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Research Article

Anti-inflammatory Potential of Glycoside Flavonoids from *Pterocarpus erinaceus* Poir. (Fabaceae) Leaves

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ABSTRACT

Background: Chronic diseases have become one of the leading causes of death worldwide in recent years. Despite tremendous advances in the treatments of diseases, several concerns remain. Those with inflammatory components are alternatively treated or completed in Burkina Faso with medicinal plants. The present study was designed to identify and evaluate the anti-inflammatory potential of glycoside flavonoids from *Pterocarpus Erinaceus* leaves. **Methods**: The flavonoids contained in *Pterocarpus Erinaceus* aerial parts were extracted, identified, and characterized. Sequential soxhlet extraction was subjected to preliminary phytochemical screening, and characterization of isolated flavonoid was done by U.V., I.R., ^{1}H & ^{13}C N.M.R. and MS. Subsequently, isolates were tested *in vitro* for their anti-inflammatory potential as well as their antioxidant capacities. **Results**: Five compounds were determined as corresponding to 3',4',5,7-tetrahydroxy flavone (luteolin); quercetin-3-O- sophoroside; quercetin-3-0- β -glucose (isoquercitrin); Kaempferol-3-O-sophoroside and 3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside (rutin). Concentrations of quercetin-3-O- sophoroside and quercetin-3-0- β -glucose that inhibit 50% of pro-inflammatory enzymes' activities were expressed in µg/mL. They were respectively 18.07 ± 0.78 and 32.27 ± 2.02 for xanthine oxidase, 1.12 ± 0.018 and 11.53 ± 0.52 for lipoxygenase, 26.91 ± 0.34 and 19.54 ± 0.25 for acetylcholinesterase. Prevention of the degradation of deoxyribose test gave respectively 19.32 ± 1.08 µg/mL and 26.21 ± 2.25 µg/mL for quercetin-3-O- sophoroside and quercetin-3-O- β -glucose while the anti-DPPH free radical potential was 3.41 ± 0.82 and 2.90 ± 0.18 µg/mL. **Conclusion:** These results may justify the traditional uses of *P. erinaceus* in treating diseases with an inflammatory component.

Key words: Enzymes, Antioxidant, Compounds, Medicinal plant.

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INTRODUCTION

The difficulty of early detection, the limits of current treatments, and new lifestyles are among the factors increasing the incidence of chronic diseases worldwide.[1] In recent years, several physiological aspects related to chronic diseases have been highlighted thanks to research programs. Non-proper regulation of the inflammation process associated with the overproduction of free radicals was among the main factors that promote the chronicity of diseases.[2] Limitation of the overproduction of free radicals by supplementation of antioxidant compounds is often recommended to prevent the development of chronic diseases.[3] Plants are one of the natural reservoirs of antioxidant compounds endowed with therapeutic potentials;[4] specifically, those used for medicinal purposes have shown great potential for preventing the development of many diseases, most of which have an inflammatory component. Therefore, medicinal plants containing antioxidants with anti-inflammatory properties are a promising way to reduce chronic diseases incidence. *Pterocarpus Erinaceus,* a medicinal plant belonging to Fabaceae botanical family, is known and used traditionally to treat several inflammatory diseases such as rheumatism, gastric ulcer, malaria, and infectious diseases. Previous biological investigations of Pterocarpus species have revealed antimicrobial activity,[5] antioxidant activity,[6,7] anti-malaria activity.[8]

This study aimed to carry out phytochemical analysis on *P. erinaceus* leaves and investigate the anti-inflammatory capacity of identified compounds.

MATERIALS AND METHODS

Plant collection

The plant material was consisted of the leaves of *Pterocarpus erinaceus* Poir. (Fabaceae). They were collected in Gourcy, a town located 150 km from Ouagadougou (Burkina Faso), and authenticated in the Herbarium of University Joseph KI-ZERBO. Besides, a voucher specimen was deposited there under the number ON-01.

Reagents and chemical

All the reagents used were of analytical grade. They were supplied by Sigma- Aldrich (St Louis MO, USA). Xanthine oxidase bovine milk, allopurinol, acetylcholine esterase (AChE) from electric eel (type VI-S lyophilized powder) Diphenyl boric aminoethanol (DBA), Physostigmine, acetylthiocholine; 5,5-dithiobis [2-nitrobenzoic acid] (DTNB), bovine serum albumin (BSA), NaCl, $MgCl_2$, acetylcholine iodine (ATCI). Commercially available thiobarbituric acid, KH_2PO_4, Na_2HPO_4, and EDTA, were also from Fluka Chemica AG (St. Gallen, Switzerland) and were of the highest quality ($\geq$ 99.0%). Silica gel F_{254} plates Merck silica gel 60 (40-63 µm, Sephadex LH20, and Lichroprep RP18 were purchased from Merck KGaA (Germany).

