



Research Article

Phytochemical Composition, Evaluation of Physicochemical and Antifungal Properties of a Syrup Formulated with *Jatropha Curcas* L. (*Euphorbiaceae*) Extracts

Composition Phytochimique, Évaluation des Propriétés Physicochimiques et Antifongiques d'un Sirop Formulé à Base d'Extraits de Jatropha curcas L. (Euphorbiaceae)

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ABSTRACT

Introduction. The widespread use of medicinal plants in the treatment of various pathologies, particularly infectious and oxidative diseases, is justified by their pharmacological properties and low toxicity. Thus, *Jatropha curcas* leaves are known for their antimicrobial properties and are used in the traditional treatment of infectious diseases, notably fungal infections. **Methodology.** *Jatropha curcas* leaves collected in the Central region, Lekie Department, Obala District (Cameroon) was used for preparation of aqueous and hydro-ethanolic extracts using maceration method. Phytochemical screening was carried out on the both extracts to identify the presence of secondary metabolites. Subsequently, a spectrophotometric assay of total polyphenols and flavonoids was carried out, and antioxidant activity was determined by the scavenging of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. The micro-dilution assay was used to evaluate the antifungal activity of hydro-ethanolic extract and formulated syrup against *Candida albicans* strain. **Results.** The study of *Jatropha curcas* leaves demonstrated an optimal extraction yield of 38% for the aqueous extract, identifying key bioactive compounds such as polyphenols, flavonoids, and alkaloids. These hydro-ethanolic extracts exhibited remarkable pharmacological potency, with an antioxidant activity (IC₅₀) of 1.135 µg/ml and an antifungal efficacy against *Candida albicans* that was four times greater than fluconazole. The resulting syrup formulation was characterized by a Brix value of 65 and a pH of 8, aligning strictly with CODEX STAN 247 standards. Furthermore, microbiological analysis confirmed a high safety profile with the total absence of pathogens, including *E. coli*, *S. aureus*, and molds. **Conclusion.** *Jatropha curcas* show strong antifungal potential, particularly against *Candida albicans*, and could be useful against fungal infections and gastroenteritis.

RÉSUMÉ

Introduction. L'utilisation répandue des plantes médicinales dans le traitement de diverses pathologies, notamment infectieuses et oxydatives, se justifie par leurs propriétés pharmacologiques et leur faible toxicité. Ainsi, les feuilles de *Jatropha curcas* sont reconnues pour leurs propriétés antimicrobiennes et sont utilisées dans le traitement traditionnel des maladies infectieuses, en particulier les mycoses. **Méthodologie.** Des feuilles de *Jatropha curcas*, récoltées dans la région centrale, département de Lekie, district d'Obala (Cameroun), ont servi à la préparation d'extraits aqueux et hydroéthanoliques par macération. Un criblage phytochimique a été réalisé sur les deux extraits afin d'identifier la présence de métabolites secondaires. Un dosage spectrophotométrique des polyphénols totaux et des flavonoïdes a ensuite été effectué, et l'activité antioxydante a été déterminée par piégeage du radical stable DPPH (2,2-diphényl-1-picrylhydrazyle). Un test de microdilution a été utilisé pour évaluer l'activité antifongique de l'extrait hydroéthanolique et du sirop formulé contre une souche de *Candida albicans*. **Résultats.** L'étude des feuilles de *Jatropha curcas* révèle un rendement d'extraction optimal de 38 % en phase aqueuse et la présence de composés bioactifs majeurs (polyphénols, flavonoïdes, alcaloïdes). Les extraits hydro-éthanoliques présentent une activité antioxydante remarquable (IC₅₀ de 1,135 µg/ml) et une efficacité antifongique contre *Candida albicans* quatre fois supérieure au fluconazole. Le sirop formulé affiche des paramètres physico-chimiques conformes aux normes CODEX STAN 247 (pH 8, Brix 65) et une stabilité microbiologique totale, garantissant l'absence de germes pathogènes. **Conclusion.** Le sirop à base de *Jatropha curcas* présente un fort potentiel antifongique, notamment contre *Candida albicans*, et pourrait être utile contre les infections fongiques et les gastro-entérites.

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HIGHLIGHTS FOR READERS IN A HURRY

What is known about the subject. The medicinal use of *Jatropha curcas* leaves is justified by their low toxicity and proven pharmacological properties, particularly their traditional efficacy against infectious and fungal diseases.

What question this study addressed. It evaluates the phytochemical composition, physicochemical stability, and antifungal properties of a syrup formulated from *Jatropha curcas* L. (*Euphorbiaceae*) leaf extracts.

What this study adds to our knowledge. This study demonstrates that the *Jatropha curcas* leaf syrup combines potent antioxidant and antifungal efficacy (four times greater than fluconazole) with physicochemical stability and microbiological safety compliant with CODEX STAN 247 standards.

Implication for practice. *Jatropha curcas* show strong antifungal potential, particularly against *Candida albicans*, and could be useful against fungal infections and gastroenteritis.

INTRODUCTION

Fungal diseases are on the rise worldwide, but attention has tended to focus primarily on benign conditions, such as nail or scalp fungal infections; these infections cause the deaths of more than 1.5 million people each year. The incidence of fungal infections has risen dramatically in recent decades, with candidiasis being the most common fungal infection in humans; its prevalence in Africa ranges from 33% to 47% of opportunistic infections [1–4]. Fungal infections of the glabrous skin are among the most common dermatological conditions and gastroenteritis. Worldwide, their prevalence is estimated at between 20% and 25% depending on the region, representing a public health problem.

Conventional treatment of fungal infections relies on the use of molecules belonging to four families. These act via three cellular mechanisms: polyenes and azole derivatives, which act on ergosterol; echinocandins, which inhibit fungal cell wall synthesis; and fluorocytosines, pyrimidine base analogues that inhibit fungal growth by disrupting protein synthesis and DNA replication. Unfortunately, the intensive use of these molecules has led to an increase in the incidence of resistance to antifungals such as amphotericin B; resistance to this molecule remains exceptional and is linked to the recognition of the molecule by membrane ergosterol receptors. Resistance to 5-fluorocytosine can develop rapidly and may be due to a failure of intracellular penetration or a failure to convert to 5-fluorouracil, which is the active molecule. Finally, resistance to miconazole and itraconazole is due to a mutation in the gene encoding the enzyme responsible for ergosterol synthesis. Furthermore, synthetic drugs are expensive and have side effects associated with their use, such as teratogenic and iatrogenic effects [4–5].

