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RESEARCH ARTICLE

PHYTOCHEMICAL PROPERTIES AND IN *VITRO* ANTIOXIDANT ACTIVITY OF *ACACIA NILOTICA* (L.) WILLD.EX DEL. EXTRACTS

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Abstract

The aim of this research was to investigate the phytochemical composition and the antioxidant activity of the *Acacia nilotica* extracts. The samples (fruits and trunk bark of *Acacia nilotica* L.) were collected in Kayes (Mali). The phenolic compounds of the different plant extracts were assayed by spectrophotometry and identified by HPLC. The antioxidant efficacy of the extracts was evaluated by using 2, 2-azinobis-3-ethylbenzothiaz oline-6-sulfonic acid (ABTS) and 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH)radicals scavenging capacities. The obtained results revealed that all the extracts are rich on polyphenols, flavonoids, tannins and anthocyanins. The contents of total polyphenols varied from 147 to 215 mg EAG/100 g, of total flavonoids from 1123 to 3037 mg EC /100 g, of total tannins from 67 to 289 mg EC/100 g and of total anthocyanins from 57 to 144 mg ECG / 100g of dry matter (DM). The highest values of those compounds were recorded in fruit extracts with respectively 215 mg EGA, 3037 mg EC, 289 mg EC and 144 mg ECG/100g (MD).HPLC analysis of fruit extracts revealed the presence of a flavonoid, named rutin, and four phenolic compounds, gallic, protocatechuic, chlorogenic and caffeic acids, which are recognized as bioactive compounds. All of our studied extracts displayed highest ABTS and DPPH radical scavenging capacities. The values varied from 72 to 350 mg EVC/100g (DM). The antioxidant activities of extracts were significantly correlated to their high contents of total phenolic and total flavonoids compounds. These justify the medicinal properties of the plant.

Introduction:-

Acacia nilotica L. belongs to the family Fabaceae, subfamily Mimosoideae, native to East Africa and West Asia. It is also known by the vernacular name of *Nile Acacia*. *Acacia nilotica* is a thorny shrub up to 20 meters tall (in humid areas) with a straight cylindrical bole; and its trunk can reach 60 cm in diameter. It has a dense crown, a bark of dark brown to black in color and deeply fissured. Its rosy gray edge exudes a reddish gum. These twigs, brownish in color, bear straight, fine thorns arranged at the base of the leaves (Arbonnier, 2002).

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The 4 to 10 cm long leaves are alternate, bipinnate, with 3 to 6 pairs of pinnules each bearing 10 to 30 pairs of leaflets. A gland is located at the bottom of the last pair of pinnae. The plant bears yellow globose flowers 1.2 to 1.5 cm in diameter.

It bears long fruits in flat or cylindrical, hairless yellow-brown pods (Baravkaretal., 2008). *Acacia nilotica* is a medicinal plant with multiple uses. The different parts of the plant (leaves, bark of the trunk, roots, flowers and fruits) contain natural sources of the active ingredients known for their therapeutic properties.

In Mali, known as "*Bouana*", *Acacia nilotica* is used by women in traditional treatments for genital infections and in the treatment of some children infections (http://bamakonews.net/2019/07). In Burkina Faso, the decoctions of the bark and pods as well as the gum of *Acacia nilotica* are used against dysentery (Séréméet al., 2008). Powder of the bark is also used to treat colds, bronchitis, diarrhea, bleeding and leukoderma(Rajbir&Saroj, 2009). The roots and tender stems are used as toothbrushes (Meenaetal., 2006). A decoction of the powder of barks and fruits is used for the treatment of urino-genital diseases (Yoganarasimhan, 1996). *Acacia nilotica* has been recognized to be an effective medicine in the treatment of malaria, throat (aerial part), and toothache (barks) (Kubmarawaetal., 2007).

Polyphenols form an immense family of natural compounds. Many studies are in favor of a positive impact of their consumption for good health. Indeed, polyphenols could prevent much pathology such as cancer (Brown et al., 1998), degenerative and cardiovascular diseases (Kong et al., 2003). Today, herbal treatments are coming back to the front as the effectiveness of drugs such as polyphenols and flavonoids which are powerful antioxidants that can inhibit the formation of free radicals and oppose the oxidation of macromolecules (Iserinetal., 2001). View the different uses of the plant according to our environment.

The objective of this study was to identify the phytochemical constituents of the fruit and bark extracts of the trunk of *Acacia nilotica* and to evaluate their antioxidant activities, which will explain the use of the plant in traditional medicine.