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Phytochemical investigations

Spots of TLC were visualized under UV light and by spraying with diphenyl boric aminoethanol (DBA). Silica gel 60 F_{254} plates were used for analytical TLC. Polyamide (MN- polyamide – DC6), and silica gem GF_{254} were used for preparative TLC. Column chromatography was carried out using Merck silica gel 60 (40-63 µm), Sephadex LH 20 resin, and Lichroprep RP18 (Merck). ^{1}H-NMR and ^{13}C-NMR, COSY; HMQC and HMBC spectra were recorded in CD_3OD on a Brucker spectrometer operating respectively at 300 and 100 MHz.

Extractions and isolations

1 Kg of powdered dry leaves was macerated in methanol (MeOH) (4x 2.5 L) at room temperature under magnetic stirring for 48 hours to obtain 111.8 g of MeOH extract after filtration and concentration under vacuum.

72 g of MeOH extract from the leaves were dissolved in 500 mL of MeOH/ CHCl3 (50/50; v/v). The resulting solution was placed in a funnel, and then, a gradual addition of distilled water yielded two phases. After concentration under vacuum, the lower phase (7.7 g) contained chlorophyll, and the upper stage was the flavonoid fraction (64 g).

41 g of the flavonoid fraction dissolved in 600 mL of distilled water was partitioned successively with dichloromethane (DCM), ethyl acetate (EtOAc), and then, butanol. DCM fraction (50 mg), EtOAc fraction (814 mg), and butanol fraction (5,2 g) were obtained thanks to liquid-liquid partition.

The EtOAc fraction was subjected to vacuum liquid chromatography (VLC) and eluted with hexane/EtOAc (50/50 to 0/100), then EtOAc/MeOH (100/0 to 0/100), to yield three fractions (M.1 – M.3). The fraction M.2 was subjected to purification on a reverse phase cartridge (RP18 Lichroprep, Merck) and eluted with H20/MeOH (0/100 to 100/0); and 12 fractions (M.2.1-M.2.12) were obtained from fraction M.2. Fraction M.2.11 was purified on a Sephadex LH 20 column to yield compound 2 (3.1 mg).

The butanol fraction was fractionated by Medium Pressure Liquid Chromatography (MPLC) with Lichroprep (RP18, 40-63 µm, Merck) to obtain 18 fractions (N.1- N.18), using H20/ MeOH (100/0 to 0/100) as eluent. Fraction N.11 was subjected to a silica gel column to yield five fractions (N.11.1 – N.11.5) eluting with EtOAc/MeOH/H2O (114/15/1; 100/21/9 ten 100/16.5/13.5). The fraction N.11.3 was purified on preparative TLC silica gel to afford compound 3 (6.5 mg) with EtOAc/MeOH/H_2O (70/17/13) as eluent. The fraction N.12 was chromatographed on silica gel (40-63 µm, Merck) column to obtain 13 fractions (N.12.1-N.12.13), using EtOAc/MeOH/H_2O (85/8/7, 70/17/13 then 65/20/15) as eluent. The purification of fraction N.12.13 on the Sephadex LH 20 column permitted to isolate the compound 4 (32 mg). The fraction N.12.7 was subjected to polyamide (MN Polyamide DC 6) preparative TLC to afford the compound 5 (9 mg), thanks to the elution with H_2O /MeOH/Methyl Ethyl Ketone/Acetylacetone (13/3/3/1). The fraction N.12.8 was subjected to Sephadex LH 20 column and eluted with MeOH to isolate compound 6 (7.7 mg). The isolated compounds were identified with analysis of ^{1}H and 2D NMR spectroscopic data and comparison with literature.