People have always turned to traditional medicine for treatment, as herbal remedies cause fewer side effects; Enhanced Traditional Medicines (ETMS) therefore represent a vital alternative in terms of healthcare costs and accessibility for most African countries. Consequently, Cameroon's flora is rich in a wide variety

of plants, many of which are used in traditional medicine for their antifungal properties, such as *Ageratum conyzoides*, *Bidens pilosa*, *Callistemon*, *Citrinus*, *Cymbopogon citatus*, *Erigeron floribundus*, *Ocimum gratissimum*, *Tephrosia vogelii* and *Jatropha curcas* [5]. This is because these plants are rich in secondary metabolites responsible for their activity.

It is against this backdrop that this study, which focuses on the formulation of an improved traditional medicine (an antifungal syrup) for the treatment of fungal infections, takes place. The choice of plant was motivated by its medicinal potential. Indeed, *Jatropha curcas* is known in traditional medicine for its antimicrobial effects, and is widely used in the treatment and prevention of various infectious diseases, particularly fungal infections. Furthermore, the literature review reveals very few studies on the antifungal activity of *Jatropha curcas* leaves.

METHODOLOGY**Plant material**

For this experiment, the plant material consisted of *J. curcas* leaves collected in the Central region of the Lekie department, Obala district; they were hand-picked early in the day at around 10 am in the presence of a botanist during the month of April, and then transported to the Genemark laboratory (4°05'56.914" north latitude and 9°68'49.649" east longitude) where they were dried away from light for seven days.

The plant was identified at the National Herbarium of Cameroon as *J. curcas*, compared to specimen No. 33592 SRF/Cam.



Figure1. *Jatropha curcas*

Microbiological Material

The study was conducted on a fungal strain composed of *C. albicans*. This strain is frequently implicated in various infections and poses problems due to resistance to conventional antifungals. Clinical isolates of this strain were provided by the Douala General Hospital (DGH) in the Littoral region, Wouri department, Douala 5th district, and were preserved in nutrient broth at 0°C. Fluconazole was used as the reference antifungal; dimethyl sulfoxide (DMSO) was used as a negative control; and Sabouraud chloramphenicol culture medium was used as the specific culture medium.

Extraction

The drug, having already been pulverised, will undergo the preparation of extracts. The method used for extraction is maceration. This involves leaving a substance, from which soluble constituents are to be extracted, to stand in a solvent at room temperature. Two types of extract will be prepared: a hydro-ethanolic extract and an aqueous extract.

Solvents	Description	Types of extracts
95% ethanol	100 g of <i>J. curcas</i> powder macerated in 1 L of 95% ethanol for 72 hours Filtration through Whatman paper	Ethanolic extract
Distilled water	100g of <i>J. curcas</i> powder macerated in 1L of water for 72 hours Filtration through Whatman paper	Aqueous extract

Hydro-ethanolic maceration: Weigh 100 g of *J. curcas* leaf powder using a balance. Place this quantity of powder into an Erlenmeyer flask, then add 1 L of 95% ethanol. Leave to macerate for three days; the resulting homogenates are then filtered through cotton wool, followed by Wattman filter paper. The filtrates are then concentrated to dryness using a rotary evaporator under pressure at a temperature of 50°C. The resulting concentrate is placed in an oven at 37°C to obtain the crude extract [6].

Maceration in distilled water: Weigh out 100 g of *J. curcas* leaf powder using a balance. Place this quantity of powder in a container, then add 1 L of distilled water. Seal the container and leave to macerate for three days at room temperature under magnetic stirring. The resulting homogenates are filtered through cotton wool, then through Wattman filter paper. The filtrates are then concentrated to dryness using a rotary vacuum evaporator at 55°C to reduce the volume. The resulting concentrate is placed in an oven at 37°C to obtain the crude extract [6].

• Extraction yield

The yield is the quantity of extract obtained from the plant powder. It is expressed as a percentage using the following formula:

$$Rd = (m \times 100) / M.$$

Rd: extraction yield as a percentage,

m: mass in grams of the dry extract,

M: mass in grams of the drug powder

Qualitative analysis: phytochemical screening

Phytochemical screening was carried out to identify the main classes of compounds present in the crude extract. The following tests were therefore performed: the Liebermann-Burchard test for the identification of sterols and terpenes [6–8]. 1 mL of the extract was diluted in 1 mL of chloroform, after which a few drops of acetic anhydride and concentrated sulphuric acid were added to the mixture. The Shinoda test for the identification of flavonoids [6–8]. For this test, 0.2 g of the extract was

shaken for 5 to 10 minutes in 10 mL of methanol; subsequently, 1 mL of concentrated HCl, 0.2 g of magnesium shavings and a few drops of concentrated sulphuric acid were added to the methanol mixture.

Dragendorff's test for the identification of alkaloids [6–8]. Dragendorff's reagent is prepared by mixing equal volumes of solution A (a solution containing 0.85 g of bismuth nitrate dissolved in 10 ml of acetic acid and 40 ml of distilled water) and solution B (a solution containing 8 g of potassium iodide in 20 ml of distilled water). 1 mL of the extract is dissolved in methanol, and a few drops of Dragendorff's reagent are added.

Quantitative analysis

It was deemed appropriate to quantify the total polyphenols and flavonoids in the various hydro-ethanolic extracts of *J. curcas*, given their high abundance as revealed by qualitative tests of the extracts and the biological properties generally attributed to them in the literature.

• Total polyphenol content

The total polyphenol content of each hydro-ethanolic extract of *J. curcas* was determined using the Folin-Ciocalteu reagent as described by [9], with some modifications. The crude extract (50 mg) was mixed with 1 ml of Folin-Ciocalteu reagent and 7.5 ml of distilled water. The mixture was left at room temperature for 5 minutes, after which 10 ml of 7% sodium carbonate was added to the mixture, followed by incubation for 90 minutes at room temperature. After incubation, the absorbance against a reagent blank was measured at 760 nm using a JENWAY 7305 UV/visible spectrophotometer. The total phenolic content of the extracts was expressed in mg/g gallic acid equivalent using the gallic acid calibration curve. All samples were analysed in triplicate and the results are expressed as the mean \pm standard deviation.

• Total flavonoids

The total flavonoid content was determined in each hydro-ethanolic extract of *J. curcas* according to the protocol described by [9], adapted with some modifications. Specifically, 0.833 ml of 80 μ g/ml extract was added to 120 μ l of 5% sodium nitrite. After 5 minutes, 120 μ l of 10% aluminium chloride was added, followed by the addition of 800 μ l of 1 M NaOH after 6 minutes. The mixture was stirred vigorously and the absorbance of the reaction mixture was measured immediately at 510 nm against a blank using a UV/visible spectrophotometer. The total flavonoid content of the plant was expressed in mg/g of quercetin equivalent using the quercetin calibration curve at different extract concentrations (0.5, 0.4, 0.3, 0.2, 0.1 and 0.05 mg/ml). All samples were analysed in triplicate.