Materials and Methods:-

Materials:-

The samples (fruits and trunk bark) of *Acacia nilotica* were collected in Kayes (Mali). They have been transported and identified to the Department of Traditional Medicine (DMT) under the number (0498). Folin-Ciocalteu, Gallic, protocatechuic, chlorogenic, caffeic acids, lawsone, rutin, apigenin, quercetin, kaempferol. ABTS and DPPH were provided by the companies SIGMA-Aldrich (France) and Across organics (Belgium). All other chemicals and solvents used were obtained from a commercial source and were of analytical grade.

Methods:-

SamplePreparation

After the initial cleaning process, the samples were dried in the shade and at room temperature in the Natural Substances laboratory of the FST. After drying, the samples were pounded using a laboratory scale hammer miller and the resulting powder sieved until a fine powder was obtained.

Preparation of extracts

The preparation of the sample extracts was carried out by the method described by (Abu Bakaret al., 2009) with slightly modified.To 5 g of plant powder were added 2 times 20 mL of a 50/50 (V/V) hydromethanolic solution for UV-Visible Spectroscopy and HPLC.The mixtures were stirred for 6 hours, and then filtered through a 0.45 µmmilliporemembrane.The filtrates collected were centrifuged at 1500 × g for 20 min.The extracts obtained have been cooled and conserved at (+ 4°C) in bottles before analysis.

Dosage of polyphenolic compounds

Total phenolic content (TPC)

The Folin-Ciocalteu method was used to measure the total phenolic content of the samples according to (Dzingiraetal., 2007) with some modifications.To (100 µL) of each extract, has been added 2 ml of distilled water in a test tube (Eppendorff tube), followed by 1 ml of FolinCiocalteu's reagent at 1 N and sodium carbonate (20%).After incubation for 40 minutes at room temperature, the absorbance was readed at 765 nm by a spectrophotometer against a blank prepared with methanol.The results were compared to a calibration curve previously established

before analysis with gallicacid.The total phenolic contents were expressed in mg equivalent gallic acid per 100 g dry matter (mg EGA / 100 g (DM)).The calibration curve has been established with a correlation coefficient R2 = 0.9757.All samples were analyzed at least three times.

Total flavonoid content (TFC)

The content of total flavonoids was evaluated by calorimetric according to (Kim et al., 2003).To 250 µL of each extract, 1 ml of distilled water and 75 µL of NaNO2 at (5%) were added.After 5 minutes, then 75 µL of AlCl3 at (10%) was added.After 6 minutes, 500 µL of NaOH (1N) and 600 µL of distilled water were added to the stirred mixture.The Absorbance of the mixture was determined at 510 nm relative to a blank prepared with water.The calibration curve was developed with standard solutions of catechins prepared at different concentrations.Total flavonoids are expressed in mg equivalents catechin per 100 g of dry matter (mg EC/100 g (MD)).The calibration curve has been established with a correlation coefficient R2 = 0.9901.All samples were analyzed at least three times.

Total tannin content (TTC)

The total tannin content was determined according to the method used by ([Villareal-Lozoyaetal., 2007) with a slight modification. In a test tube containing 1.5 mL of concentrated sulfuric acid, 50 µL of extract and 3 ml of a 4 % methanol-vanillin solution were added. The mixture was left to stand for 15 minutes. Absorbance has been measured at 500 nm against a blank prepared with methanol. The calibration curve was developed with standard solutions of catechins prepared at different concentrations. The calibration curve has been established with a correlation coefficient R2 = 0.9899. The results are expressed in mg equivalent catechin per 100 g of dry matter (mg EC/100 g DM). All samples were analyzed at least three times.

Total anthocyanin content (TAC)

Total anthocyanin compounds (TAC) were evaluated by the differential pH method according to (Abu Bakaretal., 2009; Lakoet al., 2007). The method used is based on a variation of absorbances using two buffers: one containing potassium chloride (KCl) (pH = 1) at 0.025 M and the other sodium acetate (CH3COONa) (pH= 4.5) at 0.4 M. 200 µL of extract samples were mixed with 1.8 mL of each of the buffer solutions. The absorbance of the solution has been determined at 510 nm and at 700 nm against a blank made with methanol. The change in absorbances was calculated by the following formula.

$\Delta A = [(A_{510}-A_{700})x\ pH_1]-[(A_{510}-A_{700})x\ Ph_4]$ (1)

The concentration of anthocyanin pigment in the extract was expressed in mg equivalent cyanidin-3-glycoside per liter of solution.

The calibration curve was established with cyanidin-3-glycoside.

