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Original Article

Phytochemical Screening and Antioxidant Effect of Ethanol Leaf and Trunk Bark Extracts of *Cordyla pinnata* (Lepr. Ex A. Rich.) Milne-Redh. (*Caesalpiniaceae*)

Alioune Dior FALL[1,*], Serigne Ibra Mbacké DIENG[1], Abdou SARR[1], Mbaye DIENG[1]

ABSTRACT

Introduction: The aim of this study was to investigate the phytochemical composition and the antioxidant effect of ethanol leaf and trunk bark extracts of an African wild plant, *Cordyla pinnata* (Lepr. Ex A. Rich.) Milne-Redh. *C. pinnata* is used in Senegalese folk medicine to treat asthenia, spasm and various infections. **Methods**: The phytochemical investigation of the extracts was done using physico-chemical reactions while antioxidant effect was assessed by DPPH and FRAP assays. **Results:** Tannins, flavonoids, cardiotonic heterosides and triterpenoids were present in leaf and trunk bark extracts of *C. pinnata*. Meanwhile, anthracenic derivatives were detected in the leaf extract of *C. pinnata*. In DPPH assay, IC_{50} values of the leaf, trunk bark extracts and ascorbic acid were respectively 21.07 ± 0.11 µg/ml, 19.53 ± 0.42 µg/ml and 0.33 ± 0.11 µg/ml. In FRAP assay, the leaf and trunk bark extracts and ascorbic acid reduced significantly ferric ion. Ascorbic acid was seen to be more active in FRAP assay than the leaf and trunk bark extracts of *C. pinnata*. **Conclusion**: Ethanol leaf and trunk bark extracts of *C. pinnata* had revealed antioxidant activity.

Key words: *Cordyla pinnata*, Phytochemical screening, Antioxidant, Leaf, Trunk bark.

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INTRODUCTION

Cordyla pinnata is a tree commonly found in areas of dry forests and tree savannas from Senegal to Niger, northern Nigeria and northern Cameroon.[1] Its leaves are imparipinnate with 10 to 20 leaflets alternate to almost opposite. Its trunk is often straight and cylindrical, fissured and scaly at the surface. Trunk bark extracts are used in folk medicine to treat constipation, liver diseases and also as tonic while leaf extracts are reputed for their anthelmintic and antispasmodic properties.

Oxidative stress due to free radicals overproduction has been involved in neurodegenerative disease (Alzeimer and Parkinson diseases), diabetes, cardiovascular disease, atherosclerosis, rheumatoid arthritis etc.[2,3]

There is a paucity about pharmacological and chemical composition investigations of leaf and trunk bark extract of *C. pinnata*. What makes us to assess the phytochemical composition and the free radicals scavenging effect of the leaf and trunk bark extracts of this plant.

MATERIAL AND METHODS

Plant collection

Leaves and trunk barks of *C. pinnata* were collected at Gossas (Senegal). The plant was identified and authenticated by Dr William Diatta (Herbarium of the Botanical Garden of the Faculty of Medicine, Pharmacy and Odontology; Cheikh Anta DIOP University of Dakar, Senegal) where voucher specimens were kept. Plant leaves and trunk barks were air dried at room temperature and ground.

An amount of 30 g of powdered leaves of *C. pinnata* was decocted twice for 30 minutes using 300 ml of ethanol and filtered through Whatman No. 1 filter paper. Ethanol was removed under reduced pressure using a rotary evaporator and lead to the ethanol leaf dried extract. The same process was used to obtain the trunk bark extract.

Phytochemical composition

To assess the phytochemical composition of the ethanol leaf and trunk bark extracts of *C. pinnata*, standard phytochemical analyses were used. Chemical tests were carried out on ethanol leaf and trunk bark extracts of the powdered specimens using standard procedures for the detection of alkaloïds (Bouchardat, Valser-Mayer and Dragendorff's reagents tests), tannins (Stiasny test followed by ferric chloride test), flavonoids (Shibata's reaction), saponins (foaming index), sterols and triterpenoids (Liebermann-Buchard reaction), carotenoids (Carr and Price reaction) anthracenic glycosides (Borntraeger test), cardiac glycosides (Baljet, Kedde and Raymond-Marthoud reagents tests), in order to identify the presence of phytoconstituents.[4]

DPPH assay

The determination of the DPPH free radical scavenging activity of samples was done using the described method.[5] An ethanol solution of DPPH was prepared by dissolving 4 mg in 100 ml of ethanol. An aliquot of each sample (0.8 ml) at

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appropriate concentration was added to 3 ml of ethanol solution of DPPH.

