*Original Research Article*

****Phytochemical characterization, antioxidant potential, and anthelmintic activity of****Desmodium triflorum****(L.) DC. (Fabaceae) from Côte d'Ivoire****

.

ABSTRACT

|  |
| --- |
| **Aims:** This study aims the pharmacological potential of Desmodium triflorum (Fabaceae), a medicinal plant from Côte d'Ivoire.  **Methodology:** For this purpose, aqueous and selective (organic) extracts of the whole plant were subjected to phytochemical screening using detection (color reaction and precipitation) tests and thin-layer chromatography (TLC), antioxidant evaluation via DPPH (free radical scavenging) and FRAP (ferric reducing antioxidant power) assays (compared to quercetin), and anthelmintic testing on earthworms (Lumbricus terrestris) with albendazole as the reference standard.  **Results:** The selective extracts revealed a richness in secondary metabolites (phenols, flavonoids, coumarins, tannins, terpenoids, saponins, anthocyanins, sterols, and polyterpenes). Concerning the quantitative profile of extracts, the decoction gave the best total phenol (TP) and total flavonoid (TF) contents (37214.29 µg GAE/g and 85805.56 µg QE/g respectively). In terms of hydrolysable tannins (HT) and condensed tannins (CT), the decoction and the macerate had practically the same contents. A dose-dependent antioxidant potential was observed (DPPH EIs and FRAP reducing power), though lower than that of quercetin. The anthelmintic activity against earthworms was notable but less pronounced compared to albendazole.  **Conclusion:** D. triflorum exhibits a diversified phytochemical profile and promising bioactivity, justifying further studies on its mechanisms of action and potential applications in animal or human health. |

*Keywords:* Desmodium triflorum*, phytochemical screening, antioxidant activity (DPPH/FRAP), anthelmintic activity, albendazole.*

1. INTRODUCTION

Helminthiasis, intestinal parasitic diseases caused by parasitic worms, is a major public health issue. Their management relies on medical treatments or traditional remedies [1,2]. According to epidemiological projections [3], chemoprevention coverage is expected to expand in the coming years. However, access to anthelmintic medications remains limited for certain populations, while their intensive use promotes the emergence of resistance to the main therapeutic classes [4,5]. In the face of these challenges, the search for plant-derived active principles that are both non-toxic and effective represents a promising alternative for controlling these parasitic infections. It is in this context that our study on *Desmodium triflorum* L. (Fabaceae) is situated, a plant used in traditional medicine against helminthic infestations. This work aims to characterize the phytochemical, antioxidant, and anthelminthic profiles of *D. triflorum* extracts.

2. material and methods

**2.1 Plant Material**

The material studied includes the whole plant (leaves, stems, and roots) of *Desmodium triflorum* L., harvested in June 2019 on the campus of Nangui ABROGOUA University (5°23′21″N, 4°01′09″W, Abidjan, Côte d'Ivoire). Botanical authentication was carried out at the National Center for Floristics (CNF) in Abidjan, referencing a herbarium voucher specimen. After harvesting, the plant was washed with running water to remove impurities and then dried for 10 days in a climatic chamber maintained at 16 °C. The dried parts were ground using a grinding machine (MOULINEX, LM 2201) to obtain a homogeneous powder. This powder was stored in airtight glass jars, protected from light and moisture, for subsequent extraction steps.

**2.2 Methods**

**2.2.1 Preparation of extracts**

**Microwave extraction:** Five grams (5 g) of plant powder were mixed with 50 ml of distilled water and subjected to microwave-assisted extraction (NASCO microwave oven, MM720CQM-S), set to low temperature. The mixture was brought to a boil for 3 minutes, followed by a cooling phase at room temperature. The solution was then vacuum filtered using a Büchner funnel (with Whatman No. 1 filter paper), and the filtrate obtained was concentrated by rotary evaporation (BUCHI R-114 rotavapor with B-480 Waterbath, T = 40–45 °C, P = 200–300 mbar). The extract was finally dried in an oven at 45 °C until a dry residue, designated as D, was obtained.

**Aqueous maceration:** Five grams (5 g) of plant powder were macerated in 50 ml of distilled water for 2 hours. The solution was then vacuum filtered using a Büchner funnel (with Whatman No. 1 filter paper), and the filtrate obtained was concentrated by rotary evaporation (rotary evaporator (BUCHI R-114 rotavapor with B-480 Waterbath, T = 40–45 °C, P = 200–300 mbar). The extract was finally dried in an oven at 45 °C until a dry residue, designated as M, was obtained. The extraction yield was calculated using the following formula:

Extraction yield (%) = () × 100

**Selective extracts:** Gradient extraction is a method of separating and purifying chemical compounds in a mixture, allowing the isolation of fractions based on their polarity. In this case, a quantity of crude extracts (from two sources, D and M) was dissolved in 10 ml of distilled water. This solution was then subjected to a series of extractions using solvents of increasing polarity: normal hexane (C6H14) was first used to extract the least polar compounds. Three extractions of 5 mL each were performed, resulting in the hexane fractions D1 and M1. Next, chloroform (CHCl3) was used to extract compounds with intermediate polarity, yielding the chloroform fractions D2 and M2. Ethyl acetate (AcOEt) was then employed to extract the more polar compounds, producing fractions D3 and M3. Finally, normal butanol (n-BuOH) was used to extract the most polar compounds, resulting in fractions D4 and M4.

**2.2.2 Qualitative tests**

***2.2.2.1 Phytochemical screening by detection (color reaction and precipitation) tests***

Phytochemical screening to identify the phytocompound families present in *D. triflorum* crude extracts was firstly carried out on crude extracts using either precipitation and color reaction tests [6-10].