Compound 1: TLC: Silica RP-18 (MeOH/H_2O, 70/30), Rf = 0.33. Spot: orange with DBA reactive; physical aspect: yellow powder. ^{1} H NMR (300 MHz, in aceton-d6): δ 6.6 (s, 1H), 6.2 (d, J = 2.1 Hz), 6.5 (d, J = 2.1 Hz), 6.9 (d, J = 8.3 Hz), 7.4 (d, J = 2.2 Hz), 7.5 (dd, J = 2.2 Hz, J = 2.2 Hz). ^{13}C NMR (300 MHz, in aceton-d6): δ 165.5 (C 2), 104.5 (C 3), 183.4 (C 4), 163.7 (C 5), 100.2 (C 6), 165.4 (C 7), 95.1 (C 8), 159.1 (C 9), 105.6 (C 10), 123.9 (C 1'), 114.4 (C 2'), 146.9 (C 3'), 150.7 (C 4'), 116.9 (C 5'), 120.4 (C 6')

Compound 2: TLC: Silica (AcOEt/MeOH/H_2O; 70/17/13), Rf = 0.47; Spot: yellow with DBA reactive; physical aspect: yellow powder. ^{1}H NMR (300 MHz, in CD_3OD): δ 4.7 (d, J = 7.2 Hz), 5.3 (d, J = 7.6 Hz), 6.2 (d, J = 2.0 Hz), 6.4 (d, J = 1.9 Hz), 6.9 (d, J = 8.5 Hz), 7.5 (d, J = 2.1 Hz), 7.7 (d, J = 2.1 Hz). ^{13}C NMR (300 MHz, in CD_3OD): δ 158.0 (C 2), 135.1 (C 3), 179.8 (C 4), 158.8 (C 5), 99.9 (C 6), 163.2 (C 7), 94.7 (C 8), 158.6 (C 9), 105.1 (C 10), 123.1 (C 1'), 117.8 (C 2'), 145.9 (C 3'), 149.8 (C 4'), 116.2 (C 5'), 121.0 (C 6'), 98 (C 1"), 82.9 (C 2"), 75.6 (C 3"), 70.9 (C 4"), 77.9 (C 5"), 62.4 (C 6"), 105.8 (C 1"'), 70.9 (C 4"'), 78.0 (C 5"'), 62.7 (C 6"').

Compound 3: TLC: Silica (AcOEt/MeOH/H_2O; 70/17/13), Rf = 0.70; Spot: yellow with DBA reactive; physical aspect: yellow powder. ^{1}H NMR (300 MHz, in CD_3OD): δ 7.7 (s), 7.5 (d, J = 8.4 Hz), 6.8 (d, J = 8.5 Hz), 6.3 (s), 6.1 (s), 5.2 (d, J = 7.3 Hz), 3.7 (d, J = 2.1 Hz), 3.6 (d, J = 5.2 Hz), 3.2 (m). ^{13}C NMR (300 MHz, in CD_3OD): δ 159.1 (C 2), 135.6 (C 3), 179.6 (C 4), 163.3 (C 5), 99.8 (C 6), 166.0 (C 7), 94.7 (C 8), 158.5 (C 9), 105.7 (C 10), 123.2 (C 1'), 117.6 (C 2'), 145.8 (C 3'), 149.7 (C 4'), 116.0 (C 5'), 123.3 (C 6'), 104.4 (C 1"), 75.7 (C 2"), 78.3 (C 3"), 71.2 (C 4"),78.1 (C 5"), 62.5 (C 6").

Compound 4: TLC: Silica RP-18 (MeOH/H_2O, 50/50), Rf = 0.40. Spot: yellow with DBA reactive; physical aspect: yellow powder.^{1}H NMR (300 MHz, in CD_3OD): δ 8.1 (d, J = 8.8 Hz), 6.9 (d, J = 8.9 Hz), 6.4 (d, J = 1.7 Hz), 6.2 (d, J = 1.8 Hz), 5.4 (d, J = 7.5 Hz), 4.8 (d, J = 7.3 Hz).^{13}C NMR (300 MHz, in CD_3OD): δ 158.3 (C 2), 134.9 (C 3), 179.7 (C 4), 163.1 (C 5), 99.8 (C 6), 165.3 (C 7), 94.9 (C 8), 158.3 (C 9), 104.7 (C 10), 122.8 (C 1'), 132.2 (C 2'), 116.2 (C 3'), 161.2 (C 4'), 115.2 (C 5'), 132.3 (C 6'), 99.8 (C 1"), 82.5 (C 2"), 77.9 (C 3"), 71.1 (C 4"), 77.9 (C 5"), 62.5 (C 6"), 104.7 (C 1"'), 75.5 (C 2"'), 78.2 (C 3"'), 71.3 (C 4"'), 77.9 (C 5"'), 62.0 (C 6"').