Evaluation of the antioxidant activity of hydro-ethanolic extracts from *Jatropha curcas* leaves

The hydro-ethanolic extract from *J. curcas* leaves was evaluated to determine its antioxidant potential. Consequently, the DPPH inhibition method was used to assess both the free radical scavenging activity and the antioxidant capacity.

• Evaluation of free radical scavenging activity based on the DPPH radical scavenging capacity of *Jatropha curcas* leaves

This test is designed to measure the ability of extracts to scavenge the relatively stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The scavenging of this radical causes the initial solution to change colour from dark purple to yellow as a result of its reduction reaction (diphenyl-picrylhydrazine). This method is based on the reduction of an alcoholic solution of DPPH^o in the presence of an antioxidant that donates a hydrogen atom or an electron. The non-radical form DPPH-H is formed (Figure 2). Indeed, DPPH is characterised by its ability to produce stable free radicals; the presence of these DPPH^o radicals results in a dark purple colouration of the solution. The reduction of DPPH^o radicals by an antioxidant agent results in absorbance at 517 nm, and in this way the antioxidant potential of a substance or plant extract can be determined [10].

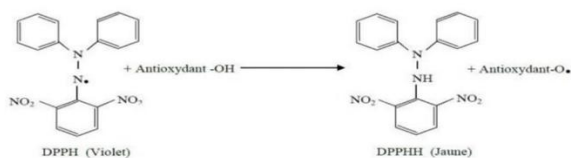


Figure2. Equation for the DPPH radical converted to DPPH^o
Source [11]

Method: The protocol used is that described by [11] and adopted by [10], with some modifications. The 0.04 mM DPPH solution: 20 mg of DPPH was completely dissolved in 500 ml of methanol using a magnetic stirrer. The extract and BHT solution In 10 ml of methanol, 40 mg of extracts and BHT were dissolved separately to form stock solutions of 4 mg/ml concentration. A series of solutions with increasing concentrations (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1 mg/ml) was subsequently prepared from the stock solution by dilution in methanol. Preparation of the reaction mixture and determination of the trapping percentage.

To tubes containing 2 ml of a 0.04 mM methanolic DPPH solution, 0.5 ml of each of the previously prepared hydro-ethanolic crude extract solutions of *J. curcas* and BHT at the concentrations specified above were added. After vortex mixing, the tubes were placed in the dark at room temperature for 30 minutes. The readings were taken by measuring the absorbance at 517 nm. The negative

control, consisting of 0.5 ml of methanol and 2 ml of DPPH methanol solution, was included in the series and evaluated under the same conditions. The percentage of DPPH radical scavenging by each extract at different final concentrations of 0.01; 0.02; 0.03; 0.04; 0.05; 0.06; 0.07; 0.08; 0.09 and 0.1 mg/ml was calculated using the following formula:

$$\text{Trapping rate} = \frac{\text{Control DO} - \text{Test DO}}{\text{Control DO}} \times 100$$

Each assay was performed in triplicate and the percentage scavenging values were expressed as the mean \pm standard deviation. From the percentage scavenging values, the 50% inhibitory concentrations (IC₅₀) of the various extracts tested were calculated using the equations derived from each regression curve. The effective concentration 50 (EC₅₀) is the concentration required to reduce the initial concentration of DPPH. It is derived from the IC₅₀ and is expressed in g/mol of DPPH.

$$\text{CE}_{50} = \frac{\text{IC}_{50}}{\text{Molar concentration of the DPPH solution}}$$

The anti-radical activity (ARA) was also calculated as the inverse of the effective concentration.

The higher the value, the greater the anti-radical activity [13].

$$\text{PA} = \frac{1}{\text{CE}_{50}}$$

Evaluation of antifungal activity

Evaluation of the antifungal activity of hydro-ethanolic extracts from *J. curcas* leaves against a fungal strain containing *C. albicans*.

Table II: characteristics of the germ to be tested

Microscopic fungi	Nature	Culture medium	Characteristics of the colonies	Temperature and incubation time
<i>C. albicans</i>	Yeasts	Sabouraud medium supplemented with chloramphenicol	Colonies of creamy white yeast	37°C nach 48 Stunden

The well method involves the diffusion of test substances of known concentration, which are impregnated into wells. These wells are carved into a solid culture medium poured into Petri dishes, which have been previously inoculated with a fungal inoculum.

Procedure: Preparation of fungal suspensions. The Petri dishes are incubated under conditions appropriate for the organism under study. The further the agent diffuses, the lower its concentration becomes, thereby creating

a concentration gradient around the wells. After incubation, if the substance in the well has antifungal activity against the inoculated organism, a zone of growth inhibition will be observed around the wells.

Inoculation of the fungal strain: The fungal strains will be inoculated into Petri dishes containing the specific agar, then incubated at 37°C for 2 to 6 days.

Preparation of hydro-ethanolic extracts for testing: For this study, hydro-ethanolic extracts of *J. curcas* leaves will be used. Solvents such as deionised water and 10% dimethyl sulfoxide (DMSO) will be employed. The hydro-ethanolic extract is dissolved in deionised water. Each extract will be prepared to yield stock concentrations of 200 mg/ml (50/50), 150 mg/ml (60/40) and 100 mg/ml (70/30).

Preparation of wells: Wells are made using sterilised tips. Each well will have a diameter of 2 mm, into which 50 µL of each *J. curcas* extract at different concentrations will be added; fluconazole solutions will also be prepared at a concentration of 10 mg/ml and added to a well corresponding to the control.

Preparation of the agar: For this study, chloramphenicol Sabouraud agar is used. The agar is prepared according to the manufacturer's instructions and, after being heated until completely dissolved, is autoclaved at 121°C for 20 minutes. After autoclaving, they are poured into sterile Petri dishes using a graduated burette before cooling. 15 ml of the corresponding agar is measured and poured into each sterile Petri dish, which has been pre-labelled and identified using a marker according to the type of agar to be used and the strain to be inoculated. The agar plates are then left to stand and solidify.

Preparation of the inoculum: From a culture aged between 2 and 6 days, 1 to 3 well-isolated colonies are picked up with a Pasteur pipette and suspended in a few millilitres of sterile 0.9% NaCl solution for each germ. The turbidity of each suspension is adjusted to that of a MacFarland scale of 0.5. This value corresponds approximately to 1.5×10^8 CFU/ml.