* **Phenolic compounds:** a few drops of a 2 % (w/v) aqueous solution of ferric chloride (FeCl3) are added to 2 ml of the crude extract (taken up with ethanol). The appearance of a blue-black or green-black color indicates the presence of polyphenols.
* **Flavonoids**: 4 tests were used to detect them;
* **Shinoda and Pew (or cyanidine reaction) tests**: 5 to 7 drops of concentrated HCl and 2 to 5 chips of Mg (Shinoda test) / Zn (Pew test), are added to 2 ml of the crude extract (taken up with ethanol). After 3 to 5 minutes, a pinkish-orange coloration is observed. To accelerate the reaction and intensify the color, the reaction mass is heated in a water bath for 2 to 3 minutes;
* **Ammonia test**: 3 to 5 drops of a 10% ammonia solution are added to 1 ml of the crude extract (taken up with ethanol). The appearance of a yellow coloration when cold, or orange or red when hot, indicates the presence of flavones, flavanones, flavonols, flavononols. Red coloration when cold indicates the presence of chalcones and aurones, while blue or purple coloration indicates the presence of anthocyanins;
* **Vanillin test**: 3 to 5 drops of an acidic vanillin solution (1 g vanillin in 50 ml concentrated HCl) are added to 1 ml of the crude extract (taken up with ethanol). The appearance of a strawberry red color indicates the presence of catechins (fluoglucine and/or resorcinol derivatives).
* **Coumarins** **(lactone cycle test)**: 2 ml of the crude extract (taken up with ethanol) are poured into 2 test tubes. To one tube is added 0.5 ml of NaOH (10%, m/v), then the 2 tubes are heated in a water bath until boiling. After cooling, 4 ml distilled water are added to each tube. If the liquid in the tube to which the alkaline solution is added is transparent yellow compared with the liquid in the tube containing no alkali, the reaction is positive. By acidifying the transparent solution with a few drops of concentrated HCl, it loses its yellow color, becomes cloudy or forms a precipitate.
* **Tannins (Stiasny test)**: In a test tube, 5 ml of Stiasny reagent (30% CH2O in concentrated HCl 2/1 (v/v)) is added to an aliquot of the extract taken up in 5 ml of methanol and then heated. The formation of flakes after cooling indicates a positive reaction. The solution was then filtered and saturated with sodium nitrate (NaNO3). A few drops of FeCl3 (2%, m/v) are added to this mixture. The reaction is positive for gallic tannins when a blue-blackish color appears.
* **Quinones**: an aliquot of the crude extract taken up with ethanol is dissolved in 5 ml of 1:5 diluted HCl and heated in a boiling water bath for 30 minutes. After cooling, it is extracted with 20 ml of CHCl3. To the chloroform phase is added 0,5 ml of NH4OH diluted to 50 % (v/v). The appearance of a red to purple color indicates a positive reaction.
* **Alkaloids**: To 1 ml of the crude extract (taken up with distilled water), are added a few drops of picric acid solution, Bürchard's reagent, Dragendorff's reagent or Wagner's reagent. The appearance of intense colored precipitates indicates a positive reaction.
* **Sterols and polyterpenes**: an aliquot of the crude extract is dissolved in 1 ml of acetic anhydride (CH3CO3CH3) in a hot test tube. Then 0.5 ml of concentrated H2SO4 is slowly poured down the sides of the tube. The appearance of a purple color that turns blue and then green indicates a positive reaction.
* **Cardiotonic glycosides**: an aliquot of the crude extract taken up with ethanol is dissolved in glacial acetic acid to which is added the concentrated mixture of CH3CO3CH3 and H2SO4 in the ratio 50:1 (v/v). After a few minutes, a pink to green color develops, and blue if the test is positive.
* **Proteins**: an aliquot of the crude extract is taken up in 2 ml of 20 % (w/v) aqueous NaOH, to which 2 to 3 drops of a 2 % (w/v) aqueous solution of copper sulphate (CuSO4) are added. The appearance of a purple coloration, sometimes with a reddish tinge, indicates a positive reaction.
* **Saponosides**: their presence is detected by determining the foam index (lm), which corresponds to the degree of dilution of an aqueous extract that produces a persistent foam under given conditions. For example, if the height of the foam is less than 1 cm in all the tubes, the lm sought is less than 100. If it is 1 cm in one of the tubes, the dilution sought in that tube is the Im.
* **Oligosaccharides**: 2 tests were used to detect them;
* **Molisch test (α-naphthol test)**: Molisch reagent is prepared by dissolving 0.25 g α-naphthol in 50 ml EtOH and 50 ml 20% H2SO4. 2-3 drops of the reagent are added to 2 ml of sample. Then, 2 to 3 ml of concentrated H2SO4 are slowly added. If the reaction is positive, the addition of H2SO4 produces a purplish-red coloration in the form of a ring.
* **Seliwanoff test**: Seliwanoff's reagent is a mixture of resorcinol (0.05%) and HCl (25%). Resorcinol crystals have been dissolved in water in equal amounts with HCl. 3 ml of reagent is added to 0.5 ml of sample. The reaction mass is kept in a boiling water bath for 15 minutes. The appearance of a red color indicates a positive reaction.

