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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, ANTIOXIDANT, ANTI-INFLAMMATORY, AND ANTIPLASMODIAL ACTIVITIES OF LEAF EXTRACTS OF *GREWIA BICOLOR*

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Abstract

Background and aim: *Grewia bicolor*, *Malvaceae* family, widespread in tropical Africa, is traditionally used in the treatment of various infections. The aim of this study was to investigate the total phenolic and flavonoid contents, the *in vitro* antioxidant, anti-inflammatory and antiplasmodial activities of *Grewia bicolor* leaf extracts, and to isolate and characterise some bioactive molecules.

Methods: The extraction was carried out by maceration of *G. bicolor* leaves successively in acetone and methanol. The methanol extract separated by repeated chromatographic columns on silica gel provided three flavonoids, which were characterized by spectroscopic techniques. Spectrophotometric methods determined total phenolic and flavonoid contents. The DPPH free radical scavenging assay allowed the evaluation of antioxidant activity. Abovine serum albumin denaturation inhibition study evaluated anti-inflammatory activity. The SYBR Green method was used for antiplasmodial activity on chloroquine-sensitive (3D7) and chloroquine resistant (Dd2) *Plasmodium falciparum* strains.

Results: The methanol extract with the highest values for antioxidant activity (IC_{50}=21.80±0.23 µg/mL) and inhibition of bovine serum albumin denaturation (IC_{50}=20.95±0.56 µg/mL), exhibited the highest total phenolic and flavonoid contents. This extract also showed the highest antiplasmodial activity, with IC_{50}=49.07±0.10 and 43.61±0.08 µg/mL for chloroquine-sensitive and chloroquine-resistant strains respectively. The three isolated flavonoids were identified as catechin (1), leucocianidol (2) and isoquercitrin (3) using one- and two-dimensional nuclear magnetic resonance and mass spectroscopy. Compound (2) showed the most promising anti-inflammatory activity compared to other compounds.

Conclusion: The results of this study support the use of *G. bicolor* in traditional medicine to treat inflammatory disorders and malaria. Furthermore, this plant could be a promising source of natural antioxidants. Antioxidant and anti-inflammatory activities of *G. bicolor* can be correlated to the presence of flavonoids.

Keywords: Anti-inflammatory, antioxidants, antiplasmodial, flavonoids, *Grewia bicolor*.

INTRODUCTION

Grewia bicolour Juss, Mavalceae family, is a shrubby widely distributed in sub-Saharan Africa, the Asian subcontinent and the Arabian Peninsula countries[1]. *G. bicolour* is used ethnobotanically in folk medicine by local African populations to treat various ailments such as diarrhoea, skin lesions, syphilis, intestinal inflammations, colds, mental illnesses, snake bites and female infertility[2]. Previous studies have described the anthelmintic, diuretic, antidiarrhoeal[3], tranquillizing[1], and anti-inflammatory activities of this plant[4]. *G. bicolour* extracts contain compounds such as phenolics, flavonoids, tannins, and alkaloids[5].

Free radicals generated by oxidative stress damage biomolecules and cause many pathologies including diabetes, cancer, Alzheimer's disease, atherosclerosis, ageing and various cardiovascular diseases[6,7]. Since synthetic antioxidants have been shown to have adverse effects, safer, more effective plant-based

alternatives have been developed as replacements[8]. Plants have antioxidant properties due to their phenolic compounds, which neutralise the free radicals responsible for oxidative stress[9].Compounds such as flavonoids can exert their antioxidant role by reducing the production of reactive oxygen species[10]. Secondary metabolites synthesized by plants such as polyphenolic compounds and flavonoids can combat oxidative stress by preventing the formation of free radicals involved in the inflammatory process[10,11].