Compound 5: TLC: Silica (AcOEt/MeOH/H_2O; 70/17/13), Rf = 0.51; Spot: orange with DBA reactive; physical aspect: yellow powder.^{1}H NMR (300 MHz, in CD_3OD): δ 7.7 (m), 6.9 (d, J = 8.2 Hz), 6.4 (s), 6.2 (s), 5.1 (d, J = 7.4 Hz), 4.5 (s).^{13}C NMR (300 MHz, in CD_3OD): δ 158.5 (C 2), 135.6 (C 3), 179.4 (C 4), 159.3 (C 5), 99.9 (C 6), 166.0 (C 7), 94.9 (C 8), 162.9 (C 9), 105.6 (C 10), 123.1 (C 1'), 116.1 (C 2'), 145.9 (C 3'), 149.9 (C 4'), 117.7 (C 5'), 123.6 (C 6'), 104.7 (C 1"), 75.7 (C 2"), 78.2 (C 3"), 71.4 (C 4"), 77.2 (C 5"), 68.6 (C 6"), 102.4 (C 1"'), 72.1 (C 2"'), 72.2 (C 3"'), 73.9 (C 4"'), 69.7 (C 5"'), 17.9 (C 6"').

Enzymatic inhibitions and the ability to prevent the degradation of deoxyribose by isolated molecules

Inhibition of Lipoxygenase (LOX); The inhibitory activity of LOX was determined according to the spectrophotometric method described by Malterud and Rydland[9] with slight modifications. 146,25 µL of lipoxygenase solution (820.51 U / mL) prepared in boric acid buffer (0.2 M; pH 9.0) was mixed with 3,75 µL of extracts (8 mg/ mL) in the 96-well microplate and then incubated at room temperature for 3 min. The reaction was initiated by adding 150 µL of the substrate (1,25 mM of linoleic acid), and the absorbances were recorded for 3 min at 234 nm with a spectrophotometer (Epoch Biotek Instruments, U.S.A.). All tests were performed in triplicate, and Zileuton was used as a reference compound. The percentage of lipoxygenase inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \{[(V_b - V_s) / V_b] \times 100\}$$

Where V_b represents the enzymatic activity without inhibitor; V_s the activity of the enzyme in the presence of a sample or reference compound

Inhibition of xanthine oxidase (XO): The XO inhibitory activity was measured using the method described by Hudaib et al.[10] with slight modifications. Briefly, the substrate and the enzyme solutions were prepared immediately before use. The reaction mixture contained an 80 mM sodium pyrophosphate buffer (pH = 8.5), 0.120 mM xanthine, and

0.1 unit of XO. The absorption at 295 nm, indicating the formation of uric acid at 25°C, was monitored using a 96-microplate reader (Epoch, BioTeck instruments, USA), and the initial rate was calculated. The methanolic dried extract, initially dissolved and diluted in the buffer, was incorporated in the enzyme assay to assess its inhibitory activity. The concentration of the tested sample that inhibits 50 % of the enzyme activity (IC_{50}) was also determined using different concentrations of the extract (10, 20, 50, 75, and 100 µg/mL). All assays were run in triplicate; thus, inhibition percentages are the mean of three observations. A negative control was prepared to contain the assay mixture without the extract. Allopurinol was used as a positive control in the assay mixture. The XO inhibitory activity was expressed as the percentage inhibition, calculated as follows:

Inhibition (%) = {[(Blank inclination – Test inclination) / Blank inclination] x 100}

Where Test inclination was the linear change in the absorbance per minute of the test material, and Blank inclination was the linear change of absorbance per minute of the blank.

Inhibition of acetylcholine esterase (AChE): AChE activity was measured using a modified 96-well microplate assay[11] based on Ellman's method.[12] The enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate. 50 mM Tris–HCl pH 8.0 was used as a buffer throughout the experiment. AChE used in the assay was from electric eel (type VI-S lyophilized powder, 518 U/mg solid, 844 U/mg protein). Further enzyme dilution was done in 0.1% BSA in the buffer. DTNB was dissolved in the buffer containing 0.1 M NaCl and 0.02 M $MgCl_2$. ATCI was dissolved in deionized water. In the 96-well plates, 100 ml of 3 mM DTNB, 20 mL of 0.26 U/mL of AChE, and 40 mL of buffer (50 mM tris pH 8.0), 20 mL of each extract in various concentrations (25, 50, 100, 250 and 500 mg/ml) dissolved in the buffer containing not more than 10% methanol were added to the wells. After mixing, the plate was incubated for 15 min at 25°C, and then the absorbances were measured at 412 nm with a microplate reader (Epoch, BioTeck instruments, USA). The enzymatic reaction was initiated by adding 20 mL of 15 mM ATCI and the hydrolysis of acetylthiocholine was monitored by reading the absorbances every 5 min for 20 min. Physostigmine was used as a positive control. All the reactions were performed in triplicate. The percentage inhibition was calculated as follows:

Inhibition (%) = $\{[(V_b - V_s) / V_b] \times 100\}$

Where $\mathbf{V_b}$ is the activity of the enzyme without inhibitor and $\mathbf{V_s}$, is the activity of the enzyme with the isolated compounds/ reference. The concentration that inhibits 50 % of the enzyme activity (IC_{50}) value was calculated from the % inhibition values of different concentrations of each tested extract.

Deoxyribose degradation assay: The ability of tested extracts to prevent the degradation of the deoxyribose was determined using the method described by Kizil et al[13] and adapted by Thiombiano et al.[14] Briefly, freshly prepared 2-desoxyribose (100 µL, 28 mmol in phosphate buffer 50 mmol, pH 7.4), EDTA (500 µL; 1.04 mmol), $FeCl_3$ (100 µL; 100 mmol), H_2O_2 (100 µL, 1.0 mmol) and ascorbic acid (100 µL, 1.0 mmol) was mixed and incubated at 37 °C for 1 hour with extract (100 µL; 1 mg/mL). Thiobarbituric acid (1 mL of 1% aqueous solution) and trichloroacetic acid (1 mL of 2.8 % aqueous solution) was added to the mixture and incubated at 100 °C for 20 min. The resultant mixture was centrifuged (3000 g for 10 min). The absorbance of the organic layer containing thiobarbituric acid reactive substances was measured at 532 nm against a blank (methanol) with a microplate reader (Epoch, BioTeck instruments, USA). Inhibition of deoxyribose degradation was calculated as follows:

Inhibition (%) = {[(Blank inclination – Test inclination) / Blank inclination] x 100}

The IC_{50} value was calculated from the % inhibition values of different concentrations of each tested extract. Quercetin was used as a reference.

DPPH radical scavenging activity: DPPH radical scavenging ability of extracts was assayed as described by Velaquez *et al.*[15] A cascade dilution of the extract and Quercetin (reference substance) was performed from a concentration of 1 mg / mL. For this purpose, 200 µL of DPPH solution freshly prepared in methanol (4 mg / 100 mL) was mixed with 100 µL of each dilution in the 96-well microplate. The mixture was incubated for 30 min at ambient temperature and the absorbances were measured at 517 nm against a blank (methanol) with a spectrophotometer (Epoch Biotek Instruments, U.S.A.). The DPPH radical inhibition was calculated as follows:

% Inhibition = [{(Ab – As) /Ab x 100]}

A_b: absorbance of blank; A_s: absorbance of sample/reference compound

The IC_{50} value was calculated from the % inhibition values of different concentrations of each tested extract. Quercetin was used as a reference.

Statistical analysis

The statistical analysis was performed with GraphPad Prism 5.03 for Windows (Graph Pad Software, Inc., California USA), using One-way variance (ANOVA). All experiments were carried out in triplicate and data as mean standard deviation. Dunnett's test for comparisons with the control group was used to analyze differences between tested samples/groups and controls. P-values less than 0.05 ($P < 0.05$) were considered significant.

RESULTS

Identification of isolated compounds

Compound 1 was obtained as a yellow powder. Analysis of the ^1H NMR of compound 1 showed protons of flavonoids ring B at δH 7.52, 6.98 et 7.45. Analysis of ^{13}C NMR and DEPT spectra showed 15 carbons including six signals corresponding to six tertial carbons. Compound 1 was identified as luteolin according to the spectroscopic analysis and comparison of NMR spectroscopic data with the literature[16] (Figure 1).