***2.2.2.2 Phytochemical screening by TLC***

The identification of bioactive compounds was also performed using Thin-Layer Chromatography (TLC) following protocols described in the literature [7, 11]. For hexane extracts, a solvent system of hexane/ethyl acetate (4:1.5; v/v) was used, while CHCl₃ extracts migrated in a mixture of CHCl₃/ethyl acetate/hexane (2:3:1; v/v/v). Ethyl acetate and *n*-butanol (*n*-BuOH) extracts were respectively developed with mobile phases composed of *n*-BuOH/CHCl₃/acetic acid (4:3:0.3; v/v/v) and *n*-BuOH/CHCl₃/acetic acid (7:5:1.5; v/v/v). The TLC plates were visualized by spraying specific reagents: Liebermann-Burchard reagent (sterols, terpenoids), 5% KOH solution (coumarins), ammonia (coumarins, anthocyanins), 1% AlCl₃ (flavonoids), 2% FeCl₃ (polyphenols, tannins), sulfuric vanillin reagent (terpenoids, phenols), and Dragendorff reagent (alkaloids). Observations were made under UV light (254 and 365 nm) and visible light, using analytical standards as references.

**2.2.3 Quantitative tests**

***2.2.3.1 Determination of total phenol (TP) contents***

Total phenols were quantified by spectrophotometry using the Folin-Ciocalteu colorimetric method. For each crude extract, 0.005 g was dissolved in 10 ml of distilled water to prepare a stock solution, which was then diluted at a ratio of 1/10. From this diluted solution, 1 ml was mixed with 0.5 ml of Folin-Ciocalteu reagent (0.5 N) and 1.5 ml of sodium carbonate (Na2CO3) at 17% (w/v). The mixture was incubated in darkness for 30 minutes. Absorbance was measured at 760 nm, using distilled water as a blank. A calibration curve was established using gallic acid and quercetin at different concentrations. Results were expressed in micrograms per gram of extract as gallic acid equivalent (µg GAE/g) and quercetin equivalent (µg QE/g) [12, 13].

***2.2.3.2 Determination of total flavonoid (TF) contents***

To determine the concentration of total flavonoids, 0.005 g of each crude extract is dissolved in 10 ml of distilled water to prepare a stock solution, which is then diluted at a 1/10 ratio. To 2 ml of this diluted solution, 100 μl of Neu reagent is added. Absorbance is measured at 404 nm and compared to that of quercetol (standard at 0.05 mg/ml) treated with the same amount of reagent. The percentage of total flavonoids is then calculated as quercetol and gallic acid equivalents [14].

***2.2.3.3 Determination of hydrolyzable tannins (HT) and condensed tannins (CT) contents***

**Hydrolyzable tannins**: The method used is based on the reaction between tannins and ferric chloride (FeCl3), which results in the formation of a red-violet colored complex [15]. For this analysis, 0.005 g of each crude extract is dissolved in 10 ml of 80% ethanol. Then, 1 ml of this solution is taken, and 3.5 ml of an FeCl3 solution (0.01 M in 0.001 M HCl) is added. Absorbance is then measured at 660 nm using a UV spectrophotometer.

**Condensed tannins**: The quantification of condensed tannins (or catechins) is performed using the method described by Broadhurst & Jones [16] as well as Heilmer et al. [17]. To 400 µl of each crude extract (300 mg/ml), 3 ml of vanillin solution (4% in methanol) and 1.5 ml of concentrated HCl are added. The mixture is incubated for 15 minutes, and absorbance is measured at 500 nm. The concentrations of condensed tannins are determined from calibration curves established with catechin (0-500 µg/ml) and are expressed in micrograms of catechin equivalent per milligram (µg CE/mg).

**2.2.4 Evaluation of antioxidant potential of crude extracts**

***2.2.4.1 DPPH test***

The method applied to assess the antioxidant activity of *D. triflorum* crude extracts underwent some modifications in terms of concentrations. Nine solutions of varied concentrations (3; 2; 1.5; 1; 0.75; 0.67; 0.5; 0.375; 0.25 mg/ml) of crude extracts (D and M) and quercetin (used as antioxidant reference) were prepared in ethanol. Additionally, the ethanolic solution of DPPH (0.03 mg/ml) was prepared in a dark place. For the test, the reaction mixture consisted of 1 ml of extract and 2.5 ml of DPPH solution, placed in the spectrophotometer cell. The absorbance of the mixture was measured at 517 nm using a UV-visible spectrophotometer every 30 seconds over a period of 1800 seconds, with a blank prepared under the same conditions as the extract but without DPPH [18, 19].

***2.2.4.2 FRAP (Ferric Reducing Antioxidant Power) test***

The reducing capacity of the crude extracts was assessed using the FRAP method, which is based on the reduction of Fe³⁺ cation to Fe²⁺ by antioxidant compounds. The TPTZ-Fe³⁺ complex added to the sample is reduced to TPTZ-Fe²⁺, which displays a blue color, and its absorbance is measured at 593 nm. Trolox is used as a positive control, and its absorbance is measured under the same conditions as the samples. The FRAP reagent used for the analysis is a mixture of three solutions: sodium acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM prepared in a 40 mM HCl solution), and FeCl₃ solution (20 mM), in a volumetric ratio of 10:1:1. This reagent, freshly prepared, is heated to 37°C in a water bath. Then, 100 µl of Trolox (at concentrations of 0.187 mM; 0.375 mM; 0.75 mM; and 1.5 mM) and samples at various concentrations (from 0.3125 to 5 mg/ml) are added to 3 ml of FRAP reagent. The absorbance of the complex (TPTZ-Fe³⁺) is then measured at 593 nm after 4 minutes. A standard curve is established from calibration with Trolox, and results are expressed in mM Trolox equivalents [20, 21].