Tissue and cell damage caused by pathogens, dangerous stimuli, or physical injury leads to inflammation of the body[12]. Manifestations of the inflammatory process include the appearance of clinical signs such as heat, redness, oedema, and loss of normal physical function[13]. Anti-inflammatory activity was studied using various models, such as inhibition of egg albumin and bovine serum albumin (BSA) denaturation[14]. Heat treatment denatures serum albumin, resulting in a modification of its tertiary structure. Non-steroidal anti-inflammatory drugs (NSAIDs) including diclofenac sodium and aspirin prevent the denaturation of BSA at pathological pH[15]. Phenolic compounds and flavonoids are secondary metabolites that exert their anti-inflammatory activities by reducing the production of reactive oxygen species (ROS) and down-regulating several anti- and pro-inflammatory mediators[10]. The anti-denaturation activity of BSA by phenolic compounds and flavonoids isolated from Date palm (*Phoenix dactylifera L.*) has been reported previously[16].

The inflammation isa key feature of malaria, caused by the infection of the host's erythrocytes by the *Plasmodium* parasite. Oxidative stress, which is common in malaria, has been identified as an effective trigger for the inflammatory activation of macrophages[17]. Malaria parasites cause oxidative stress in the host but also have antioxidant effects on the host's defences. Anti-malarial medicines therefore have an antioxidant effect on the host and/or a pro-oxidant effect on the parasite. Consequently, molecules with antioxidant properties would be potential anti-inflammatory and antiplasmodial remedies[18].

Although *G. bicolor* has beneficial properties in traditional medicine, very few studies have focused on phytochemical analysis and its biological activities *in vitro*. This is the first study to report the isolation of anti-inflammatory flavonoids from *G. bicolor* and to study the correlation between antioxidant, protein denaturation inhibition and antiplasmodial activities and total phenolic and flavonoid content. The results could justify the use of *G. bicolor*in traditional medicine for treating certain chronic illnesses and inflammations.

MATERIALS AND METHODS

Leaves of *G. bicolor* collected in February 2021 in Poli, in the North of Cameroon (8°28′60″North, 13°15′0″East), were identified at the Department of Biological Sciences of the Faculty of Sciences of the University of Ngaoundere. The cleaned and dried plant material was ground and stored in a hermetic container at an ambient temperature of 25°C before extraction.

Extraction procedure

For extraction, leaf powder (1 kg) contained in a container was mixed with 4 L of acetone and left to macerate for 72 hours at room temperature, with occasional stirring. The mixture was then filtered and concentrated on a vacuum rotary evaporator at a temperature of 40°C to give the acetone extract. The residue obtained was extracted with methanol using the same protocol as described above to give the methanol extract. All extraction operations were repeated three times.

Phytochemical screening

The various secondary metabolites present in acetone and methanol extracts were characterised using standard methods for phytochemical screening. The presence or absence of compounds such as phenols, terpenoids, tannins, saponins, flavonoids, alkaloids, etc. was therefore determined using phytochemical screening tests of the extracts[19].

Determination of Total Phenolic Content (TPC)

The total phenolic content of acetone and methanol extracts from leaves was determined using the method reported by Alara *et al.*[20]. Briefly, 500 µl of a 10% (v/v) Folin-Ciocalteu reagent solution was mixed with 100 µl of solutions diluted to different concentrations (10-100 µg/ml) of the extract and gallic acid, the standard. After 5 minutes of incubation of the mixture in the dark, 600 µL solution of 7.5% (w/v) Na_2CO_3 was added to the previous mixture. The mixture was then left for 30 minutes and the absorption at 760 nm was measured using a spectrophotometer (Optizen POP, Korea), with methanol as the blank. The mean ± SD was calculated after analyses were carried out in triplicate. The gallic acid calibration curve was used to calculate the total phenolic content of both extracts in milligrams of gallic acid equivalents per gram of dry weight (mg gallic acid/g).

Determination of Total Flavonoid Content (TPC)

The spectrophotometric method of Al-Rimawi *et al*. (2022), was used to determine the total flavonoid content[21]. A solution of 500 µL diluted extract (1 mg/mL) was mixed with 2 mL distilled water and 150 µL $NaNO_2$ (15%). After leaving the mixture at ambient temperature for six minutes, a solution of 150 µL of 10% $AlCl_3$ was added, followed by incubation for a further six minutes at room temperature. After adding 2 mL of a 4% NaOH solution, the mixture was left to incubate for 15 minutes at room temperature. The absorbance of the mixture was then determined at 510 nm using a UV-vis spectrophotometer (Optizen POP, Korea), with methanol as the blank. The result was expressed in milligrams of catechin equivalents per gram of dry weight of the sample (mg Catechin/g) obtained from the catechin calibration curve. Analyses were performed three times.

