPERIMENTAL SECTION

2.1. *Plant material*

The aerial parts of *Posopis africana* (leaves) were supplied as powdered and dried. It was harvested from Sikasso: a city 380 Km south the capital Bamako in Mali. It was identified by the Head of Botany Department of the Faculty of Pharmacy of Bamako where herbarium specimens were deposited.

2.2. *Preparation of plant extract*

Methanol and aqueous extracts of *Prosopis africana,* was prepared according Sarr et al. 2015. 500 grams of the plant powder was extracted with ethanol (1L, 95°) for ethanolic extract and water (1L, 90°C) for water extract. The extract solution was concentrated to dryness in a rotary evaporator. The crude extract was conserved at 4°C until use. The required concentrations were prepared with extracts for absorbance measurements [12].

2.3. Antioxidant activity determinations ($ABTS^{\bullet+}$)

For $ABTS^{\bullet+}$ assay was done according to the method of Arnao et al. (2001) with some modifications. The stock solutions were prepared by dissolving 38.4 mg of $ABTS^{\bullet+}$ and 6.75 mg potassium persulfate each with 2.5 mL of distillated water. The working solution was then prepared by mixing the two stock solutions ($ABTS^{\bullet+}$ and potassium persulfate) completed to 10 mL and allowed to react for 12 hours at room temperature in the dark. This solution was diluted by mixing of methanol to obtain an absorbance of 0.70 at 734 nm. For each assay, 2 mL of alcohol extract, aqueous extract, quercetin and ascorbic acid at different concentration (1, 2, 3, 4, 5µg/mL) were allowed to react with 2 mL of $ABTS^{\bullet+}$ solution diluted and left for 2 minutes. The absorbance was measured at 734 nm using the spectrophotometer [13].

2.4. Antioxidant activity determinations (DPPH•)

The DPPH• assay was done according to the method of Brand-Williams et al. (1995) with some modifications. The stock solution was prepared by dissolving 4 mg DPPH• with 100 mL of methanol for 12 hours in a dark. The working solution was tested at 517 nm. For each assay, 0.8 mL of alcohol extract, aqueous extract, quercetin and ascorbic acid at different concentration (1, 2, 3, 4, 5µg/mL) were allowed to react with 3.2 mL of the DPPH• solution in dark. 30 mn after the absorbance was measured at 517 nm using the spectrophotometer.

The measurements of absorbance were made in triplicates. The ability to inhibit absorbance of ABTS•+ or DPPH• radical was calculated by the following formula (1):

$$PI = \frac{\mathrm{A1} - \mathrm{A2}}{A1} * 100$$

A1: absorbance of ABTS•+ or DPPH• solution; A2: absorbance of ABTS•+ or DPPH• solution after adding the extract [14,15].

2.5. Total phenolic estimation determination

The total phenolic of alcoholic or aqueous extracts were determined by the Folin-Ciocalteu (FC) method using Gallic acid as a standard (5-25 µg/mL) by modifying the protocol of Anvitha, (2015). Different concentrations of standard as well as the extracts (50-250 µg/ml) were taken and 1 mL of Folin-Ciocalteu reagent (1:1 dilution) was added, 3-5 mn after 2.0 mL of sodium carbonate was added and the mixture was allowed to stand for 45 mn under dark condition. After absorbance of standard and samples were read at 670 nm using a spectrophotometer. The concentration of total phenolic was expressed in terms of µg/mL GAE (Gallic acid equivalence) [16,17].

RESULTS AND DISCUSSION

3.1. Antioxidant activity determinations ABTS•+ and DPPH• tests

Both ethanolic and aqueous extracts inhibited the absorbance of DPPH• and ABTS•+ radical depending on the different concentrations of each solution. The ethanol extract presented higher activity compared to aqueous extract with ABTS•+ and DPPH•. It is also possible to observe, that the inhibitory activity of each extract, is greater with ABTS•+ has DPPH•. Percentages of inhibition (PI) of the two extracts are almost similar to those of quercetin and ascorbic acid, used as a standard. The PI of the high concentrations of ethanolic extracts tends to reach 100% values. In opposite, inhibition of DPPH• radical with the aqueous extract has a constant tendency between 26.39 and 30.94%. With concentrations extracts tested, all the curves show a non-linear domain with the depletion of standards concentrations (Figure 1, 2, 3 and 4).