**2.2.5 Evaluation of anthelmintic activity**

The anthelmintic activity of crude extracts was evaluated on ***Lumbricus terrestris*** (earthworms), used as a biological model, collected on the campus of Nangui ABROGOUA University (Abidjan, Côte d’Ivoire). The experimental protocol, adapted from Guissou et al. [22], involved three groups:

* **Control group** (n = 6 worms) treated with 10 ml of distilled water.
* **Treated group**: exposed to three concentrations of extracts (100, 20, and 15 mg/ml), with 6 worms per concentration.
* **Reference group** (n = 6 worms) treated with albendazole (reference drug [23]) at concentrations of 20 and 15 mg/ml.

The earthworms were placed in Petri dishes containing 10 ml of test solution, maintained at 25 ± 1 °C. Mortality was monitored every 2 hours for 24 hours. The lethal time 100% (the time required to induce total worm mortality) was determined for each condition.

3. results and discussion

**3.1 Extraction yields**

The extraction yields of 5 g of plant powder obtained were 12.4% for maceration and 7.6% for microwave extraction. The extraction yield of phytocompounds depends on several parameters: solvent(s), pH, temperature, extraction time and the nature and composition of the sample [24]. Water soluble substances are present in good proportions when cold (maceration). This is thought to be one of the reasons for the increase in yield associated with the water extraction method [7]. Extraction time also has an effect, being shorter for microwave extraction (3 min) than for maceration (2 h). Water increases the permeability of plant tissues and favours mass diffusion during the extraction phase [25].

**3.2 Qualitative phytochemical profile**

**3.2.1 Phytochemical profile using detection tests**

Based on the identification of phytoconstituents using appropriate reagents, the detection tests were able to identify a number of phytocompound families by the appearance of specific colours visible to the naked eye. The results of the detection tests are summarised below (Table 1). Six families of metabolites were detected in *D. triflorum* crude extracts: polyphenols, flavonoids, coumarins, tannins, proteins and oligosaccharides. Sterols and polyterpenes are present in the macerate and absent in the decoction. This may be due to the heat sensitivity of sterols and polyterpenes. Saponins are present in the decoction (Fi = 200), as a sample contains saponins when Fi is greater than or equal to 100. Saponosides have haemolytic, antimicrobial and anti-inflammatory activities and are known for their cytotoxic properties [26].

The presence of all these phytocompounds in *D. triflorum* extracts would justify the pharmacological properties attributed to it in non-conventional medicine. Indeed, according to the work of Bhosle [27], the anticonvulsant activity of the species could be attributed to the presence of alkaloids, steroids, flavonoids, tannins and saponins in the aqueous extract. Studies have also shown that alkaloids and flavonoids possess numerous biological properties, including analgesic and anti-inflammatory activities [28] atributed to the species. However, we note the absence of quinones, alkaloids and cardiotonic glycosides. These results are in agreement with previous work carried out on the aqueous extract of *D. triflorum* (Taiwan) which also showed the presence of polyphenols, flavonoids, sterols, triterpenes, reducing sugars, alkaloids, proteins, steroids, phytosterols, saponins and tannins [29 ; 27]. These identified phytoconstituents have a variety of biological properties, which may explain the use of *D. triflorum* in non-conventional medicine for the treatment of various pathologies [30].

Table 1. Phytochemical composition of *D. triflorum* crude extracts by detection tests

|  |  |  |  |
| --- | --- | --- | --- |
| **Phytoconstituents / Tests** | | **Decoction (D)** | **Macerate (M)** |
| Phenolic compounds | FeCl3 | + | + |
| Flavonoids | Shinoda (Mg) / HCl | + | + |
| Pew (Zn) | + | + |
| NH4OH | + | + |
| Vanillin | + | + |
| Coumarins | HCl | + | + |
| Tannins | Stiasny | + | + |
| Quinones | Borntraëger | - | - |
| Alkaloids | Bürchardat | - | - |
| Dragendorff | - | - |
| Wagner | - | - |
| Picric acid | - | - |
| Sterols & polyterpenes | H2SO4 | - | + |
| Saponosides | Fi | + ( 200) | |
| Cardiotonic glycosides | Liebermann-Burchard | - | - |
| Proteins | Biuret | + | + |
| Oligosaccharides (oses, diholosides) | Molish | + | + |
| Seliwanoff (ketoses) | - | - |

(+): positive ; (-): negative ; Fi: Foam index

**3.2.2 Phytochemical composition by TLC**

**The results obtained through TLC (Table 2) highlight an in-depth analysis of the chemical composition of the studied extracts. They reveal several key points:**

* **Diversity of phytocompounds**: the identification of sterols, terpenes, lupane-type triterpenes, coumarins, flavonoids, tannins, and anthocyanins reflects significant phytochemical richness. These compounds often play various biological roles, ranging from antioxidant activity to medicinal effects [31, 32].
* **Absence of detected alkaloids**: although the use of Dragendorff’s reagent allowed the visualization of orange molecular fingerprints characteristic of alkaloids, none were detected in the analyzed extracts [33]. This contrasts with previous studies conducted on a Taiwanese species, where alkaloids were identified in methanolic and n-butanolic extracts that also contained flavonoids [34]. This absence could be attributed to the specific pedoclimatic conditions of Côte d'Ivoire, which influence the biosynthesis of secondary metabolites.
* **Hypothesis of a potential presence:** Chemical research indicating the presence of indole-3-alkylamine-type alkaloids in other parts of the plant (leaves and roots) suggests that their absence in these extracts might be linked to the methodologies or plant parts selected for the analysis [35]. A targeted extraction would therefore be essential to confirm their potential presence.