Inoculation of culture media: A sterile swab is dipped into the fungal culture suspension and used to inoculate Petri dishes containing Sabouraud's medium by making a series of closely spaced streaks. Wells containing plant extracts and/or antifungal agents are placed in each Petri dish using forceps.

Formulation of the antifungal syrup

For this formulation, the choice of excipients was made in accordance with the manufacturing standards defined in the general regulations and monographs, as well as in the preambles of the National Formulary of the European Pharmacopoeia, in line with the objective of producing syrup [14]. The proportions were determined experimentally after several trials. The final formula adopted is as follows: Formulate two batches of syrups based on *J. curcas* extract (10 g and 20 g).

Method: the preparation of the syrup took place in several stages. The first stage involved making the sugar syrup. The preparation of the syrup took place in two phases; the first involved dissolving the sucrose: this involved placing 470g of distilled water and 500 g of sugar in a food-grade

stainless steel saucepan and heating to a temperature of 65°C (check the temperature using a dip thermometer) for 20 to 25 minutes until a viscous consistency, a clear appearance and a pleasant flavour were achieved. The second phase involves adding the active ingredients (plant extract), preservatives and food additives; this was carried out by adding, in batches and whilst stirring, a 20 g/ml concentration of *J. curcas* leaf extracts into the mixture (sugar syrup), followed by homogenisation and the incorporation of preservatives and food additives. The final product was packaged in sterile 100 ml containers and then hermetically sealed. In this study, the antifungal syrup was formulated using two preparations, which differ in terms of the quantity of plant extracts and the type of sugar, as shown in the table below: In this study, the antifungal syrup was formulated using two preparations, which differ in terms of the quantity of plant extracts and the type of sugar, as shown in the table below:

The antifungal syrup was formulated in accordance with the European Pharmacopoeia method, specifying the quantity of extract in each batch.

Table III: Formulation of the antifungal syrup

Formulation	Sugar	Extracts <i>J. curcas</i>
Antifungal syrup Batch No.1	White	10g
Antifungal syrup Batch No.2	White	20g

Evaluation of the antifungal activity of the formulated syrup

The well method described previously in section 2.7 will be identical for the antifungal syrup activity formulated with hydroethanolic extracts of *J. curcas*. For this study, the syrup is dissolved in demineralized water. Stock solutions will be prepared at concentrations of 10 mg/ml, 5 mg/ml, and 2.5 mg/ml.

Quality control test:

• Physicochemical parameters:

pH: The pH measurement was carried out using potentiometry in accordance with the NF V05-108 reference protocol, using a Mettler Toledo Tester HI 98107 pHmeter. To carry out the test, the pH meter is first immersed for 10 minutes in 50 ml of distilled water, then in a phosphate buffer solution of pH 7. The probe is then rinsed in distilled water and re-immersed in a phosphate buffer solution of pH 5.5. The probe, rinsed once more, is then directly immersed in 50 ml of the syrup sample. The pH value is read directly from the device's display. The procedure was carried out in three trials.

Density: The density of the formulated antifungal syrup was determined by measuring the mass of 10 ml of the syrup, then dividing this by the weight of the same volume of water contained in a volumetric flask, using a precision balance. The analysis was carried out three times and the results obtained are expressed as an average. The density of the syrup represents the mass in kg per 1 litre of syrup. **Titrateable acidity:** the acidity of the syrup is attributed to the quantity of organic acids added or derived from the raw material, primarily citric acid. It is determined in syrups by titrimetry in accordance with the reference

protocol NF V04 – 206. This involves taking 2.5 ml of syrup and diluting it in 22.5 ml of distilled water. Subsequently, 2 to 3 drops of phenolphthalein (1% solution prepared in 95% ethanol) are added to the solution. The solution is titrated against a 0.1N sodium hydroxide (NaOH) solution dispensed from a graduated burette. The equivalence point is determined when the colour of the sample turns to a persistent pink for 30 seconds. The titratable acidity of each syrup is determined using standard citric acid solutions of 0.05, 0.025, 0.0125 and 0.00625 M, which are in turn titrated with 0.1 M NaOH.

Brix degree: the Brix scale (in °B or °Bx) is used to measure the sucrose content in syrups; in other words, it indicates the sucrose concentration or the percentage of soluble dry matter. The higher Brix degree, the sweeter sample. The °Bx of syrups is measured using a pre-calibrated KERN Optique refractometer. To do this, a drop of each sample to be analysed, corresponding to a volume of 50 µl, is placed on the refractometer's prism, and the sample cover plate is tilted to cover the sample. The instrument is then directed towards the light source and the illumination window is opened to read the result, after adjusting the contrast until the horizontal contrast line corresponds to the lower half of the adjustment circle. The Brix degree and refractive index of each sample were recorded [15].

• Microbiological testing

The microbiological quality of the syrup was assessed by isolating and counting microorganisms after culturing the sample on selective and identification agar plates (solid culture medium). The analyses were carried out according to standard methods and the determined Colony-Forming Unit (CFU)/g values were compared with normal values.

Total aerobic mesophilic flora (TAMF): deep plating of dilutions.

Fungal flora: Yeasts and moulds spread on Sabouraud agar, incubated for 1–2 days.

Total coliforms: (E. coli)

Specific organisms: S.aureus, Escherichia coli

Specific media: Sabouraud, EMB and Mannitol Salt Agar.

Microbiological limits

Aerobic organisms $\leq 10^3$ CFU/g

Yeasts $\leq 10^2$ CFU/g

Absence of S. aureus and Escherichia coli in 1 g

• Stability testing

It is not possible to reliably correlate a stability period under selected conditions with a stability period under accelerated conditions. However, if a syrup withstands these severe conditions, it is highly likely to remain stable until use. In our study, we will examine the effect of storage at +40 °C and +10 °C for 7 days and centrifugation at a speed of 1800 rpm.

Data analysis

During the course of our work and whilst drafting this thesis, we used Microsoft Word 2019 for data entry and Microsoft PowerPoint 2019 for presentations. Microsoft Excel was used for data analysis and plotting diagrams.

RESULTS

Extraction

Extracts of *J. curcas* were prepared in various solvents from the plant's leaves, and extraction yields were calculated by dividing the mass of extract obtained by the mass of the initial powder. The table below shows the yields obtained from the aqueous and hydro-ethanolic extracts.

Table IV: Characteristics of the extraction obtained

Type of extract	Aqueous	Hydro-ethanolic (v/v)			
		50/50	60/40	70/30	
Features	Brown dry	Dark brown	Slightly pasty	Slightly pasty	Dark brown Slightly pasty
Extraction yield	38%	17%	14,5%	16,7%	

Key: 50/50 v/v: 50% ethanol and 50% water; 60/40 v/v: 60% ethanol and 40% water; 70/30 v/v: 70% ethanol and 30% water.