Antioxidant activities measured in methanol and aqueous extracts obtained using ABTS•+ and DPPH• assays were measured three times to test the reproducibility of the assays. The standard and extracts curves were nonlinear between 1 and 5 µg/ml. The DPPH• assays with quercetin and ascorbic acid showed no significance differences among determinations, while the ABTS•+ assays with these standards differed among runs. The values of the PI with quercetin are slightly greater than those of ascorbic acid in DPPH• test. However, these values are much higher than those of ascorbic acid in ABTS•+ test. The PI coincides when the concentrations of the two standards almost completely neutralize those DPPH• and ABTS•+ to very strong concentrations (Figure 1 and 2). Both extracts, aqueous and ethanolic, behave differently with ABTS•+ and DPPH•. The PI values of the methanol extract are higher with the ABTS•+ test. This difference is most notable with the aqueous extract. Activities of both extracts are similar to those of ascorbic acid with ABTS. A higher quercetin activity can be observed compared to extracts with the same radical. In DPPH• test, the extracts have a very similar activity to that standards but ethanolic extract was more active than aqueous extract. Figures 1, 2, 3 and 4 provide more information on these aspects mentioned above.
Absorbance is inversely proportional to the PI (formula 1). The calculated PI saw strong fluctuations at low concentrations of the extracts and standards. This fluctuation becomes waste to very high concentrations of extract. At over 90%, this fluctuation is explained by the concentration of the extracts and standards. The correlation of the two parameters (PI and concentration) is performed in a growing manner until the complete neutralization of the concentration of ABTS•+ and DPPH•. Conversely, absorbance and concentration move in opposite directions that is to say when the absorbance is high is that the standard or sample concentration is low.

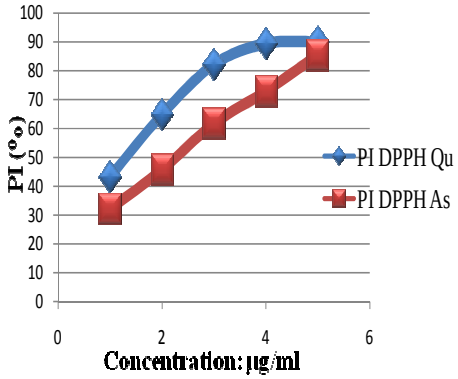

Figure 1: PI of the free radical DPPH• by ascorbic acid and quercetin.

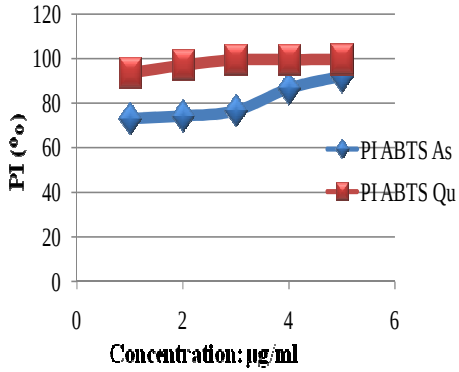

Figure 2: PI of the free radical ABTS•+ by ascorbic acid and quercetin.

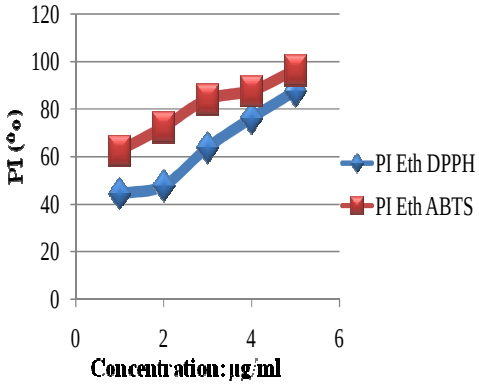

Figure 3: PI of the free radical DPPH• and ABTS•+ by the ethanol extract.

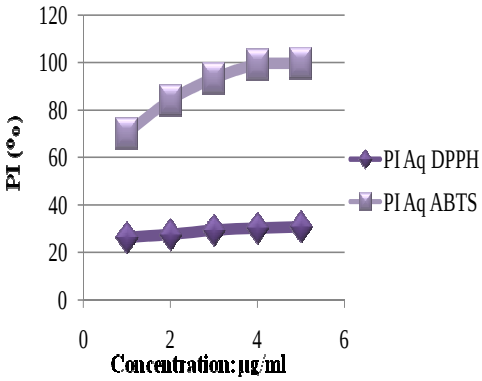

Figure 4: PI of the free radical DPPH• and ABTS•+ by the aqueous extract.