**These results highlight the importance of environmental and methodological factors in the expression of secondary metabolites. They also underline the need for more focused exploration to better understand the specific chemistry of this species in Côte d'Ivoire. This could pave the way for broader comparative studies and potential pharmacological applications.**

Table 2. Phytochemical composition of *D. triflorum* selective extracts by TLC

|  |  |  |
| --- | --- | --- |
| **Selective extracts** | | **Secondary metabolites identified : [Rf], color** |
| **C6H14** | **D1** | **Sterolse :** [0.14] Ge ; [0.20] Ge ; [0.26] Ge ; [0.38] Ge ; [0.45] Ge ; [0.64] Ge ; [0.70] Ge ; [0.75] Ge ; [0.85] Ge  **Terpenesf :** [0.93] Pf;  **Triterpènes lupanee** : [0.58] Oe ; [0.93] Oe;  **NI :** [0.15] Gre ; [0.30] Be ; [0.51] Be |
| **M1** | **Sterolse :** [0.06] Ge ; [0.23] Ge ; [0.28] Ge ; [0.45] Ge ; [0.51] Ge ; [0.65] Ge ; [0.70] Ge ; [0.75] Ge ;  **Terpenesf :** [0.75] Pf; [0.93] Pf;  **Triterpènes lupanee :** [0.59] Oe ; [0.93] Oe;  **NI :** [0.33] Be ; [0.40] Be ; [0.85] Be ; |
| **CHCl3** | **D2** | **Coumarinsa:** [0.12] Ba ; [0.16] Ba ; [0.21] Ba ; [0.29] Ba ; [0.37] Ba ; [0.47] Bfla ; [0.57] Bfla ; [0.82] Bfla ; [0.93] Ba  **Flavonoidsb:** [0.12] Pb ; [0.16] Gb ; [0.21] Gb ; [0.29] Gb ; [0.37] Gb ; [0.47] Gb ; [0.50] Gb ; [0.57] Gb ; [0.62] Gb ; [0.67] Gb ; [0.78] Gb;  **Taninsc:** [0.69] Grc |
| **M2** | **Coumarinsa:** [0.12] Ba ; [0.18] Ba ; [0.25] Ba ; [0.30] Ba ; [0.39] Ba ; [0.47] Bfla ; [0.57] Bfla ; [0.59] Ba ; [0.67] Ba ; [0.88] Ba ;  **Flavonoidsb:** [0.12] Gb ; [0.18] Gb ; [0.25] Gb ; [0.30] Rb ; [0.39] Gb ; [0.47] Gb ; [0.49] Gb ; [0.57] Bflb ; [0.67] Gb ;  **NI :** [0.92] Ra-Rb |
| **AcOEt** | **D3** | **Coumarinsag:** [0.16] Bra ; [0.20] Brg ; [0.26] Bra ; [0.33] Brg ; [0.36] Bra ; [0.49] Bra ; [0.61] Brg ; [0.64] Ba ; [0.75] Brg ; [0.81] Va ; [0.91] Bfla ; [0.98] Yg  **Flavonoidsb:** [0.18] Yb ; [0.34] Yb ; [0.45] Yb ; [0.64] Bflb ; [0.76] Pb  **Tanninsc:** [0.21] Grc ; [0.31] Grc ; [0.44] Grc ;  **Anthocyaninsg :** [0.69] Pg ; [0.86] Gg ; [0,94] Bflg |
| **M3** | **Coumarinsag:** [0.16] Ya ; [0.26] Bra ; [0.33] Brg ; [0.35] Bra ; [0.49] Bra ; [0.59] Brg ; [0.68] Brg ; [0.71] Brg ; [0.86] Brg ; [0.98] Yg  **Flavonoidsb:** [0.18] Yb ; [0.28] Yb ; [0.33] Yc ; [0.34] Yc ; [0.45] Yc ; [0.63] Yc ; [0.78] Pc  **Tanninsc:** [0.14] Grc ; [0.22] Grc |
| **n-BuOH** | **D4** | **Coumarinsag :** [0.28] Bra ; [0.31] Brg ; [0.39] Bra ; [0.43] Brg ; [0.59] Bra ; [0.61] Brg ; [0.88] Ga  **Flavonoidsc :** [0.28] Yc ; [0.39] Yc ; [0.59] Yc |
| **M4** | **Coumarinsag :** [0.28] Bra ; [0.31] Brg ; [0.39] Bra ; [0.44] Brg ; [0.59] Bra ; [0.63] Brg ; [0.65] Bra ; [0.71] Brg  **Flavonoidsc :** [0.28] Yc ; [0.39] Yc ; [0.59] Yc ; [0.65] Yc ; [0.89] Gc |

a : KOH ; b : AlCl3 : c : FeCl3 ; e : Liebermann-Bürchard ; f : sulfuric vanillin ; g : ammoniac ; O : orange, B : blue, Y : yellow, Bfl : fluorescent blue, Br : brown, P : purple, G : green, R : red, Gr : gray

**3.3 Quantitative phytochemical profile**

**3.3.1 TP and TF contents**

The concentrations of total phenols in the crude extracts were quantified using calibration equations established with gallic acid standards (y = 0.0196x - 0.0149; R² = 0.999) and quercetin standards (y = 0.0072x + 0.0037; R² = 0.9997). The results are expressed as micrograms equivalent of gallic acid (μg GAE/g) and quercetin per gram of extract (μg QE/g) (Figures 1). The concentrations of total phenols (gallic acid and quercetin) vary, being higher in the decoction (37214.29 µg GAE/g sample and 85805.56 µg QE/g sample) compared to those obtained using the maceration method. These values are consistent with those reported for a species in Taiwan, where the crude methanolic extract showed 36600 µg catechin E/g sample and the aqueous extract 22800 µg catechin E/g sample [34]. Furthermore, microwave extraction resulted in a slight increase in total phenol content compared to the maceration technique, a finding also corroborated by previous studies on olive leaves, which revealed higher total phenol concentrations when extraction was microwave-assisted [36]. Overall, the results suggest that the extraction method plays a decisive role in the chemical composition of the extracts. This opens avenues for optimizing extraction techniques based on research objectives or industrial applications.

