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# Pharmacognostic Evaluation and Antioxidant Potential of *Celtis integrifolia* Leaves

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**ABSTRACT**

***Background****: Celtis integrifolia* Lam is commonly known as hackberry or nettle tree from the family of Ulmaceae. It's renowned for its medicinal properties having a history of traditional use. This study aimed to investigate some pharmacognostic characteristics, phytochemical constituents and antioxidant potential of the leaves of *C. integrifolia.* Methods: Pharmacognostic standardization of the leaf was assessed based on the chemomicroscopic and some physicochemical parameters. The powdered leaf was extracted using methanol, concentrated and used for the phytochemical analysis and antioxidant assays.

***Results:*** The chemomicroscopy evaluation showed the presence of Cellulose, lignified cell wall, calcium oxalate, inulin and tannin. Physicochemical parameters such as moisture content/loss on drying (6.59 %), total ash value (21.93%), acid insoluble ash (14.1%), water-insoluble ash (17.5%) and water had high extractive value of (17.1%) compared to methanol which had extractive value (10%). Qualitative phytochemical analysis of methanol extract of *C. integrifolia* leaves revealed the presence of carbohydrate, flavonoids, steroids, cardiac glycoside, tritepenes, tannins, alkaloids and saponins. The methanol extract of *C. integrifolia* leaves demonstrated antioxidant activity.

***Conclusion:*** The Pharmacognostic parameters observed in this study will be of help in correct identification and quality control of *C. integrifolia*. The presence of bioactive chemicals and exhibition of antioxidant activity of *C. integrifolia* leaves justified its uses the traditional medicine.

***Keywords***: Antioxidant, *Celtis integrifolia*, Chemo microscopy, Physicochemical parameters, Standardization,

## 1. INTRODUCTION

The use of herbal medicine for treating illnesses and maintaining health is the oldest and most widely practiced form of healthcare known to humanity. It has been an integral part of all cultures throughout history and remains a fundamental aspect of traditional medicine [1]. Herbal medicines incorporate therapeutic knowledge and practices that have been passed down through generations, offering valuable insights into the selection, preparation, and application of herbal formulations for therapeutic benefits [2]. However, a major limitation to the widespread acceptance of medicinal herbs is the lack of standardization, which includes proper identification, quality control, and quality assurance. The evaluation of herbal drug standards can be achieved through macroscopic, microscopic, and physicochemical analyses [3,4]. *Celtis integrifolia* Lam, commonly known as hackberry or nettle tree, is widely distributed across warm temperate regions of the Northern Hemisphere, including Southern Europe, Southern and Eastern Asia, and North and Central America. It is also found in parts of South and Central Africa, as well as Northern and South America, but is predominantly present in Africa [6]. In Nigeria, it is known as *Zuwo*, *Nguzo*, and *Wanco* in Hausa, Kanuri, and Fulfulde languages, respectively [5]. The plant belongs to the Ulmaceae family and typically grows to a height of about 26 meters. Its leaves are alternate, simple, ovate, 3–15 cm long, and have evenly serrated margins [7]. *C. integrifolia* has been traditionally used in the management of epilepsy, mental disorders, general weakness, and pain relief. It is also employed in the treatment of chickenpox, measles, gout, diarrhea, sore throat, and as an ecbolic agent. Additionally, it is used for wound healing, controlling bleeding, and as a spice and aphrodisiac in

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Northern Nigeria [8,9]. Despite its extensive medicinal applications, there is currently no documented information on the pharmacognostic parameters and quality control of *C. integrifolia* leaves. Therefore, this study was conducted to establish key pharmacognostic parameters and evaluate the antioxidant potential of *C. integrifolia* leaves. These findings will contribute to standardizing the plant for quality, purity, and validation of its traditional medicinal uses.

## 2.0 MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Biological Materials

*Celtis integrifolia*

*2.1.2 Chemicals and Reagents*

Zinc Chloride, Hydrochloric Acid, Ferric Chloride, Molisch's Reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH)

### 2.2 Methods

*2.2.1 Collection and preparation of plant material*

The plant of *C. integrifolia* was collected from Igabi Local Government Area of Kaduna State, which is located between latitude $10^0$ 47'0"N and longitude $7^0$ 46'0"E. It was identified at the Herbarium unit, Department of Plant Biology, Bayero University, Kano State, where accession number BUKHAN 0102 was given. The leaves were dusted, and cleaned, and all foreign matter was removed. They were then air-dried and ground to powdered form, stored in an airtight container for subsequent use.

*2.2.2 Extraction of plant material*

The powdered leaf of *C. integrifolia* was extracted using cold maceration technique with methanol in a glass jar for 3 days (72 hours) at room temperature. The extract was filtered; the filtrate was further concentrated by using a rotary evaporator and finally evaporated to dryness using a water bath set at a temperature of 50°C.