The ethanol leaf and trunk bark extracts of C. *pinnata* and ascorbic acid were tested at different concentrations. The absorbance of each sample was measured at 517 nm after 30 min using a spectrophotometer. Each experiment was done in triplicate and the absorbance of the initial ethanol DPPH solution did not change after 30 min. The antioxidant activity related to the DPPH free radical scavenging effect was expressed as IC_{50} (concentration of sample required to scavenge 50% of free radicals). IC_{50} values were determined with Statgraphics Plus 5.0 software

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing power was determined according to the described method.[6] An aliquot of 0.20 ml of each sample (ethanol leaf and trunk bark extracts of *C. pinnata*) at appropriate concentration was mixed with 0.5 ml of phosphate buffered saline (0.2 M; pH 6.6) and 0.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 30 min and 0.5 ml of 10% trichloroacetic acid was added. After centrifugation for 10 minutes at 3000 rpm, the supernatant (0.5 ml) was mixed with distilled water (0.5 ml) and 0.1% ferric chloride (0.1 ml). The experiments were done in triplicate. Absorbance was measured at 700 nm using a spectrophotometer and ascorbic acid was used as positive control. Absorbance increasing relatively to that of concentration represented the reducing capacity of tested sample.

Stastical analyses

Data were expressed as mean ± SEM. For comparison of results, analyses of variance (ANOVA) were done using Fischer's test. Statistical significance was set at $p<0.05$.

RESULTS

Extraction

From 30 g of dried powdered leaves of *C. pinnata*, 5.69 g of dried extract were obtained corresponding to a yield of 18.96%. From the same amount of dried trunk bark, 4.86 g of dried extract were got yielding 16.2 %.

Phytochemical composition

The main phytochemical groups identified both in the leaf and trunk bark extracts were: tannins, flavonoids, cardiac glycosides, sterols and triterpenoids. As shown in Table 1, Anthracenic derivatives were only met in the leaf extract.

DPPH assay

According to the Figure 1, all tested samples had shown significative ability to scavenge the free radical DPPH ($p<0.05$ versus negative control). The IC_{50} value of ascorbic acid (0.33 ± 0.11 µg/ml) was lowest than those of the ethanol trunk bark extract (19.53 ± 0.42 µg/ml) and leaf extract (21.07±0.11 µg/ml) of *C. pinnata* ($p<0.05$).

FRAP assay

For ethanol leaf and trunk bark extracts of *C. pinnata* and ascorbic acid, increasing absorbance were noticed relatively to the concentration variations as shown in Figure 2 ($p<0.05$ *versus* negative control). Ascorbic acid had shown better ability to reduce the ferric ion than the plant extracts ($p<0.05$). However, the trunk bark extract had exhibited similar ferric reducing capacity than the leaf extract at all tested concentrations ($p>0.05$).

The absorbance values of leaf extract varied from 0.011 ± 0.005 at 6.25 µg/ml to 0.399 ± 0.012 at the concentration of 200 µg/ml while those of trunk bark extract were respectively 0.016 ± 0.007 and 0.039 ± 0.007 at the same concentrations.

For ascorbic acid, the absorbance values increased significantly from the concentration of 6.25 µg/ml to 200 µg/ml (respectively 0.072 ± 0.006 and 0.75 ± 0.007; $p<0.05$).

Table 1: Phytochemical groups identified in leaf and trunk bark extract of *C. pinnata*.

Phytochemical groups	Leaf extract	Trunk bark extract
Alkaloids	-	-
Tannins	+	+
Flavonoids	+	+
Sterols and triterpenoids	+	+
Saponins	Foaming index <100	Foaming index <100
Anthracenic glycosides	-	+
Cardiac glycosides	+	+

+: presence, -: absence

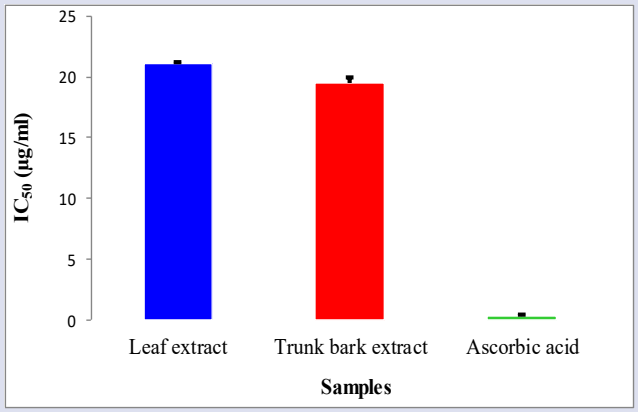

Figure 1: Free radical scavenging effect of tested samples.

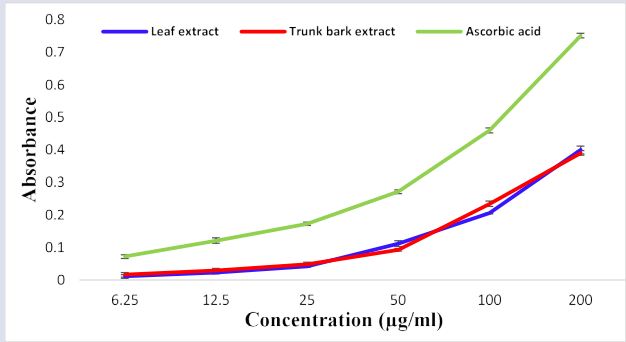

Figure 2: Absorbance increasing of different tested samples.