**Figures 1: Total phenol and total flavonoids contents**

**3.3.2 HT and CT contents**

The obtained data, presented in the form of histograms (Figure 2), show that both HT (hydrolyzable tannins) and CT (condensed tannins) are effectively extracted by both methods. The values for HT and CT do not show differences, indicating a comparable efficiency of the maceration and decoction methods. However, the levels of CT are consistently higher than those of HT, suggesting a predominance of condensed tannins in the crude extracts of *D. triflorum*. The increased presence of CT compared to HT may be related to the chemical structure and solubility of the tannins in the solvents used. These results are consistent with previous observations from phytochemical screenings that confirmed the richness in tannins of this species. The absence of differences between the extraction methods highlights the effectiveness of these techniques in extracting bioactive compounds. The results of this study underscore the richness in tannins of *D. triflorum*, with a predominance of condensed tannins. These compounds may contribute to the pharmacological properties of the plant, and their effective extraction by the methods used could be leveraged in therapeutic applications. Further studies on the biochemical and pharmacological activity of *D. triflorum* crude extracts are recommended to better understand the potential of this plant.

**Figure 2: Hydrolyzable and condensed tannins contents**

**3.4 Antioxidant profile**

**3.4.1 DPPH antioxidant potential**

The assessment of antioxidant activity using the DPPH radical method revealed significant variations in the percentage of reduction (PR) of *D. triflorum* crude extracts, depending on concentration and incubation time. The results obtained, with PR values of 83.36% for the macerate and 82.09% for the decoction at a concentration of 3 mg/ml after 1800 seconds, indicate a notable antioxidant potential of these extracts. It is important to note that, although *D. triflorum* crude extracts show valid antioxidant activity, their PR remains lower than that of quercetin, a flavonoid well-known for its antioxidant properties (85.42% at 0.1 mg/ml). This may indicate that, while *D. triflorum* crude extracts contains antioxidant compounds, their effectiveness is lesser compared to a recognized standard like quercetin. This raises the possibility that the active compounds in *D. triflorum* may require higher concentrations or specific synergies to achieve comparable levels of effectiveness. The slight decrease in PR observed in the decoction compared to the macerate may be attributed to the degradation or loss of thermosensitive phytocompounds during the boiling process. This underscores the importance of the extraction method in preserving the bioactive properties of plants. Macerations, which involve infusing plants in a solvent at room temperature, may better preserve certain thermosensitive compounds, while decoctions may extract other compounds, but at the potential cost of degradation. The efficiency index (EI) is a relevant indicator for assessing antioxidant activity. In this case, the results show that the EIs of *D. triflorum* crude extracts are higher than those of quercetin, which could indicate a relatively stronger effectiveness of *D. triflorum* crude extracts in relation to their concentration. However, the observed trend, with lower EIs for the macerate compared to the decoction, raises questions about the mechanisms of action of the compounds present in these extracts. This could also suggest that different mechanisms of action or types of antioxidant compounds are involved in the two extraction methods. Thus, the results highlight the antioxidant potential of the aqueous raw extracts of *D. triflorum* while emphasizing the importance of the extraction method on the preservation and efficacy of bioactive compounds.

**Figure 3: Evolution of the percentages of reduction as a function of time and of the efficacy indices for crude extracts of *D. triflorum* and quercetin**

**3.4.2 FRAP antioxidant potential**

The results obtained from the antioxidant activity measured by the FRAP test (Figure 4) indicate that the decoction has a higher reducing capacity than the macerate. In contrast, data from the DPPH test reveal that the macerate exhibits a greater antioxidant efficiency than the decoction. This divergence can be attributed to the different reduction mechanisms involved in these tests. Indeed, the DPPH test relies on a hydrogen transfer mechanism, while the FRAP test is based on an electron transfer mechanism [36]. It is interesting to note that the results from the FRAP and DPPH tests may sometimes seem contradictory, but they highlight the complexity of the antioxidant compounds present in plant extracts. In the case of the FRAP test, which evaluates the total reducing capacity of the samples, the decoction, through its extraction methods, may have released compounds with a high capacity to donate electrons, which explains its superior performance in this test. On the other hand, the DPPH test specifically measures the capacity of antioxidants to donate hydrogens to neutralize the DPPH free radical. The macerate, likely containing compounds that act more effectively as hydrogen donors, thus shows better efficiency in this test. These results underscore the importance of choosing the appropriate test for evaluating antioxidant capacities based on the action mechanisms of the present compounds. This also means that a combined approach using multiple tests could provide a more comprehensive assessment of the antioxidant activity of plant extracts.