The observation of two doublets at δH 6.2 and 6.4 in the ^1H NMR spectrum indicated the presence of meta substituted A ring. Two anomeric protons at δH 4.7 (d, J= 7.2 Hz) and 5.3 (d, J= 7.6 Hz) and proton multiplet between 3.2 and 3.8 pp showed the presence of two

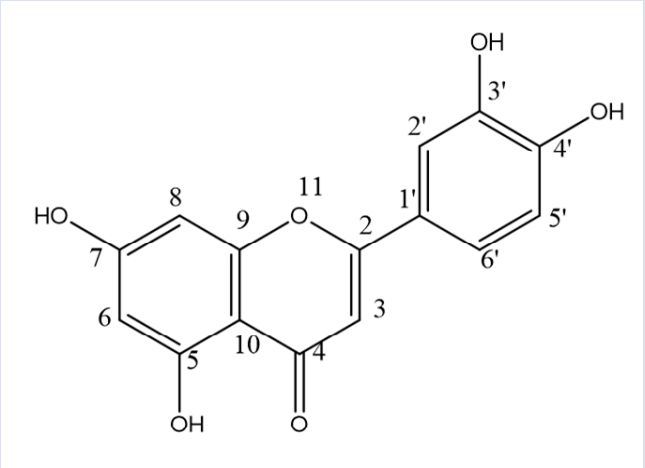

Figure 1: Structure of luteolin

sugars. Analysis of the HMBC spectrum established a correlation rang coupling between H-1''' (δ 4.74) and C-2'' (δ 82.9), and between H-1'' (δ 5.34) and C-3 (δ 135.1). The comparison of NMR spectroscopic data,[17] was permitted to identify compound 2 as quercetin-3-O-sophoroside (Figure 2).

In the ^{1}H-NMR spectrum, aromatic protons H-6 and H-8 of ring A of flavonoid were seen at δH 6.14 et 6.32 ppm, respectively. Protons at δH 7.67 (s), 7.53 (dd, J = 8.4, 1.6 Hz), 6.82 (d, J = 8.5 Hz) were characteristic of ring B of flavonoids. The presence of sugar was indicated by the speak at δH 5.20 (d, J = 7.3 Hz). NMR spectroscopic data were compared with the literature,[18,19] and compound 3 was identified as quercetin-3-O-β-glucose (isoquercitrin) (Figure 3).

Compound 4 was obtained as a yellow powder that appeared on TLC yellow spot after treatment with DBA. In the ^{1}H NMR spectrum, aromatic protons observed at δH 8.05 (d, J= 8.8 Hz) and 6.92 (d, J=8.9 Hz) suggested for the ring B of flavonoids. However, the protons appeared at δH 6.40 (d, J=1.7 Hz) and 6.21 (d, J=1.8 Hz), corresponding to the ring A. The signals of two anomeric protons observed at δH 5.44 (d, J=7.5 Hz) and 4.77 (d, J=7.3 Hz) showed the presence of two sugars. DEPT and ^{13}C NMR spectra indicated the presence of 27 carbons. Those spectroscopic data compared with literature[18,20] identified compound 4 as kaempferol-3-O-sophoroside (Figure 4).

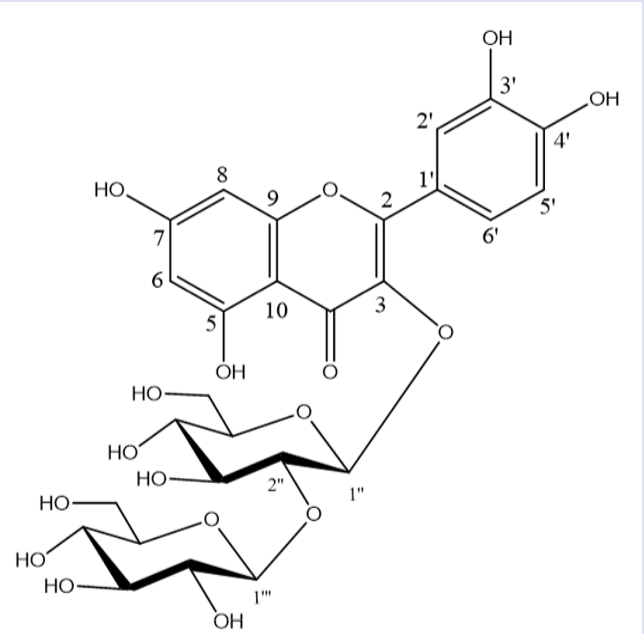

Figure 2: Structure of structure of quercetin-3-O-sophoroside

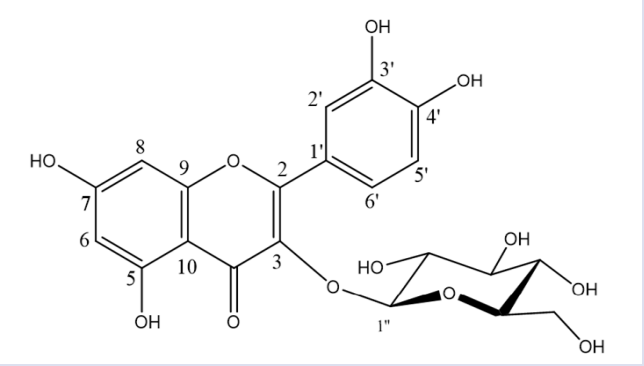

Figure 3: Structure of quercetin-3- β- O-glucose (Isoquercetin)