$C(\text{mg/L})=\Delta A \times Mm \times Df \times (100/Ma)$ (2)

ΔA is the change in absorbances, Mm the molecular mass of cyanidin (449.2 g/mol),Df is the dilution factor, Ma the molecular absorptivity (26.900).

HPLC analysis

The HPLC analysis was carried out according to the method described by (Muandaetal., 2009) with a slight modification, using an elution gradient consisting of three phases.Solvent A: 50 mM ammonium phosphate (NH4H2PO4) at pH 2.6 (adjusted with ortho phosphoric acid), solvent B: (80/20 (v/v)) acetonitrile/solvent A, and solvent C:200 mMortho phosphoric acid (H3PO4) at pH 1.5 (pH was adjusted with 0.1 M NaOH).After preparation, the solvents were put in an ultrasonic device for 10 min for homogenization.The profile of the gradient used for 60 min is presented in Table 1.

The elution flow rate was 1 mL/min and the injection loop capacity 20 µL.Detection was performed at 280 and 320 nm.Standard phenolic compounds (9 standards) were prepared by dissolving 2 mg/mL.In each sample, the phenolic compound was identified by the retention time of the corresponding standard and the concentration of the phenolic compound was calculated by comparing the peak areas.The samples were analyzed at least three times.After each cycle, the system was reconditioned 10 minutes before a new analysis.

Table 1:- Profile of the gradient used for 60 min.

T(mn)	A%	B%	C%
0-4	100	0	0
4-10	92	8	0

10-22.5	0	14	86
22.5-27.5	0	16	84
27.5-50	0	25	75
50-55	0	20	80
55-60	100	0	0

Antioxidant Activity Assay

The scavenging capacity of ABTS radicals

The method developed by (Kim etal., 2002) slightly modified has been used in this experiment.1.0 mM AAPH was mixed with 2.5 mM ABTS using buffer.The buffer solution consists of 100 mM potassium phosphate (pH 7.4) containing 150 mMNaCl. The mixture was heated in a water bath at 68°C for 20 minutes until the concentration of the blue-green ABTS•+ radical complex gives an absorbance of between 0.65 ± 0.02 at 734 nm. To 60 µL of the sample has been added 2.94 mL of the radical blue-green solution of ABTS. The mixture was incubated in a water bath at 37°C for 20 minutes. The control consists of 60 µL of methanol and 2.94 ml of ABTS•+ and was checked for each series of samples. The absorbance decay was measured at 734 nm. Stable radical scavenging activity in the ABTS test of phenolic compounds was expressed in mg equivalent vitamin C (mg EVC / 100(DM)). The radical solution was prepared daily. All samples were analyzed at least three times

The scavenging capacity of DPPH radicals

The DPPH radical scavenging activity was determined using the method of (Hosteet al., 2006) with some modification. To 2.90 ml of an aqueous solution of 50% methanol (100 mM of DPPH), 100 µL of plant extract were added. The mixture has been heated in a water bath at 20°C away from light for 40 min. the blank was prepared with (100 µL of 50% methanol and 2.90 mL of the DPPH solution) and checked for each series of samples. The decrease in absorbance was measured at 517 nm 40 minutes later.The free radical scavenging activity in the DPPH test of total phenolic compounds was expressed in mg equivalents vitamin C (mg EVC / 100 g (DM)).The radical solution has been prepared daily.All samples were analyzed at least three times.

Statistical Analysis:

All experiments were conducted at least in triplicate with SPSS software (version 16.0, the predictive Analytics Company, Chicago, U.S.A.). The data were subjected to a one way analysis of variance (ANOVA), followed by Duncan's multiple range test.

Results and Discussion:-

Content of total polyphenolic compounds

The results of the analysis of the dosage of total polyphenolic compounds (TPC, TFC, TTC and TAC) are illustrated in Fig. 1. The two extracts (bark of the trunk and fruits) of *Acacia nilotica* analyzed, all contain phenolic compounds,flavonoids, tannins and anthocyanins.But, the best levels were observed in the fruit extract with 215 mg EGA;3037 mg EC;289 mg EC and 144 mg ECG per 100 g of dry matter, respectively. The content of total phenolic compounds in the *Acacia nilotica* fruit extract was 215 mg EGA/100g (DM) (Fig. 1), higher than what we found in the trunk bark extracts (147 mg EGA/100g (DM)). Our results were far superior to those of [Aadiletal., 2014], who noted a content of total phenolic compounds of (393.30 µgEGA) in the extract from the bark of the trunk of the same plant. Flavonoids were very present in both extracts. However, the fruit extract showed a high concentration of total flavonoids (3037 mg EC/100g (DM) compared to the trunk bark extract (1123 mg EC/100g (DM)). The total flavonoid values of our two extracts are superior to that of [Bansal&Goel, 2012], (4.20 mg EC / 100g (MD)). The extracts from the bark of the trunk and from the fruits recorded respective contents of (67 and 289 mg EC / 100g (MD)) in total tannins (Fig. 1), higher than those reported by (Séréméetal., 2008), (28.7 and 18. 9 mg EC / 100g (MD)). Several *in vivo* and *in vitro* studies have focused on the evolution of the antibacterial properties of the polyphenols contained in the different parts of *Acacia nilotica*(Ali etal., 2018).