**Figure 4: FRAP antioxidant profile for crude extracts of *D. triflorum***

**3.5 Anthelmintic activity**

The studied crude extracts exhibit anthelmintic activity, although this is less pronounced than that of albendazole (Figure 5). Indeed, albendazole caused death of all 6 earthworms after 14 minutes at 15 mg/ml and 8 minutes at 20 mg/ml. For the lowest concentration of 15 mg/ml (M15 and D15), the time required to induce lethality is prolonged, reaching 600 minutes for the macerated extracts and 960 minutes for the decocted ones. These results are consistent with data reported in previous studies [37, 23]. Research has shown that flavonoids and tannins play a significant role in anthelmintic activity. Therefore, the anthelmintic effect observed in the crude extracts of *D. triflorum* may be attributed to the presence of flavonoids and/or condensed tannins. In fact, insect larvae and nematodes can ingest condensed tannins, which bind to the intestinal mucosa and lead to autolysis of the developing larvae. Additionally, these tannins can attach to the cuticle of the larvae, which is rich in glycoproteins, thereby causing their death [38].

**Figure 5: Anthelmintic activity of *D. triflorum* crude extracts compared with albendazole**

4. Conclusion

This study highlights the phytochemical, antioxidant, and anthelmintic properties of *D. triflorum,* a plant from the Fabaceae family, native to Côte d'Ivoire. The results of this preliminary study, based on extracts obtained through maceration and microwave-assisted extraction, reveal the presence of various beneficial phytocompounds. The analyses conducted, including *in vitro* tests and thin-layer chromatography (TLC), identified different groups of active compounds in the extracts. Hexane extracts are notable for their richness in sterols and polyterpenes, while chloroformic and *n*-butanolic extracts are particularly rich in coumarins and flavonoids. Ethyl acetate extracts also contain coumarins, flavonoids, and tannins. The antioxidant properties of the extracts were assessed using the DPPH and FRAP methods, showing significant activity. Regarding anthelmintic activity, while the extracts demonstrated effectiveness, this was inferior to that of albendazole, a reference drug. Interestingly, the anthelmintic activity of *D. triflorum* decoction is more pronounced than that of the macerate. *D. triflorum* could be considered a traditional source of natural antioxidants and vermifuges, offering potential for medicinal and therapeutic applications in Côte d'Ivoire. This work paves the way for further research into the phytotherapeutic applications of this species.