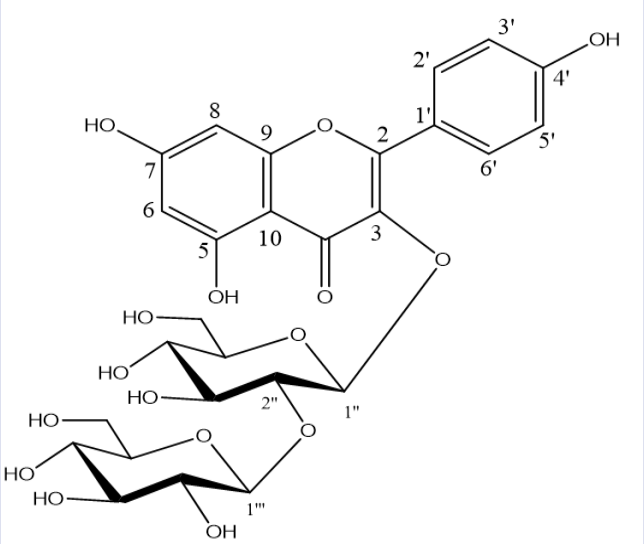

Figure 4: Structure of kaemferol-3-O-sophoroside

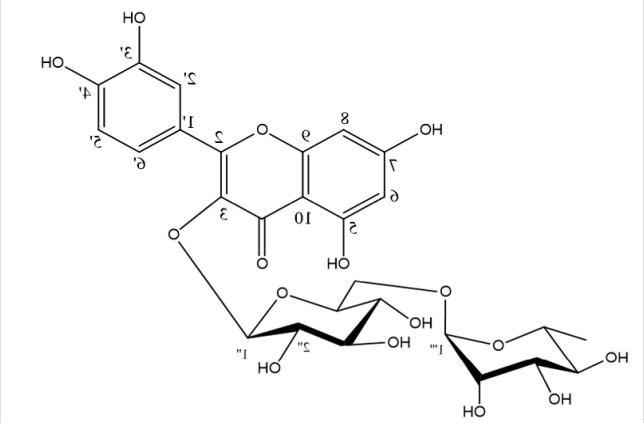

Figure 5: Structure of rutin

Compound 5 was obtained as a yellow powder. It appeared on TLC orange spot after treatment with DBA. In the ^{1}H-NMR spectrum, the protons of two aromatic rings were exhibited at δH 6.4, 6.2, 7.7, and 6.9 (d, J=8.2 Hz). The anomeric protons at δH 5.1 (d, J= 7.4 Hz) and 4.5 (s) indicated the presence of two sugar. Methyl presence at δH 1.10 and anomeric proton at δH 5.1 assigned that one sugar was Rhamnose. ^{13}C and DEPT NMR spectra displayed signals for 28 carbons. NMR (^{1}H, ^{13}C) analysis in agreement with published data[20] was assigned that compound 5 was rutin (Figure 5).

Enzymatic inhibition

Table 1 shows that all the tested samples were significant inhibitors of xanthine oxidase, Lipoxygenase, and Acetylcholinesterase. The isolated compounds were not active as the references used except quercetin-3-O- sophoroside in inhibiting of lipoxygenase. Indeed, no significant difference was observed between this compound and zileuton.

Values were expressed as mean SD of three replicates, nd: not determined. Values with a different letter in superscript are significantly different ($p < 0.05$).

Antioxidant potential

Table 2 presents the results obtained with the anti-radical test. Quercetin-3-O- sophoroside and Quercetin-3-O-β-glucose showed an

Table 1: Inhibition of Xanthine Oxidase, Lipoxygenase et Acetylcholine esterase by isolated compounds.

	Xanthine oxidase	Lipoxygenase	Acetylcholine esterase
Sample	IC_{50} (µg/mL)		
Quercetin-3-O-sophoroside	18.07 ± 0.78[b]	1,12 ± 0,018[a]	26.91 ± 0.34[c]
Quercetin-3-O-β-glucose	32.27 ± 2.02[c]	11,53 ± 0,52[b]	19.54 ± 0.25[b]
Allopurinol	2.09 ± 0.31[a]	nd	nd
Zileuton	nd	2,74 ± 0,02[a]	nd
Galanthamine HBr	nd	nd	0.23 ±0.05[a]

Table 2: Antioxidant potential of isolated compounds.