DPPH radical scavenging activity

The DPPH free radical scavenging method described by Guo *et al.*[9], was used to determine antioxidant activity. A volume of 1 mL of each extract at various concentrations (8-125 µg/mL) was mixed with 1 mL of a methanolic solution of DPPH (40 µg/mL). The

mixture was left for 30 minutes, and then its absorbance at 517 nm was determined using a UV-vis spectrophotometer (Optizen POP, Korea), with methanol serving as the blank. Ascorbic acid at various concentrations (8-125 µg/mL), was used as a standard. Percentage inhibition of DPPH was calculated using the following equation:

$$\%\ \text{inhibition of DPPH} = \frac{A0 - A1}{A0} \times 100$$

A0 = Absorbance of the blank; A1 = Absorbance of the tested extract.

The concentration of sample required to inhibit 50% of DPPH (IC_{50}) was used to express the results. Experiments were carried out three times.

Anti-inflammatory assay

Anti-inflammatory activity was assessed by measuring the inhibition of BSA denaturation by the extracts, as described in the slightly modified method of Dernouich *et al*.[22]. Total 50 µL of each extract at different concentrations (10-150 µg/mL) were added to 450 µL of BSA (1%), left to stand for 20 minutes at 37°C and heated for 3 minutes at 57°C. Once cooled, 2.5 ml of phosphate buffer solution was added to the mixture. The samples' absorbances were measured using a UV-visible spectrophotometer at 660 nm, with the positive control being Diclofenac sodium. All tests were carried out three times. The following formula was used to calculate the percentage inhibition of protein denaturation:

$$\text{Denaturation inhibition}\ (\%) = \frac{Ac - As}{Ac} \times 100$$

Where, Ac is the absorbance of the control solution and As is the absorbance of the sample. The concentration of the sample that showed 50% inhibition (IC_{50}) was determined from interpolation of the linear regression analysis.

***In vitro* antiplasmodial assay**

Chloroquine-sensitive 3D7 and chloroquine-resistant Dd2 strains of *P. falciparum* were used for evaluation of *in vitro* antiplasmodial activity of *G. bicolor* leaf extracts. The method reported by Trager and Jenssen (1976) was used to cultivate *P. falciparum*[23]. *P. falciparum* culture was maintained in fresh human erythrocytes (O+), suspended at a haematocrit of 4% in complete RPMI 1640 medium [25 mM HEPES, 0.5% albumax, 45 µg/L hypoxanthine and 50 µg/L gentamicin] and incubation was carried out at 37°C in a humidified atmosphere with 5% CO_2. The medium was changed daily with fresh complete medium until the parasitaemia level reached 1-2%.

The antiplasmodial assay of both extracts was evaluated using the previously reported SYBR Green I fluorescence assay[24]. A ring-stage synchronised parasite was obtained before each experiment using 5% (w/v) sorbitol, as described by Lambros and Vanderberg (1979)[25]. Serial dilutions of dried extracts and the reference drug (artemisinin) (0–500 µg/mL) were prepared using dimethyl sulfoxide. The incubation of sorbitol-synchronised parasites under normal culture conditions at 1% haematocrit and 2% parasitemia in the presence of prediluted extracts and artemisinin (10 µL) was for 72 hours at 37°C in microwells. A volume of 100 µL of SYBR Green I buffer [6 µL of 10,000×SYBR Green I (Invitrogen)+600 µL of red blood cell lysis buffer {Tris (25 mM; pH 7.5)}+360 µL of EDTA (7.5 mM)+19.2 µL of parasite lysis solution {saponin (0.012%; w/v)} and 28.8 µL of Triton X-100 (0.08%; v/v)}] was added to each well after incubation, mixed carefully twice and incubated in the dark at 37 °C for 1 h. The TECAN M 200 microplate reader was used to measure the fluorescence, with excitation and emission set to 485 and 538 nm, respectively. The dose-response curve was analysed to determine the fluorescence counts as a function of the logarithm of the concentration as well as the IC_{50} using GraphPad Prism 5 software. Interpretation of IC_{50} values (µg/mL) was performed according to the classification proposed by Gathirwa *et al*.: high activity (IC_{50}<10), moderate activity (10≤IC_{50}<50), low activity (50≤IC_{50}≤100) and inactive (IC_{50}>100)[26]. Experiments were performed in duplicate. For each extract and control, results were validated microscopically by examination of Giemsa-stained blood spots.