DISCUSSION

The extraction of phtytoconstituents was done using ethanol, a polar solvent, chosen for its ability to extract polar compounds such as polyphenols present in the leaves and trunk barks of *C. pinnata* (tannins and flavonoids). This could explain the extraction yields obtained for the leaf and trunk bark extracts (18.96% and 16.2% respectively).

The dried leaflets of *C. pinnata* were reported to contain about 1% of its weight in flavonoids such as quercetin and kaempferol.[7]

The antioxidant potential of plant extracts can be measured through several *in vitro* tests. However, for the complexity of plant extracts composition, a single method is not recommended for evaluating their

antioxidant activity.[8] DPPH and FRAP methods were cited as reflecting the antioxidant activity of plants extracts.[9] The DPPH assay measures the ability of the bioactive compound to scavenge free radicals while the FRAP test measures their capacity to reduce metals. DPPH can be reduced by phytoconstituents that are capable for transfering proton or donating hydrogen.[10]

In DPPH method, the ethanolic leaf and trunk bark extracts of *C. pinnata* exhibited significant antioxidant activity ($p < 0.05$).

The ethanol trunk bark extract of *C. pinnata* (IC_{50} : 19.53 ± 0.42 µg/ml) had exibited better ability to scavenge the DPPH free radical than that of the leaf extract (IC_{50} : 21.07 ± 0.11 µg/ml) ($p<0.05$). Ascorbic acid (IC_{50} : 0.33 ± 0.11 µg/ml) was seen to be more active than the plants extracts.

The FRAP method is often used to evaluate the reducing power of extracts. At all tested concentrations, ethanol leaf and trunk bark extracts of Cordyla pinnata had similar reducing power of ferric iron ($p>0.05$).

The reducing capacity of ferric ion seems to be related to the degree of hydroxylation and extend to the conjugation in phenolic compounds.[11] Thereby the reducing power of the extracts may be due to the presence of hydroxyl groups of polyphenols (flavonoids and tannins) present in these extracts.

Indeed, polyphenolic compounds can serve as electron donors.[12] Therefore, they are considered as reducing and inactivating oxidants. Other previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity.[13]

Antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins have been shown to reduce and discolor DPPH due to their ability to donate hydrogen.[14]

The polyphenols (tannins and flavonoids) of the leaf and trunk bark extracts of *C. pinnata* may be partly responsible for the antioxidant activity of these extracts. Indeed, polyphenols are among the most effective antioxidant phytoconstituants and many biological activities attributed to flavonoids are linked to their antioxidant property.[15,16] Other studies have shown that flavonoids are good inhibitors of enzymes responsible for the production of free radicals such as xanthine oxidase, which is an important biological source of the superoxide radical.[17,18] According to these studies, the antioxidant action of polyphenols is not limited only to the inhibition and deactivation of free radicals, but also to the neutralization of oxidative enzymes and the chelation of traces of metal ions inducing activated oxygen species production.[19,20]

CONCLUSION

The ethanol leaf and trunk bark extracts of *C. pinnata* contained, a part of anthracenic derivatives, the same phytochemical groups. The ethanol leaf and trunk bark extracts of the plant had also shown similar activity in DPPH and FRAP assays. For the plant preservation the use of the leaf extract must be recommended in place of the trunk bark one which may lead to its rarefaction.

CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interests.

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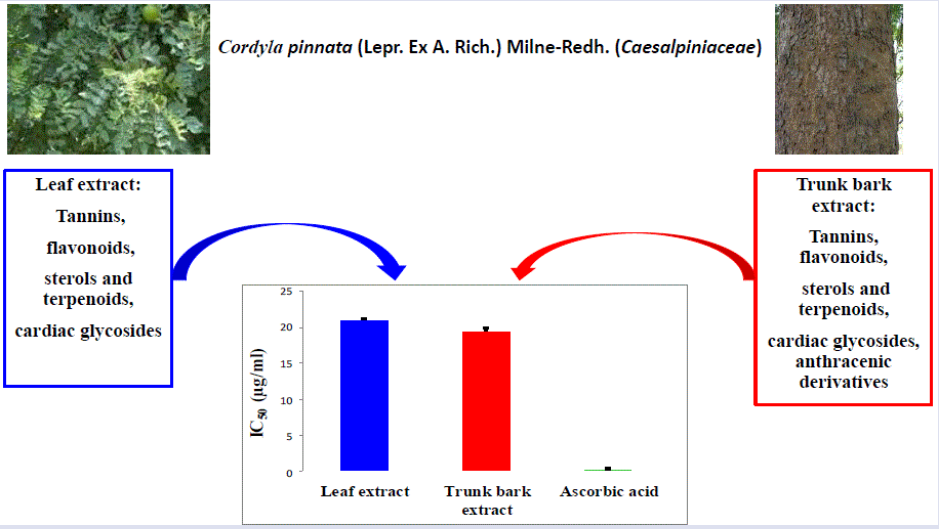
GRAPHICAL ABSTRACT



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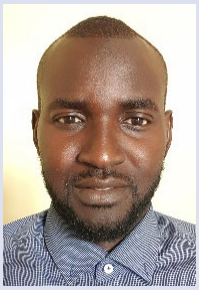

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