	Deoxyribose inhibition	Anti-DPPH test
Samples	IC_{50} (µg/mL)	
Quercetin-3-O-sophoroside	19.32 ± 1.08[b]	3.41±0.82[a]
Quercetin-3-O-β-glucose	26.21 ± 2.25[c]	2.90 ± 0,18[a]
Quercetin	10.06 ± 0.11[a]	3.12 ± 0.21[a]

Data are expressed as mean ± SEM (n= 3); values harboring different letters in superscript are significantly different (p<0.05)

IC_{50} of 3.41 ± 0.82 µg /mL and 2.90 ± 0,18 µg /mL respectively. These values are statistically close to that obtained with the quercetin used in our test as a reference.

DISCUSSION

The study made it possible to isolate at the leaf level rutin, luteolin, quercetin-3-O-sophoroside, kaempferol-3-O-sophoroside, and quercetin-3-O-β-glucose.

Flavonoid glycosides are recognized for their antioxidant, anti-bronchospastic, and anti-inflammatory activities.[17]

Comparatively, quercetin -3-O-sophoroside was more active on xanthine oxidase than quercetin -3-O-glucose, while the opposite observation was made in the case of lipoxygenase inhibition. Xanthine oxidase is one of the key enzymes implicated in producing uric acid and hydrogen peroxide (H_2O_2), and by the way in the pathologies linked to hyperuricemia, molecules isolated and tested were significant inhibitors of this enzyme.[21] The potential of the isolated molecules to inhibit the activity of xanthine oxidase may justify the traditional uses of *Pterocarpus erinaceus* in the treatment of pathologies with oxidant components and rheumatism conditions.[22]

Lipoxygenase is one of the key enzymes implicated in the biosynthesis of leukotrienes and other lipid mediators of inflammation,[23] significant inhibition was obtained when it was tested *in vitro* with isolated compounds. The treatment of inflammatory diseases with the extract of *P. erinaceus* may be justified by the presence of compounds responsible for the limitation of inflammatory mediator generation through the modulation of inflammatory enzyme activities.

Acetylcholinesterase is a hydrolase responsible for the termination of signal activity in the cholinergic system. It is directly linked to a progressive neuronal disease which is Alzheimer's disease.[24] Thus, its inhibition has been considered a means of preventing or delaying the memory loss and neuronal dysfunction seen in the elderly.[24] Different tested samples significantly inhibited acetylcholinesterase. The obtained results may yet justify the uses of *P. erinaceus* as an alternative in the treatment or prevention of the apparition of diseases related to the loss of memory and neuronal dysfunction may be justified by the activity of these molecules in the different traditional formulas elaborated with this plant.

The obtained results reveal that the isolated compounds are potent inhibitors of deoxyribose degradation. Deoxyribose is a capital component of DNA whose damage is the starting point of mutation and associated diseases.[25] Many antioxidant molecules were found to be effective in preventing deoxyribose degradation, and among them are the phenolic compounds.[25,26] Quercetin-3-O-sophoroside and Quercetin-3-O-β-glucose presented IC_{50} values of 19.32 ± 1.08 and 26.21 ± 2.25, while quercetin used as a control was two times more inhibitor of the degradation of deoxyribose (Table 2). Structural differences in the composition may explain discrepancies in obtained values of the prevention of the degradation of the deoxyribose.[26]

Most degenerative diseases afflict humanity and arise from a harmful reaction of free radicals.[27] Several studies suggest that regulating the production of free radicals can reduce the progression of these diseases.[27,28] The presence of compounds with anti-radical potential in the secondary metabolites of *P. erinaceus* could thus justify its traditional uses.

CONCLUSION

In this study, we provide further scientific evidence supporting the traditional uses of *Pterocarpus erinaceus* for medicinal purposes in Burkina Faso. Phytochemical analysis of the methanolic extract and its fractions led to the isolation and characterization of five compounds. Results suggest that the presence of bioactive compounds 1 and 2 may explain the claimed therapeutic uses in the traditional healthcare system. Antioxidant activities and the potential of the prevention of the degradation of deoxyribose and inhibition of pro-inflammatory enzymes also indicate the usefulness of this plant in the prevention of inflammation and associated diseases.

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CONFLICTS OF INTEREST

The author reports no conflicts of interest in this work.

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