Separation and isolation

The methanol extract (25 g) was separated by repeated column chromatography (CC) on silica gelwith a gradient of the increasing polarity of methanol in chloroform (1-100 %) and 150 fractions (A1-150) were collected and pooled after examination of the eluates by thin layer chromatography. The precipitate obtained from fractions A40-51 was purified by recrystallization in 100% ethyl acetate to give 20 mg of yellow powder[1]. Fractions A75-85 was subjected to CC on silica gel to produce 26 subfractions (B1-26). The combined sub-fractions (B10-15) formed a precipitate which was purified by recrystallization in 100% acetone to give 18 mg of amorphous white powder[2]. Purification of fractions A100-115 was performed by preparative thin layer chromatography on silica gel to yield 25 mg of yellow powder[3].

Statistical Analysis

SPSS (Statistical Package for the Social Sciences) version 21 was used to perform all statistics. Each analysis was carried out three times, and the resulting values were presented as the mean ± standard deviation (SD). Statistical significance was attributed to means if p<0.05. Pearson's correlation coefficient squared (R^2) was also utilised to evaluate the relationship between paired variables.

RESULTS AND DISCUSSION

Phytochemical screening

Extraction of *G. bicolor* leaves yielded 32.5 g (3.25%) acetone extract and 102.53 g (10.26%) methanol extract. The presence of flavonoids, tannins, alkaloids, terpenoids, glycosides and phenolic compounds was revealed by phytochemical analysis of both extracts. These results are similar to those described in earlier studies[1,27,28].The presence of polar secondary metabolites, such as phenolic compounds and flavonoids, was revealed by both extracts. The extracts' antioxidant and anti-inflammatory properties may be due to these compounds.

Table 1: DPPH scavenging activity of different extracts of *G. bicolor*.

Samples	Conc (µg/mL)	Inhibition±SD (%)	IC_{50} (µg/mL)
Acetone extract	8	35.52±0.06	68.75 ± 0.88
	16	37.13±0.35	
	31.25	41.07±0.02	
	62.5	48.52±0.04	
	125	63.44±0.15	
Methanol extract	8	42.38±1.0	21.80 ± 0.23
	16	46.58±0.46	
	31.25	55.17±0.64	
	62.5	72.36±0.26	
	125	100.00±0.0	
Ascorbic acid	8	38.37±0.82	33.90 ± 0.75
	16	40.35±0.49	
	31.25	44.42±0.64	
	62.5	52.54±0.17	
	125	68.79±0.55	

Determination of total phenolic and flavonoid contents

Polyphenols, including phenols, phenolic acids, flavonoids, quinones, phenylpropanoids, and tannins, are secondary metabolites present in whole plants[29]. Some phenolic compounds may have antioxidant andanti-inflammatory activities[30]. The calculation of the total phenolic content of acetone and methanol extracts in *G. bicolor* leaves was done using the gallic acid calibration curve regression equation. Methanol extract had the highest TPC (409.99±4.35 mg Gallic Acid/g of extract) than acetone extract (329.29±4.0 mg Gallic Acid/g of extract). The total flavonoid content of both extracts was determined using catechin calibration curves. The TFC of the methanol extract (126.31±0.36 mg Gallic Acid/g of extract) is higher compared to acetone extract (86.40±0.80 mg Gallic Acid/g of extract). Quantitative phytochemical analysis revealed that the polyphenol content of G. bicolor leaf extracts was higher than the flavonoid content. In addition, the methanol extract had a higher polyphenol and flavonoid content than the acetone extract, confirming the hypothesis that methanol is the best solvent for extracting polyphenols and flavonoids[31].The excellent antioxidant activity of the methanol extract of *G. bicolor* is probably due to its high TPC value (Table 3). This suggests that the most polar extract, with the highest TPC, exhibited the highest antioxidant activity. These results differ from those of a previous study which revealed that, despite having a significantly high TPC, the aqueous extract of G. bicolor berries exhibited low antioxidant activity compared to the ethanol extract[5].