Studies of the inhibiting power of phenolic compounds on bacterial growth have been demonstrated that alkaloids, flavonoids, saponins, tannins, anthraquinones and cardiotonic glycosides have a significant effect on the bacterial strain Streptococcus mutans (gene responsible for dental caries). This partly explains the use of the plant in the treatment of dental caries (Deshpande, 2013; Deshpande&Kadam, 2013). However, antimicrobial tests performed by (Bansoetal., 2009), showed a high potential for inhibition against pathogens (Escherichia coli and Salmonella strains). This explains the use of *Acacia nilotica* in traditional medicine for the treatment of infections caused by these bacteria. (Khanbabaee&Ree, 2001) reported that the different parts of the plant (leaves, trunk barks, root barks

and fruits) contain tannins. They have a certain astringent power, which explains their cardio-protective, healing, anti-helminthic and anti-diarrheal properties (Bachayaet al., 2009; Fluck, 1973). A low concentration of anthocyanins was recorded in the trunk bark extract 57 mg ECG/ 100g (DM) (Fig. 1). However, the experimental analyzes carried out by (Sadiqet al., 2015) showed the absence of anthocyanins in the various extracts of the plant. Anthocyanins are responsible for the coloring of plants, they have antiviral, antiallergic(Chatterjeeet al., 2013), anti-inflammatory, antibacterial (Li etal., 2012), antitumor properties, a protective power against cardiovascular diseases, an effect lipid peroxidation inhibitor, as well as the ability to scavenge free radicals (Kong et al., 2003).

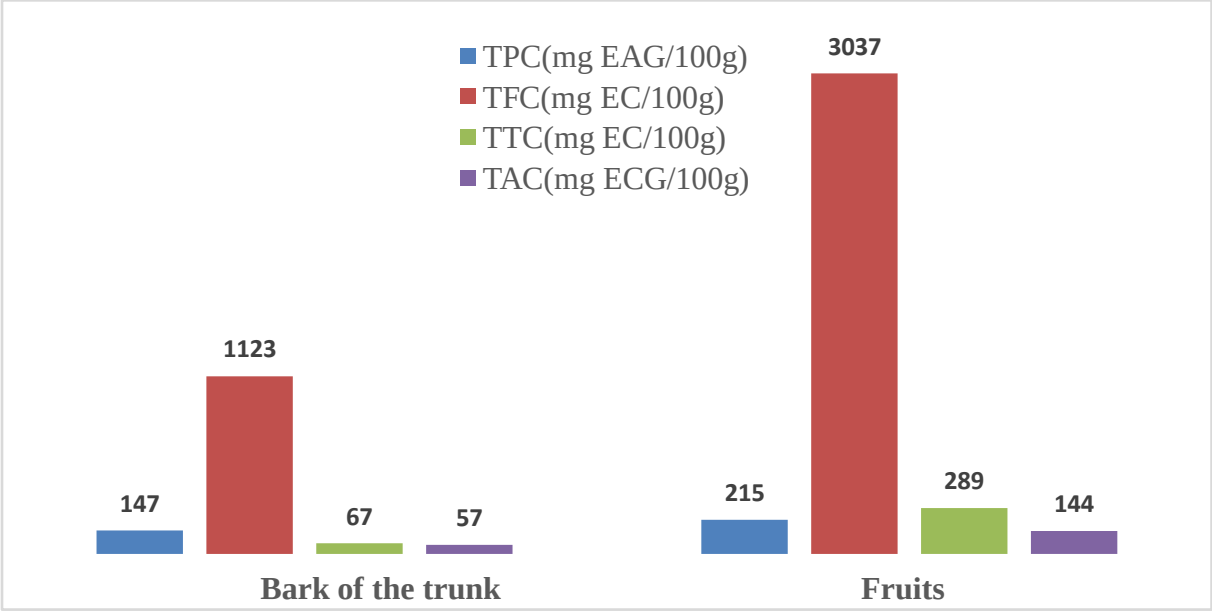

Fig. 1:- Quantitative composition of polyphenolic compounds of the two *Acacia nilotica* extracts;