Qualitative screening of *Jatropha curcas* extracts

The table below presents the results of qualitative tests carried out on the total extract, using either colour reactions or precipitation reactions. It highlights the different classes of compounds present in the extract.

Table V: Results of the phytochemical screening of *Jatropha curcas* extracts

Tests	Compound Families	Observations	Results
Dragendorff	Alkaloids	Presence of an orange-yellow precipitate	+
Foam index	Saponins	Presence of persistent foam	+
96% concentrated H ₂ SO ₄	Steroids	A brick-red colour that turns purple	+
1% iron chloride	Hydrolysable tannins	Observation of a pink flocculent precipitate	-
Fehling's reagent	Reducing sugar	A hasty observation: brick red	-
Sodium hydroxide 10 % (Shinoda)	Flavonoids	Purple or reddish-orange colour	+
Ethyl acetate and ammonia	Coumarin	Observation of intense flowering	-
Iron chloride 5%	Polyphenol and phenol	Green or blue colouring	+

Key: AE: aqueous extract; HE: hydro-ethanolic extract; +: present; -: absent; 50/50 V/V: 50% ethanol and 50% water; 60/40 V/V: 60% ethanol and 40% water; 70/30 V/V: 70% ethanol and 30% water.

Analyse quantitative

• Teneur en polyphénols totaux des extraits hydro-éthanoliques de *Jatropha curcas*

The total polyphenols were determined using Folin-Ciocalteu reagent. The graph in Figure 3 below represents the calibration curve of gallic acid, whose regression equation $Y = 1.435x - 0.0963$ ($R^2 = 0.96$) allowed the determination of the total polyphenols.

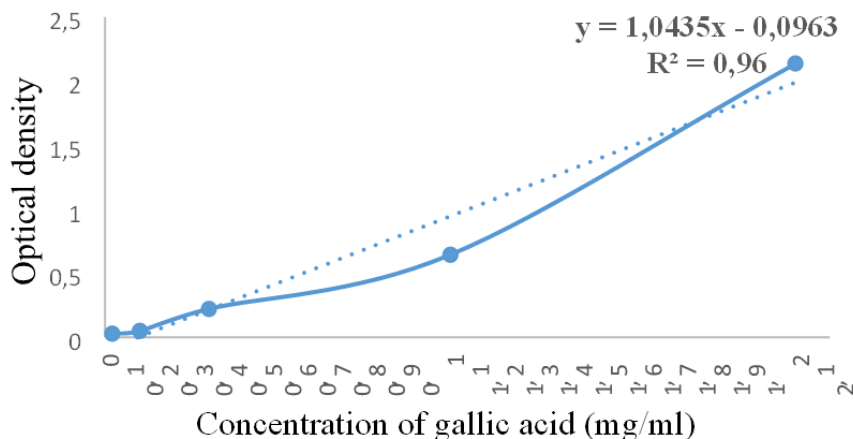


Figure 3. Calibration curve for gallic acid

The table below shows the polyphenol content of *J. curcas* leaf extracts, calculated using the equation $Y = 1.0435x - 0.0963$.

Table VI: Polyphenol content (mg EAG/g of extract) of the <i>Jatropha curcas</i> leaf extract.			
Types of extracts	Hydro-ethanolic	Hydro-ethanolic	Hydro-ethanolic
Hydro-ethanolic	50/50 v/v	60/40 v/v	70/30 v/v
Polyphenol content (mg EAG/g of extract)	0,195 ± 0	0,578 ± 0	0,886 ± 0

Key: 50/50 v/v: 50% ethanol and 50% water; 60/40 v/v: 60% ethanol and 40% water; 70/30 v/v: 70% ethanol and 30% water.

• Total flavonoid content of hydro-ethanolic extracts of *Jatropha curcas*

Flavonoids were quantified using a method based on the formation of a stable complex between aluminium chloride and the oxygen atoms present on the 4th and 5th carbon atoms of the flavonoids. Figure 4 below shows the calibration curve for quercetin, from which the regression equation was used to determine the total flavonoid content in the hydro-ethanolic extracts.

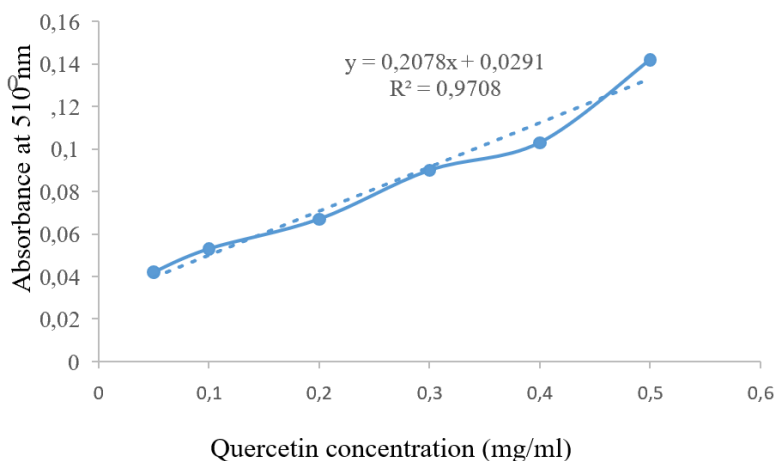


Figure 4. Quercetin calibration curve

Table VII: Polyphenol content (mg EAG/g of extract) of the *Jatropha curcas* leaf extract

Types of extracts	Hydro-ethanolic	Hydro-ethanolic	Hydro-ethanolic
Hydro-ethanolic	50/50 v/v	60/40 v/v	70/30 v/v
Flavonoid content (mg EAG/g of extract)	9.76 ± 0.054	7.86 ± 0.065	8.14 ± 0.057

Key: 50/50 v/v: 50% ethanol and 50% water; 60/40 v/v: 60% ethanol and 40% water; 70/30 v/v: 70% ethanol and 30% water.

Evaluation of the antioxidant activity of hydro-ethanolic extracts from *Jatropha curcas* leaves

The antioxidant activity of various extracts from *J. curcas* leaves and of the reference antioxidant BHT (butylated hydroxytoluene) was assessed using the DPPH radical scavenging assay.

• Evaluation of antioxidant activity based on the DPPH radical scavenging capacity of *Jatropha curcas* leaves

The figures below show the curves of DPPH radical scavenging percentages as a function of the concentration of hydro-ethanolic extract from *J. curcas* leaves and of BHT (used as a standard) respectively.