DPPH free radical scavenging activity

DPPH scavenging activity was assessed as a function of the dose of extract and ascorbic acid (Table 1). The IC_{50} values show that methanol extract (21.80±0.23 µg/mL) inhibits the DPPH free radical to a greater extent than acetone extract (68.75±0.88 µg/mL) (Table 2).

Table 2: Inhibition of bovine serum albumin denaturation of *G. bicolor* extracts and compounds.

Samples	Conc (µg/mL)	Inhibition±SD	IC_{50}(µg/mL)
Acetone extract	25	07.86±0.21	141.92±0.53
	50	16.80±0.41	
	100	35.18±0.30	
	150	52.81±0.46	
	200	71.87±0.52	
Methanol extract	25	50.85±0.54	20.95±0.56
	50	56.10±0.30	
	100	66.60±0.70	
	150	77.10±0.20	
	200	87.60±0.44	
1	25	46.75±0.07	35.85±0.75
	50	54.25±0.42	
	100	69.25±0.13	
	150	84.25±0.84	
	200	98.02±0.45	
2	25	53.28±0.45	15.24±0.32
	50	62.85±0.26	
	100	81.23±0.19	
	150	99.54±0.11	
	200	100.0±0.0	
3	25	30.93±0.82	86.92±1.36
	50	41.38±0.21	
	100	50.66±0.03	
	150	69.54±0.26	
	200	83.72±0.56	
Sodium Diclofenac	25	46.73±0.32	31.45±0.20
	50	63.58±0.23	
	100	98.82±0.26	
	150	99.91±0.08	
	200	100.0±0.0	

Values represents the mean ± standard deviation (n = 3). 1: catechin. 2: leucocianidol. 3: isoquercitrin.

Table 3: *In vitro* antiplasmodial activity of *G. bicolor*.

Samples	IC_{50} (µg/mL)*Pf*3D7	IC_{50} (µg/mL) *Pf*Dd2
Acetone extract	> 100	> 100
Methanol extract	49.07±0.10	43.61±0.08
Artemisinin	23.73±0.24	17.76±0.25

Moreover, the methanol extract is more effective in trapping the DPPH radical than ascorbic acid, which is the reference compound. The potential of both extracts to scavenge DPPH free radicals makes them promising candidates that may play an important role in destroying the free radicals responsible for oxidative stress in the human body[32]. The presence of certain classes of polyphenols in plant extracts, particularly flavonoids, is the reason for this scavenging capacity[22,33]. Indeed, studies by Gwatdzido *et al.*[28], showed the presence in *G. bicolor* fruits of phenolic compounds such as flavonoids which may be accountable for the plant's antioxidant activity.

Inhibition of bovine serum albumin denaturation

The BSA inhibition method was used to assess the anti-inflammatory activity of the extracts and isolated flavonoids. Five doses (25-200 µg/mL) were used to assess the inhibition of BSA denaturation by extracts and isolated compounds and showed a concentration-dependent inhibition (Table 2). Methanol extract showed strong inhibition of BSA denaturation with an IC_{50} value of 20.95±0.56 µg/mL compared with acetone extract (141.92±0.53 µg/mL). In addition, the activity of methanol extract was close to that of the reference drug, diclofenac sodium (31.45±0.20 µg/mL). Among the compounds, leucocianidol (2) showed the strongest inhibition of BSA denaturation with an IC_{50} value of 15.40±0.32 µg/mL compared to catechin (1) and isoquercitrin (3) with IC_{50} values of 35.85±0.75 and 154.36±2.47 µg/mL respectively.