**Disclaimer (Artificial intelligence)**

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

1. Pilly E. Infectious and tropical diseases. Edition, APPIT. Montmorency, France. (Translated from french). 2001; 654.
2. Mali GR, Mehta AA. A review of anthelmintic plants. Natural Product Radiance. 2008; 7(5): 466-475.
3. WHO. Schistosomiasis: population in need of chemoprevention and number of people treated in 2010. Weekly epidemiological record. 2012; 87(4): 37-44.
4. Waller PJ. From discovery to development: current industry perspectives for the development of novel methods of helminth control in livestock. Veterinary Parasitology. 2006; 139(1-3): 1-14.
5. Koffi YM, Kossonou YK, Kouamé AG, Kouadio NJ, Bakayoko A, Tra Bi FH, Koné MW. In vitro anthelmintic activity and tannin and flavonoid content of eight forage plants used for raising small ruminants in Côte d'Ivoire. (Translated from french). European Scientific Journal. 2018; 14(15): 434-449.
6. Ladiguina EY, Safronitch LN, Otriachenkova VE, Balandina IA, Grinkevitch NI. Chemical analysis of medicinal plants. Edition Moskva, Vischaya Chkola. Moscow (Russia). (Translated from russian). 1983; 347.
7. Békro YA, Mamyrbekova-Békro JA, Boua BB, Tra Bi FH, Éhilé E. Ethnobotanical study and phytochemical screening of Caesalpinia benthamiana (baill.) herend et zarucchi (caesalpiniaceae). (Translated from french). Sciences & nature. 2007; 4(2): 217-225.
8. Bruneton J. Pharmacognosy, Phytochemistry, Medicinal plants. 5th edition, Lavoisier Tec. & Doc. Paris (France). (Translated from french). 2016; 1487.
9. N’Gaman KCC. Phytochemical study and effect of extracts of Gmelina arborea Roxb. (Verbenaceae) from Ivory Coast on the osmotic stability of erythrocytes. PhD thesis. University of Abobo-Adjamé, Abidjan (Côte d’Ivoire). (Translated from french). 2013; 112.
10. Shashank B, Suresh D. Preliminary phytochemical screening of Ailanthus excelsa roxb.International Journal ofCurrent Pharmaceutical Research. 2012; 4(1): 87-89.
11. Brou KG, Mamyrbekova-Békro JA, Dogbo DO, Gogbeu SJ, Békro YA. On the Qualitative Phytochemical Composition of Crude Hydromethanolic extracts of the Leaves of 6 Varieties of Manihot esculenta Crantz of Ivory Coast. European Journal of Scientific Research. 2010; 45(2): 200-211.
12. Singleton VL, Ortofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Packer L (ed). Methods in enzymology Orlando Academic Press. 1999; 299: 152-178.
13. Heilerová L, Bućkova M, Tarapćik P, Silhár S, Labuda J. Comparison of antioxydative activity data for aqueous extracts of Lemon balm (Melissa officinalis L.), Oregano (Origanum vulgare L.), Thyme (Thymus vulgaris L.), and Agrimony (Agrimonia eupatoria L.) obtained by conventional methods and the DNA-based biosensor. Czech Journal Food Science. 2003; 21(2): 78-84.
14. Hariri EB, Sallé G, Andary C. Involvement of flavonoids in the resistance of two poplar cultivars to mistletoe (Viscum album L.). Protoplasma. 1991; 162(1): 20-26.
15. Dif MM, Benchiha H, Mehdadi Z, Benali-Toumi F, Benyahia M, Bouterfas K. Quantitative study of polyphenols in the various organs of Papaver rhoeas L. Phytotherapy. (Translated from french). 2015; 13(5): 324-319.
16. Broadhurst RB, Jones WT. Analysis of condensed tannins using acidified vanillin. Journal of the science of food and agriculture. 1978; 29(9): 788-794.
17. Heilmer D, Vigndini P, Dini MG, Vincieri FF, Romani A. Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food chemistry. 2006; 99(3): 464-469.
18. Savatovic SM, Ćetkovic GS, Čanadonovic-Brunet JM, Djilas SM. Kinetic behaviour of the DPPH radical-scavenging activity of tomato waste extracts. Journal of the Serbian Chemical Society. 2012, 77(10): 1381-1389.
19. Blois MS. Antioxidant determination by the use of stable free radical. Nature. 1958; 181: 1199-1200.
20. Benzie IFF, Strain J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of Antioxidant Power: The FRAP Assay. Analytical biochemistry. 1996; 239(1): 70-76.
21. Gong WL, Yan J, Zhao LX, Li C, Huang ZL, Tanq BZ, Zhu MQ. Single-wavelength-controlled in situ dynamic super-resolution fluorescence imaging for block copolymer nanostructures via blue-light-switchable FRAP. Photochemical and photobiological Sciences. 2016; 15(11): 1433-1441.
22. Guissou LP, Ouedraogo S, Sanfo A, Some N, Lompo M. Development of a biological model for parasite testing applied to medicinal plants. Pharmacopoeia and Traditional African Medicine. (Translatef from french). 1988; 10: 105-133.
23. Gavalapu VR, Kolli P, Korra SK, Kavuri MK, Avagadda C, Singam V, Vanumu Y, Kudirella H. Preliminary Phytochemical Screening and Anthelmintic Activity of Desmodium triflorum (L.) DC Leaf and Root Extracts. International Journal of Pharma Sciences. 2013; 3(1): 156-158.
24. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH. Effect of extraction solvent on total phenol content, total ﬂavonoids content, and antioxidant activity of Limnophila aromatica. Journal of Food and Drug Analysis Research article. 2013; 22(3): 1-7.
25. Arimboor R, Arumughan C. Sea buckthorn (Hippophae rhamnoides*)* proanthocyanidins inhibit in vitro enzymatic hydrolysis of protein. Journal of Food Science*.* 2011; 76(6): 130-137.
26. Sparg GS, Light ME, Van staden J. Biological activities and distridution of plant saponine. Journal of Ethnopharmacology. 2004; 94(2-3): 219-243.
27. Bhosle V. Anticonvulsant and antioxidant activity of aqueous leaves extract of Desmodium triﬂorumin mice against pentylenetetrazole and maximal electroshock induced convulsion. Brazilian Journal of Pharmacognosy. 2013; 23(4): 692-698.
28. Govindarajan R., Vijayakumar M., Rao V. C., Shirwaikar A., Kumar S., Rawat A. K. S. and Push-pangadan P. Anti-inflammatory and antioxidant activities of Desmodium gangeticum fractions in carrageenan-induced inflamed rats. Phytotherapy Research. 2007; 21(10) :975–979.
29. Belloni J. Photography: enhancing sensitivity by silver crystal doping. Radiation Physics and Chemistry. 2003; 67(3-4): 291-296.
30. Kumar S, Singhal V, Roshan R, Sharma A, Rembhotkar GW, Ghosh B. Piperine inhibits TNF-α induced adhesion of neutrophils to endothelial monolayer through suppression of NF-K and IKB kinase activation. European Journal of Pharmacology.2007; 575(1-3): 177-186.
31. Kabran G. Phytochemical screening and determination of total flavonoids of Secamone afzelii (Asclepiadaceae) leaf extracts. Master's thesis in Fundamental and Applied Sciences. University of Abobo-Adjamé, Abidjan (Côte d’Ivoire). (Translated from french). 2007; 33.
32. Boua BB. Study of the phytochemistry and bioactive potential on erectile dysfunction of four medicinal plants from Côte d’Ivoire, PhD thesis. University of Abobo-Adjamé, Abidjan (Côte d’Ivoire) (Translated from french). 2008; 225.
33. Lagnika L. Phytochemical study and biological activity of natural substances isolated from Beninese plants. PhD thesis. University of Louis Pasteur, Strasbourg (France) /University of Abomey, Calavi (Benin). (Translated from french). 2005; 280.
34. Lai SC, Ho YL, Huang SC, Huang TH, Lai ZR, Wu C, Lian KY, Chang YS. Antioxidant and Antiproliferative Activities of Desmodium triflorum(L.) DC. The American Journal of Chinese Medecine, 2010; 38(2): 329-342.
35. Ghosal S, Srivastava RS, Bhattacharya SK, Debnath PK. Desmodium alkaloids Chemical and pharmacological evaluation of D. triflorum. Planta Medica. 1973; 23(4): 321-329.
36. Apak R, Gorinstein S, Böhm V, Schaich MK, Özyürek M, Güçlü K. Methods of measurement and evaluation of natural antioxidant capacity/activity. Pure and Applied Chemistry. 2013; 85(5): 957-998.
37. Rajith NP, Ramachandran VS. Ethnomedecines of Kurichyas, Kannur district, Western Ghats, Kerala. Indian Journal of Natural Products and Resources. 2010; 1(2): 249-253.
38. Athanasiadou S, Kyriazakis I, Jackson F, Coop RL. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. Veterinary Parasitology. 2001; 99: 205-219.