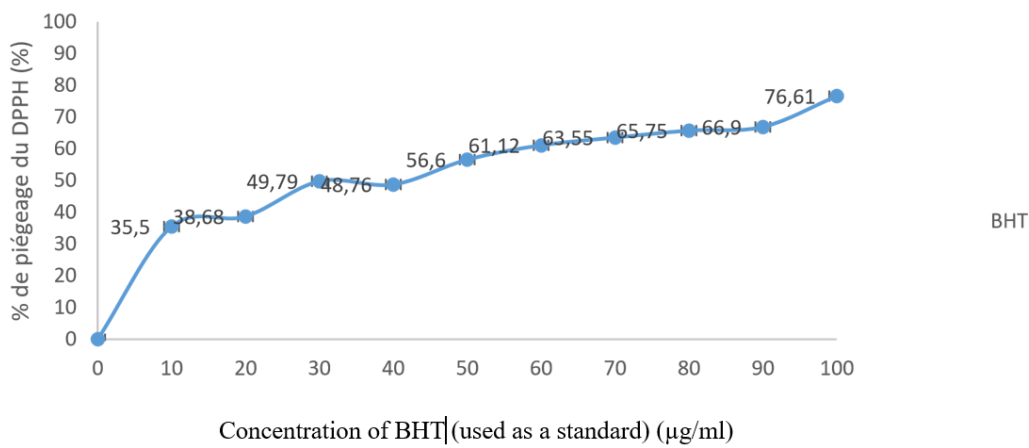


Figure 5. Percentage of DPPH radical scavenging at different concentrations of the BHT

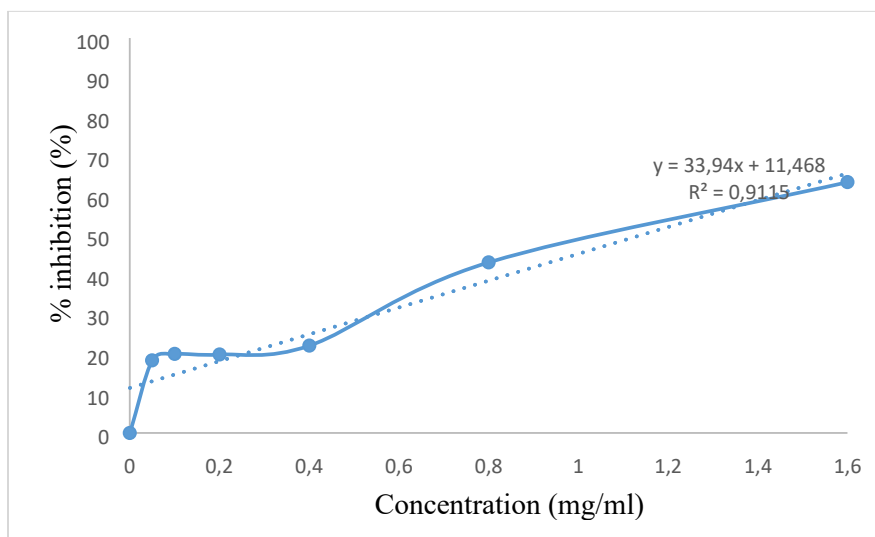


Figure 6. Regression curve showing DPPH inhibition by *Jatropha curcas* extract

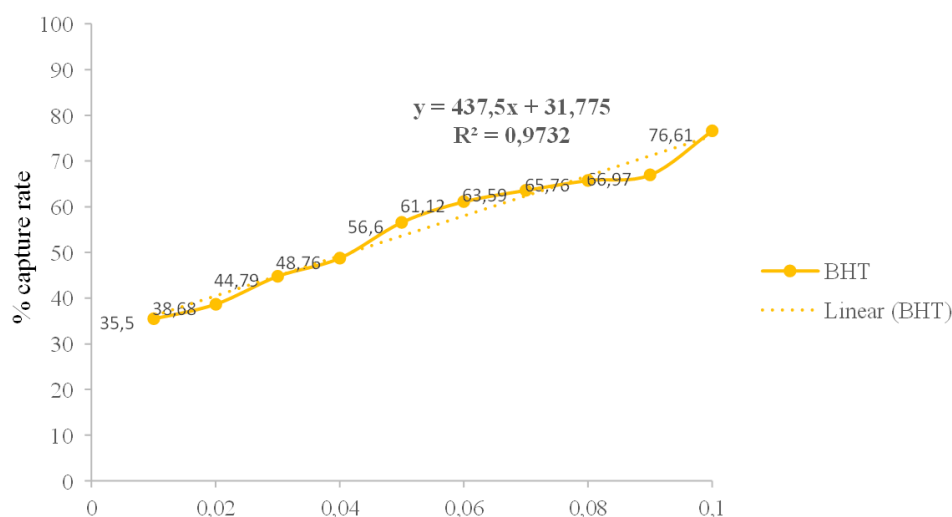


Figure 7. Regression curve showing DPPH inhibition by BHT

Table VIII: IC₅₀, EC₅₀ and PA values for *Jatropha curcas* leaf extracts and BHT

Antioxidant properties	IC ₅₀ (µg/ml)	CE ₅₀ (mg/mol)	PA (mol/mg)
<i>J. curcas</i>	1,135	28,375	0,036
BHT	0,042	1,05	0,952

Evaluation of the antifungal activity of hydro-ethanolic extracts of *Jatropha curcas*

The antifungal properties of the various *J. curcas* extracts were evaluated using a single fungal isolate. The table below shows the growth inhibition diameters (mm) measured for the test strain.

Table IX: Inhibition zone diameters of *Candida albicans* by *Jatropha curcas* extracts

Inhibition zones for each test (mm)	Types of extracts and their concentrations (mg/ml)			Reference	Report CMI/reference
	50/50 (200mg/ml)	60/40 (150mg/ml)	70/30 (100mg/ml)		
				Fluconazole	
CMI (50/50)	33	21	27	24	4,37
CMI (60/40)	30	21	27	21	3,28
CMI (70/30)	42	27	33	24	3,66

Legend: 50/50 v/v: 50% ethanol and 50% water; 60/40 v/v: 60% ethanol and 40% water; 70/30 v/v: 70% ethanol and 30% water

In general, all the test samples exhibited greater activity than fluconazole. The comparison of the test samples with the reference shows that test sample 1 exhibits four times the activity of fluconazole.