Moreover, compound (2) was found to be more active than the reference drug, diclofenac sodium (IC_{50}= 31.45±0.20 µg/mL) in inhibiting BSA denaturation. This anti-denaturation effect of BSA is due to compounds such as NSAIDs, which can prevent the denaturation of heat-treated proteins[22]. Previous studies have shown that protein denaturation is responsible for the production of autoantigens in inflammation. Stress, a compound or heat causes protein denaturation, which involves the modification of proteins' tertiary and secondary structures. This denaturation is one of the causes of inflammation and leads to the loss of the biological functions of most proteins[34]. Many earlier investigations have shown that flavonoids can interact with proteins such as BSA to improve their thermal stability, thereby preventing their denaturation. Isolated compounds belonging to the flavonoid class have been shown to inhibit the denaturation of egg albumin and BSA. These results are similar to those of several previous studies that have shown that flavonoids can interact with proteins such as BSA to improve their thermal stability, thus preventing their denaturation[22]. The chemical structure of flavonoids affects their interactions with proteins (BSA). This could explain the difference in protein denaturation inhibition potential observed for the isolated compounds[35]. Among the isolated compounds, one flavonol glycoside (isoquercitrin) has the lowest inhibition potential compared to the other two (catechin and leucocianidol) which are flavanols. Its structure differs from the other two compounds belonging to the single one by the presence, on the C ring, of a double bond connecting C2 and C3, a carbonyl at C4 and a glycoside at C3 (Figure 1). Based on the IC_{50} values, a relationship was observed between the structure of the isolated compounds and the potential to inhibit BSA denaturation. Indeed, leucocianidol, which has two OH groups, is more active than catechin (one OH group), which in turn is more active than isoquercitrin (one double bond conjugated to the CO group), suggesting that the thermal stability of BSA increases with the number of OH groups on the C-ring of flavonoids[36].

***In vitro* antiplasmodial activity**

The *in vitro* antiplasmodial potentials of acetone and methanol extracts of *G. bicolor* leaves were evaluated for *P. falciparum* strains 3D7 and Dd2. The methanol extract appeared to be more active than the acetone extract, according to *in vitro* tests (Table 3). Among the two extracts, only the methanol extract showed antiplasmodial activity (IC_{50}=49.0±0.10 and 43.61±0.08 µg/mL for strains 3D7 and Dd2, respecti vely). In contrast, the acetone extract with IC_{50} values greater than 100 µg/mL showed no activity for both strains[37]. The results of this in vitro study revealed that the methanol extract exhibited anti-plasmodial activity against both chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains of *P. falciparum*. This suggests the presence of bioactive compounds in the methanol extract. The traditional use of *G. bicolor* in the treatment of malaria is supported by these findings. The methanol extract's effectiveness against the Dd2 strain suggests it could be an effective treatment for chloroquine-resistant malaria.

Correlation analysis

The correlation between antioxidant, anti-inflammatory and antiplasmodial activities and TPC and TFC of the extracts was studied using Pearson's correlation coefficient. A strong correlation was revealed by the analytical data between DPPH radical scavenging potential and total phenolic content (TPC) and total flavonoid content (TFC) with a coefficient (R^2) of 0.968 and 0.996 respectively (Table 4). The DPPH free radical scavenging capacity is enhanced by certain classes of polyphenols in plant extracts, in particular, flavonoids[32].The positive correlation between total phenolic content and total flavonoid content (R^2=0.972) means that the content of total phenolic compounds is proportional to that of total flavonoids. In general, polyphenols and flavonoids play an important role as antioxidants by acting as free radical scavengers and electron donors[38]. Consequently, polyphenols and flavonoids may be responsible for the variations observed between the extracts studied.

Table 4: Correlation between phenolic content, flavonoid level, antioxidant activity, and inhibition of protein denaturation.