HPLC Analysis

The results of the HPLC analysis of the two extracts (bark of the trunk and fruits) of *Acacia nilotica*are presented in Table 2. Rutin was identified in both studied extracts, but with a higher content in the fruit extract 189mg /100mL. Four acids have been also identified in the fruit extract namely gallic acid, protocatechuic acid, chlorogenic acid and caffeic acid. Thus, the main compound identified in the *Acacia nilotica* fruit extract is gallic acid with a concentration of (497.9 mg/100mL) (Table 2). Gallic acid has the ability to reduce the viability of lung cancer cells in mice in *vivo*. The combination of this acid with anticancer drugs such as cisplatin could be an effective treatment for this type of cancer (Kawada et al., 2001; Rangkadilok, etal., 2007). The chlorogenic acid content in *Acacia nilotica* fruit extract was around 170.7 mg/100 mL.Chlorogenic acid has the power to inhibit the enzymes responsible for the regeneration of ROS (reactive oxygen species) in living cells (Bouayedetal., 2007). Flavonoids are the most characteristic secondary metabolites of the genus Fabaceae(Siegleretal., 2003). However, the rutin concentrations in the two *Acacia nilotic*a extracts (trunk bark and fruit) are respectively (38.3 and 189 mg/100 mL). Rutin is reputed to be a powerful antioxidant that is used in Chinese medicine to treat high blood pressure and to inhibit damage induced by the oxidative effects of UV radiation (Borset al., 1990). It is also known for its anti-inflammatory, hepato-protective and antioxidant properties (Afsaret al., 2015).Caffeic acid has been shown to be very effective against viruses, bacteria and fungi (Banso, 2009).

Table 2:- HLPC Analysis results of the two *Acacia nilotica*extracts.

Names of compound	Rt (min)	Barks of the trunk (mg/mL)	Fruits (mg/ML)
Gallic A.	09.65	0.642±0.01	4.976±0.1
Protocatechuic A.	11.85	-	1.149±0.1
Chlorogenic A.	16.72	0.807±0.05	1.707±0.1
Caffeic A.	19.55	-	0.071±0.002
Cumaric A.	39.90	-	-
Rutin	35.50	0.383±0.05	1.89±0.1

Quercetin	39.82	-	-
Apigenin	41.42	-	-
kaempferol	42.52	-	-

Results reported are means of triplicate samples ± standard deviation. Rt: Retention time, (-):Absence

Antioxidant activity

The results of the antioxidant activity are reported in Table 3. Both tests show a high antioxidant potential in the extracts of the barks of the trunk and fruits of *Acacia nilotica*. Extracts from the trunk barks and fruits of *Acacia nilotica* displayed the highest values of antioxidant activity of ABTS and DPPH (Table 3). This anti-radical activity is linked to a high content of total phenolic compounds and total flavonoids (Dembéléetal., 2017). The high antioxidant potential of our extracts confirmed the results of HPLC analysis (Table 2), which identified four phenolic acids: gallic, protocatechuic, chlorogenic, caffeic and a flavonoid, rutin. According to (Bossokpietal., 2002), these phenolic acids have antioxidant and antiradical activities. Our results are far superior to those of (Afsaretal., 2015), who quantified by HPLC gallic acid (300.05 µg/100 mg), caffeic acid (137.43 µg/100 mg) and rutin (235.38 µg/100 mg) in the extract of *Acacia nilotica* trunk bark, who also found a fairly high antioxidant activity.

Table 3:- ABTS and DPPH radical scavenging activities of the two *Acacia nilotica*extracts.

Samples	ABTS-Test(g EVC/100g(MD))	DPPH-Test(g EVC/100g(MD))
Bark of the trunk	0.72±0.001	1.56±0.001
Fruits	2.50±0.01	3.50±0.001

Results reported are means of triplicate samples ± standard deviation.

Conclusion:-

The results of this study revealed the presence of total phenolic compounds, total flavonoids in considerable quantity, total tannins and total anthocyanins in the extracts of the barks of the trunk and fruits of *Acacia nilotica*.However, the HPLC analysis allowed us to identify in the extracts of *Acacia nilotica* phenolic acids and flavonoids.These chemical constituents are known to have the ability to scavenge free radicals from ABTS and DPPH.This explains the best antioxidant activities of our extracts.In sum, the results of phytochemistry and antioxidant activity of *Acacia nilotica* extracts revealed that it is a medicinal plant.This justifies the use of *Acacia nilotica* in the management of bacterial infections in traditional medicine.

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