Formulated antifungal syrup

Two formulated syrups were produced during preparation and differed in the quantities of sugar and excipients sqs added. The figure below shows the various formulated samples. For each sample, a number of macroscopic and organoleptic characteristics were recorded and summarised in Table X below

Table X: Macroscopic and organoleptic characteristics of the syrups

	Syrup No.1 50/50	Syrup No.2 70/30
Consistency	Viscous	Viscous
Flavour	Milled	Milled
Appearance	Clear	Clear
Colour	Brown	Brown

Both syrup samples have a good viscous consistency, with a strawberry flavour and a clear appearance. As for colour, the syrups are both brown in colour.

Evaluation of the antifungal activity of the formulated syrup

Based on agar plate cultures, the diameters of the inhibition zones for the syrups exhibiting antifungal activity (showing an inhibition zone around the well) were measured, and the values obtained are shown in the table below.

Table XI: Diameter of the zones of growth inhibition of *Candida albicans* for each syrup

	Syrup No.1 (Test 1, concentration = 200 mg/ml)	Syrup No. 2 (Test 2, concentration = 200 mg/ml)
Inhibition zone diameters for each syrup in each Petri dish	22 mm	26 mm
Fluconazole inhibition zone diameter (reference C=200 mg/ml)	24 mm	24 mm
Volume added to each well	50 µl	50 µl
Ratio Recency / CMI	1.5	1.3

Tests on the formulated syrups showed antifungal activity, with inhibition zones of 22 mm for Syrup No.1 and 26 mm for Syrup No.2, both at the same concentration (200 mg/ml). Generally speaking, the inhibition zone for the fluconazole tested was 24 mm. The comparative trial report shows that the formulated syrups are not only effective against the strains but are also 1.5 times more effective than fluconazole.

Quality control of the syrup

• Physico-chemical parameters

The antifungal syrup formulations were prepared in accordance with the European Pharmacopoeia method. After preparation, the physicochemical parameters were determined for each sample in accordance with WHO standards. The results obtained are presented in Table XII below.

Table XII: Physicochemical parameters of the syrups

Physico-chemical parameters	Standard	Syrup No.1	Syrup No.2
PH	7,02±7,11	8	8
°Brix (%)	55 - 65	65	65
Density	1,31-1,33	1,135 ± 004	1,130 ± 004
Titrateable acidity (g of citric acid per 100 ml of syrup)		0,02	0,02
Number of days worked	10	10	10

Key: (-) = parameter not specified by CODEX

• Microbiological testing of the syrup

Following culture, the colony-forming unit (CFU) counts in each culture medium were determined and then expressed as the microbial concentration in CFU/g of the original syrup sample. The results of the microbiological quality tests carried out in accordance with the European Pharmacopoeia (1992 and 2018 editions) are presented in the table below.

Table XIII: Microbiological quality of syrups

Microbiological parameters	Standards	Syrup No.1	Syrup No.2
FMAT (CFU/g)	< 10 ⁵ (NF EN ISO 4833)	00	00
Mushrooms de	< 10 ² (NF V 08-059)	00	00
Total coliforms (CFU/g)	< 10 (NF V 08-050)	00	00
<i>S. aureus</i> (CFU/g)	< 10 (NF EN ISO 6888-2)	24. 10 ²	24. 10 ²
<i>E. coli</i> (CFU/g)	< 10 (NF ISO 16649-2)	00	00

Key: UFC = Colony-forming units; FMAT = Total aerobic mesophilic flora

The Total Aerobic Mesophilic Flora (TAMF) of each of the two samples is 00 CFU/g and is therefore compliant with the standard. Furthermore, fungal contamination is absent in both syrups, with values of 00 CFU/g recorded for both. All these values also comply with the ISO 17025 standard for microbiological quality. Table XII shows that, in all the syrups, no bacteria corresponding to total coliforms or *E. coli* were detected. The *S. aureus* value in the table above is 24.10² CFU/g; this value is below the standard.

DISCUSSION

Extraction yields were 38% for the aqueous extract, and (17% for the 50/50 mixture; 14.5% for the 60/40 mixture; 16.7% for the 70/30 mixture) for the hydro-ethanolic extracts. These values are higher than those reported by Mpiana et al., who found a yield of 5% for the aqueous extract, and differ from those reported by Zhang Y et al.,

2018 [17], who found yields of 22% and 19% for aqueous and hydro-ethanolic extracts respectively [10]. These variations can be explained by differences in working conditions and the extraction techniques employed.

Phytochemical screening of *J. curcas* leaf extracts revealed numerous metabolites (alkaloids, saponins, steroids, flavonoids, phenols and polyphenols), with variations depending on the type of extract. These tests confirm the richness of *J. curcas* leaves harvested in the Central Cameroon region in bioactive substances.

The quantitative study of secondary metabolites found in hydroethanolic extracts of *J. curcas* leaves, using spectrophotometric assays, aimed to determine the total polyphenol and flavonoid content. The main reason for choosing these groups of secondary metabolites is that these groups of compounds have been described as the major components of *J. curcas* leaves and are responsible for most of the pharmacological properties attributed to

this plant. The evaluation of antioxidant activity by DPPH radical scavenging revealed that the extract exhibits high activity, with an IC₅₀ of 1.135 µg/ml, an EC₅₀ of 28.375 mg/mol, and a PA of 0.036 mol/mg. However, BHT, with an IC₅₀ of 0.042 mg/ml and an EC₅₀ of 1.05 mg/mol, proved less active than the extract. Indeed, the results of phytochemical characterization tests show that the extracts contain flavonoids, polyphenols, tannins, and alkaloids, which are phytoconstituents known for their antioxidant properties Chen F., 2014 [18]. Nevertheless, the extracts scavenging capacity remains lower compared to that of BHT, which has an IC₅₀ of 0.042 mg/ml. The results obtained differ from those of many authors, notably Asuk et al., 2015 [19], who determined an IC₅₀ of 47.34 µg/ml. This difference in activity could be due to the location and technique of plant harvesting and the phytochemical composition of the extracts.

Antifungal tests performed on *C. albicans* show that hydroethanolic extracts of the leaves possess antifungal activity. Indeed, on the tested isolate, the extracts were sensitive with an inhibition zone diameter ranging from 33 to 42 mm with a concentration of 200 mg/ml for trial 1, from 21 to 27 mm with a concentration of 150 mg/ml for trial 2, and from 27 to 33 mm with a concentration of 100 mg/ml for trial 3. Bauer et al. and Saetae et al. found an inhibition zone diameter ranging from 10 to 20 mm. The difference in activity observed between the results of these authors and those obtained in the present study could be explained by the difference in sensitivity of the multi-resistant microorganisms, the technique used, and the variation in the composition of the extracts. The evaluation of the antifungal activity of the syrups on the fungal strain responsible for fungal infections showed inhibitory activity through the inhibition zone diameters (mm) observed around the wells containing each syrup using the method of Cos et al., 2006 [20]. The results showed that the two antifungal syrups formulated with the extracts had inhibitory activity on the studied fungal strain *C. albicans*, with an inhibition zone of 22 mm for syrup No. 1 and 26 mm for syrup No. 2. These results are attributed to the antifungal properties of *J. curcas* leaves, as Bauer et al. demonstrated the antibacterial activity of this plant against fungal strains; proven in alternative medicine for its antibacterial, antifungal, and antidiabetic properties. In contrast to our syrups, the reference product with fluconazole as its active ingredient, purchased from a pharmacy, also showed inhibitory activity against the studied yeast, but with less efficacy than the plant-based antifungal syrup. Although fluconazole is effective against yeasts and fungi, it has side effects that cause drug-related illnesses (teratogenic diseases). These results could thus support the research question: the formulation of this syrup, which is not only more effective than fluconazole but can also be used for long-term treatment of fungal infections.