	DPPH	IPD	TPC	TFC
DPPH	1			
IPD	0.988	1		
TPC	0.968**	0.956**	1	
TFC	0.996**	0.988**	0.972**	1

** Correlation is significant at the 0.01 level (two-ailed). DPPH: DPPH radical scavenging activity. IPD: BSA Inhibition denaturation. TPC: total phenolic content. TFP: total flavonoid content.

The results also showed a strong positive correlation between inhibition of BSA denaturation and TPC and TFC with R^2=0.956 and 0.988 respectively. This is consistent with the study by Boukhali *et al.*, that showed the inhibition of BSA denaturation by phenolic compounds isolated from *Phoenix dactylifera* L. seeds. A strong correlation between antioxidant and anti-inflammatory activities was revealed in this study. Indeed, DPPH scavenging activity showed a relationship with inhibition of BSA denaturation (R^2=0.988). Certain compounds with free radical scavenging capacity reduce oxidative stress, thereby preventing protein denaturation[39].

Flavonoids isolation

The isolation of flavonoids was achieved using the methanolic extract, which demonstrated the highest levels of antioxidant and anti-inflammatory activity. This extract was subjected to silica gel column chromatography using a $CHCl_3$/MeOH gradient of increasing polarity yielded three flavonoids (1, 2, and 3). Spectral techniques (1H-NMR and 1C-NMR) were used to elucidate their structures shown in Figure 1.

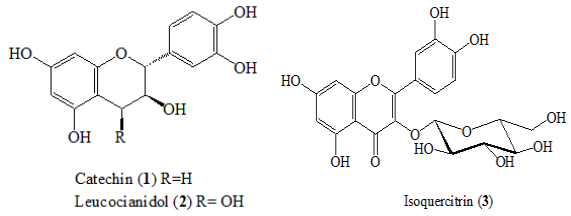

Figure 1: Structures of the isolated compounds from *G. bicolor*.

Catechin (1)

Compound 1 was found to be a pale yellow powder. The ^{1}H-NMR spectrum (600 MHz, CD_3OD) showed d/ppm: 2.73 (1H, dd, J = 16.8, 2.9 Hz, H-4b), 2.86 (1H, dd, J = 4.6, 16.7 Hz, H-4a), 4.17 (1H, m, H-3), 4.84 (1H, s, H-2), 5.91 (1H, d, J = 2.3 Hz, H-8), 5.93 (1H, d, J = 2.3 Hz, H-6), 6.75 (1H, d, J = 8.1 Hz, H-5'), 6.79 (1H, dd, J = 2.0, 8.5 Hz, H-6'), 6.97 (1H, d, J = 1.9 Hz, H-2'). The ^{13}C-NMR (151 MHz, CD_3OD) data showed at δ 29.79 (C-4), 67.65 (C-3), 80.04 (C-2), 96.02 (C-6), 96.52 (C-8), 100.21 (C-4a), 115.47 (C-5'), 116.02 (C-2'), 119.53 (C-6'), 132.44 (C-1'), 145.94 (C-3'), 146.11(C-4'), 157.53(C-8a), 157.53(C-5), 158.17 (C-7). These spectral data compared to the literature identified compound 1 as catechin[40].

Leucocianidol (2)

Compound 2 is a white powder which responded positively to the flavonoid test. The ^{1}H-NMR spectrum (300 MHz, DMSO-d6) δ 4.21 (1H, m, H-3), 5.08 (2H, d, J = 7.9 Hz, H-2, H-4), 6.04 (s, 2H, OH, OH), 6.31 (1H, d, J = 9.5 Hz, H-5'), 6.95 (1H, d, J = 2.9 Hz, H -6), 6.96 (1H, d, J = 2.5 Hz, H-8), 7.01 (s, 1H, OH), 7.05 (s, 1H, OH), 7.30 (1H, s, H-2'), 7.95 (1H, d, J = 9.5 Hz, H-6'). The ^{13}C-NMR spectrum (75 MHz, DMSO-d6) δ 60.30 (C-4), 76.83 (C-3). 78.09 (C-2, 108.42 (C-6), 108.75 (C-8), 113.23 (C-4a), 113.94 (C-5'), 115.00 (C-2'), 122.35 (C-6'), 130.13 (C-1'), 140.84, 144.54 (C-4'), 147.54, (C-3'), 148.18 (C-8a), 149.13 (C-5), 160.86 (C-7). Compound 2 was identified as leucocianidol by comparing its spectra with literature data[41].