3.2. IC_{50} value of different antioxidant activity

The simplest estimate of IC_{50} is to plot x-y and fit the data with a straight line (linear regression). IC_{50} value is then estimated using the following formula (2):

$$y = a * x + b \qquad IC50 = \frac{(0.5-b)}{a}$$

The highest IC_{50} inhibitory concentration was 20.83 g / ml. It was calculated from the results of the reaction of the ethanolic and aqueous extracts with DPPH•. An IC_{50} value was shown in Figure 5.

The calculated IC_{50} are 0.97; 2.28; 2.56 and 20.83µg/ml for the quercetin, the ascorbic acid, the ethanol and aqueous extracts respectively. These results show that the quercetin and ascorbic acid are more active than the extracts with the ABTS•+ assay.

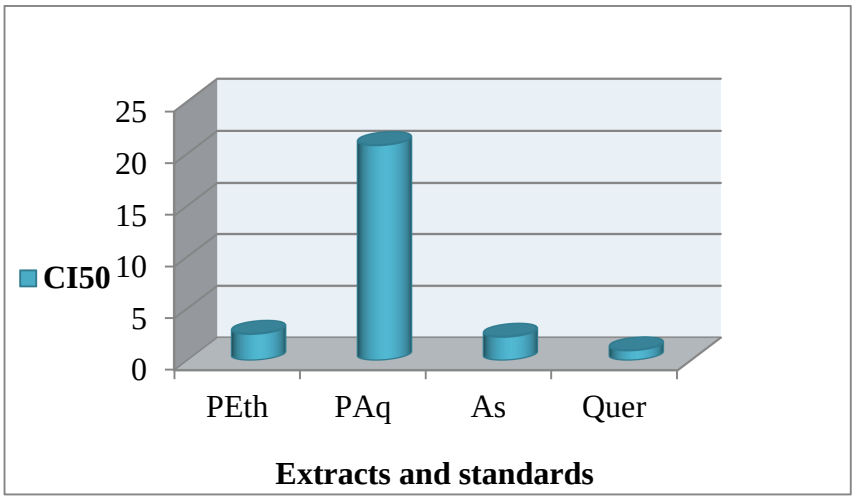

Figure 5: IC_{50} of standards, ethanolic and aqueous extracts (DPPH•)

3.3. Total phenolic determination

The ethanolic and aqueous extracts of *Prosopis africana* leaves contained moderate phenolic compounds respectively 34.19 and 33.02µg/ml.

3.4. Phytochemical Screening

The phytochemical of ethanolic and aqueous extracts revealed that ethanolic extract contained alkaloids salts, saponins, tannins, flavonoids, while aqueous extract contained all that ethanolic extract had except flavonoids (Table 1). The phytochemical of ethanolic and aqueous extracts revealed that ethanolic extract contained alkaloids salts, saponins, tannins, flavonoids. Study of extracts from the leaves of *Prosopis africana*, the results showed the presence of a compound whose chemical formula is: $C_{16}H_{14}O_6$. This compound was isolated from the ethyl acetate extract of *Prosopis africana*. Based on the spectral data and by the comparison with literature, the structure of this compound was identified as 7,3',4'-trihydroxy-3-methoxyflavanone (1) reported for the first time in this plant [2]**.** These results fully confirm the presence of alkaloids highlighted by the phytochemical screening done during this work. Most of these chemical constituents have been known to be associated with particular pharmacological activities [18,19]. These pharmacological principles are the phytochemical like alkaloids, saponins, tannins, steroids [20,21,22].

Table 1: Phytochemical screening of ethanolic and aqueous extracts

Phytochemical compound	Ethanolic extract	Aqueous extract
Tanins	++++	++++
Polyphenols	+++	+++
Saponosides	++++	++++
Alkaloid salts	+++	-

++++ = Abundant; +++ = moderately present; - = absent.

CONCLUSION

After this study, we can say that all biological activity is dependent on the presence of secondary metabolites in plant tissues. The plant studied contains polyphenols, alkaloids, saponins and flavonoids. It also has some antioxidant activity and can be considered as an antioxidant plant. This antioxidant activity is very high and similar to that of quercetin and ascorbic acid.

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