Regarding syrups, MTAs in syrup form are highly valued due to their ease of use by patients Sanogo et al., 2006 [21]. The two antifungal syrups based on *J. curcas* extract showed satisfactory results, identical to those formulated by Sanogo et al., 2014 [22], who had formulated antimalarial syrups based on *Argemone mexicana* L.

Traoré extracts, 2010 [23]. The syrups obtained also had a good appearance, without sediment, a strawberry taste, and a brown color. They had an alkaline pH, with values of 8 for both trials. These pH values all conform to the Codex Stan standard for plant-based syrups, which suggests that the pH of a syrup should be between 7 and 8. This alkalinity helps combat acidity in the body and thus contributes to acid-base balance. This corroborates the pH results obtained by Abbès et al., 2014 [24], ranging from 7.33 to 7.80 for date syrups. The pH values of the syrups formulated in this study are also higher than those found by Raiesi et al., 2014 [25] for date syrup ice cream, which were around 4.20. This difference in results can be explained by several factors, such as the type of formulation and the production process, and food additives like strawberry flavoring; strawberries are a product with an alkaline pH. Furthermore, the °Brix results obtained show a value of 67% for syrup No. 1. 65% for syrup No.2. Syrup No. 1 has a value exceeding the CODEX STAN 247-2005 standard, unlike syrup No.2, which complies with this standard, suggesting that the Brix value of syrup should be between 55 and 65. However, the results obtained for syrup No. 1 are accepted according to the CODEX STAN 212-1999 standard, which suggests that the Brix value of syrup should be between 65.8 and 69%.

Titrate acidity was determined by titration. The measurement of the titratable acidity of the different syrup samples yielded a result of 0.02 g of citric acid per 100 ml of syrup. These results are higher than the values obtained by AlHooti et al., 2002 [26] for date syrups made from two Kuwaiti date varieties, which were approximately 0.67 g and 0.77 g/100 g, respectively; as well as the titratable acidity results obtained by Adou et al., 2018 for mixed syrups based on ginger and pineapple juice, whose values ranged from 0.9 ± 0.01 to 0.92 ± 0.03 g/100 ml.

However, the acidity obtained in this study is lower than the values found by Djermoune et al., 2015 [27] for Biskra syrups, which ranged from 1.55 to 3.51 g/100 g. These differences may be due to various factors such as the type of raw material, the formulation conditions for each type, the extraction process, and the storage conditions of the syrups.

Overall, regarding the microbiological quality of the syrups, five (5) parameters were analyzed in each syrup sample: TMF (Total Aerobic Mesophilic Flora), total coliforms, yeasts and molds, and *E. aureus*. The results for the number of microorganisms per CFU/g for each studied flora, based on the analyzed samples from each of the two formulated tests, show that the microbial load values obtained comply with the microbiological quality standard according to the applicable ISO 17025 normative reference. These results are close to those found by Coulibaly Bakary et al., 2018 [28], who conducted a microbiological quality assessment of Improved Traditional Medicines (ITMs) sold in six communes of Abidjan (Côte d'Ivoire), obtaining similar results. Coulibaly Bakary et al., 2018 [28], suggest that the good microbiological quality of the syrups can be explained by the combined effect of the antimicrobial compounds present in the *J. curcas* extract, and a second explanation

could be adherence to GMP and GLP during manufacturing and the traceability of herbal drugs.

CONCLUSION

In conclusion, this study aimed to: formulate a syrup from *Jatropha curcas* leaf extract, identify the groups of secondary metabolites found in the different extracts (aqueous and hydro-ethanolic) of *Jatropha curcas* leaves, quantify the content of *Jatropha curcas* leaves, evaluate the in vitro antifungal activity and determine the antiradical and antioxidant properties of the hydro-ethanolic and aqueous macerates of *Jatropha curcas* leaves, formulate the antifungal syrup based on the extracts, and evaluate the antifungal activity of the syrup against yeasts and fungi responsible for fungal infections. *Jatropha curcas* leaves contain several groups of secondary metabolites depending on the solvents used for extraction, including alkaloids, polyphenols, steroids, and flavonoids; with a higher extraction yield of 38% for the aqueous extract. (17% 50/50 v/v), (14.5% 60/40 v/v), and (16.7% 70/30 v/v) for the hydroethanolic extracts.

Quantitative analysis of total polyphenols and total flavonoids revealed the abundance of these compounds, particularly in the leaves of *Jatropha curcas*. The highest polyphenol content was observed in the 70/30 v/v hydroethanolic extract, with a value of 0.886 mg GAE/g of extract. Regarding flavonoids, the highest content was observed in the 50/50 v/v hydroethanolic extract, at 9.76 mg QE/g of extract. The results of the study on antioxidant activity suggest that all extracts of *Jatropha curcas* leaves are a potential source of antioxidant substances with DPPH radical scavenging capacity, with an IC₅₀ of 1.135 µg/ml, an EC₅₀ of 28.375 mg/mol, and a PA of 0.036 mol/mg, respectively.

The evaluation of the antifungal activity of the hydro-ethanolic extracts revealed that they possess inhibitory properties against the growth of most of the tested fungal strain (*Candida albicans*).

The formulated syrup exhibits sensitivity to yeasts of the genus *Candida albicans*. The physicochemical analysis of the formulated syrups shows an alkaline pH (8). The °Brix of the syrups varies between 65.13 and 67%. The microbiological quality of the formulated syrups obtained was generally compliant with the standards of Codex STAN 247-2005, ISO 17025, and the European Pharmacopoeia (1992 and 2018 editions).

This work therefore suggests the use of the syrup formulated in this context as an effective means of managing fungal infections and gastroenteritis for long-term treatment, subject to further studies.

AUTHORS' CONTRIBUTIONS

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