Isoquercitrin (3)

Compound 3 appeared as a yellow powder. The ^{1}H-NMR spectrum showed Aglycone: (400 MHz, CD_3OD) δ 7.72 (d, J = 2.2 Hz, H-2'), 7.60 (1H, dd, J = 8.5, 2, 2 Hz, H-6'), 6.88 (1H, d, J = 8.5 Hz, H-5'), 6.40 (1H, d, J = 2.1 Hz, H-8), 6.21 (1H, d, J = 2.1 Hz, H-6). Sugar: 3.37 (1H, m, H-5''), 3.42 (1H, d, J = 9.0 Hz, H-3''), 3.43 (d, J = 6.2 Hz, H-4''), 3.48 (1H, d, J = 9.0 Hz, H-2''), 3.59 (1H, dd, J = 11.9, 5.3 Hz, H-6b''), 3.71 (1H, dd, J = 11.9, 2.4 Hz, H-2''), 3.72 (1H, dd, J = 11.9, 2.4 Hz, H-6a''), 5.26 (1H, d, J = 7.5 Hz, H-1''). The ^{13}C-NMR spectrum exhibited the characteristic 15 carbon resonances of quercetin-3-O-*β*-D glycoside at δ 61.03 (C-6''), 69.98 (C-4''), 74.15 (C-2''), 76.55 (C-3''), 77.65 (C-5''), 93.60 (C-8), 100.87, (C-6), 101, 99 (C-1''), 104.03 (C-4a), 115.97 (C-2'), 116.24 (C-5'), 121.19 (C-6'), 121.69 (C-1'), 133, 36 (C-3), 144.89 (C-3'), 148.27 (C-4'), 156.24 (C-2), 156.40 (C-8a), 161.03 (C-5),163.95 (C-7), 177.51 (C-4). These spectral data, compared with those in the literature, identified compound 3 as isoquercitrin[41].

Limitations of the study

The first part of this study focused on the isolation and characterization of flavonoids from the leaves of *G. bicolor*. Next, the correlation between total phenolic and flavonoid content and *in vitro* antioxidant and anti-inflammatory activities was investigated. The antiplasmodial activity of the extracts and the anti-inflammatory activity of the isolated flavonoids were also tested. The *in vitro* activities were evaluated by determining the IC_{50} values, which were also used to study the correlation between the variables. However, these activities were not tested *in vivo* to determine the pharmacological dosage. Similarly, the antiplasmodial activity of the isolated flavonoids was not tested to predict whether these compounds could be responsible for this activity in the plant.

CONCLUSIONS

G. bicolor leaf extracts showed antioxidant and anti-inflammatory activities close to those of the reference compounds (ascorbic acid and diclofenac sodium,

respectively). *G. bicolor* leaves containing high levels of phenolics and flavonoids showed potent antioxidant and anti-inflammatory properties through their potential to remove DPPH and prevent BSA denaturation. Phytochemical separation of *G. bicolor* leaves revealed the presence of three flavonoids, catechin, leucocianidol, and isoquercitrin. *G bicolor* leaves exhibited good antioxidant and anti-inflammatory activities. This plant could thus be utilised as a natural resource of antioxidants and anti-inflammatories.

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AUTHOR CONTRIBUTIONS

Fadimatou A: study design, conceptualization, experimental work. **Momeni J:** experimental work, revision. **Hacene FB:** designed the research, conceptualization. **Yanawa SS:** collected and prepared the plant material, fractionated and separated the extract. **Faissam LSN:** data collection, anti-inflammatory activity. **Ghalem S:** study design, revision. All authors read and approved the final manuscript.

DATA AVAILABILITY

The accompanying author can provide the empirical data that were utilized to support the study's conclusions upon request.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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