*2.2.3 Chemo microscopic examination*

The powdered leaves (5 g) leaf of *C. integrifolia* was cleared in a beaker containing 100 ml of 70% chloral hydrate solution. It was boiled on a water-bath at a temperature of 100°C for thirty minutes to remove obscuring materials. The cleared sample was mounted on a microscope slide, using dilute glycerol. Using various detecting reagents, the presence of some cell inclusions and cell wall materials was detected in accordance with [10,11].

*2.2.4 Determination of physiochemical constituents*

The powdered sample was subjected to physicochemical analysis; water and alcohol soluble extractives, total ash, acid insoluble ash, water soluble ash and moisture content were determined [10,11].

*2.2.5 Qualitative phytochemical screening*

The methanol extract of *C. integrifolia* was subjected to phytochemical tests for the detection of various chemical constituents [10].

*2.2.6 Antioxidant activity*

The antioxidant activity of methanol extract of *C. integrifolia* was measured in terms of radical scavenging ability, using a stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) according to the modified method adopted from [12]. 200 µl of 100 µM methanol solution of DPPH was added to 100 µL of various concentrations of the sample fractions in methanol (1000, 500, 250, 125, 62.5, 31.25, 15.63 and 7.8µg/ml) and made to react in dark for 30mins time at room temperature. Absorbance of the blank, test and control were recorded at 517 nm using UV spectrophotometer. The experiment was performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of inhibition.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The concentration corresponding to the 50% inhibition ($IC_{50}$) was determined using probit analysis by means of Statistical Package for Social Sciences (SPSS), IBM version 20. The $IC_{50}$ values obtained was compared with that of ascorbic acid as a standard antioxidant.

**2.3 Data analysis**

Data obtained from the study was analyzed using Statistical Package for Social Sciences (SPSS), IBM version 20. The results was expressed as mean ± standard error of mean (SEM) of three replicate.

## 3. RESULTS

Chemo microscopic examination of *C. integrifolia* leaves was conducted to examine the presence of Cellulose cell wall, lignified cell wall, calcium oxalate, inulin, and other parameters qualitatively. The results showed the presence of Cellulose cell wall, Lignin, Starch, Calcium Oxalate, Inulin, Tannins, Lignified cell wall and the absence of Calcium carbonate crystals. The physicochemical composition of C. integrifolia leaves powdered shows the percentage composition of moisture content, total ash value, acid insoluble ash, water insoluble ash and water extractive value.

Table 1: Physiochemical constituents of *Celtis integrifolia* powdered leaves

| Parameters | *Value obtained ± SEM (%w/w) |
|---|---|
| Moisture contents | 6.59 ± 0.54 |
| Total ash value | 21.93 ± 1.10 |
| Water insoluble ash | 17.5 ± 0.57 |
| Acid insoluble ash | 14.1 ± 0.58 |
| Water soluble extractive value | 17.1 ± 0.45 |
| Alcohol soluble extractive value | 10.0 ± 0.56 |

*Average values of five determinations. SEM: Standard Error of Means

The results of qualitative phytochemical screening of the methanol leaf extract of *C. integrifolia* revealed that the colour of the extract was green and revealed the presence of carbohydrates, flavonoids, steroids, cardiac glycoside, triterpenes, tannins, alkaloids, and saponins and the absence of Anthraquinones. The leaves of *C. integrifolia* exert antioxidant potentials but not as standard Gallic acid at 517nm but visible spectrophotometer was found to be higher at inhibition concentration of 1000 µg/mL which is 93.61 µg/mL and 98.75 µg/mL in methanol leaves extract and Gallic acid respectively (Table 4). The $IC_{50}$ value (required concentration to inhibit 50% of DPPH radicals of *C. integrifolia* was 74.192 µg/mL and that of Gallic acid was 0.009 µg/ml (Table 5).

Table 2: Antioxidant activity of methanol leaves of *Celtis integrifolia* using DPPH

| Analyte | Concentration (µg/mL) / % Inhibition | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1000 | 500 | 250 | 125 | 62.5 | 31.25 | 15.62 | 7.8 |
| Methanol extract | 93.62 | 91.72 | 69.62 | 53.12 | 40.32 | 26.99 | 25.14 | 18.21 |
| Gallic acid | 98.75 | 96.22 | 94.78 | 94.89 | 93.92 | 93.36 | 92.37 | 90.45 |

Table 3: Half-maximal inhibitory concentration ($IC_{50}$) value of methanol extract of *Celtis integrifolia*

| Analyte | $IC_{50}$ (µm) |
|---|---|
| Methanol extract | 74.192 |
| Gallic acid | 0.009 |

## 4. DISCUSSION

Chemo-microscopic examination of *C. integrifolia* leaves revealed the presence of cellulose cell walls, lignin, calcium oxalate, inulin, tannins, and starch. These cell wall components and inclusions play crucial roles in providing mechanical strength, protection, growth regulation, and insulation while reinforcing vascular structures to prevent toppling [13]. The observed changes in color, structure, and chemical reactions serve as valuable markers for identifying powdered plant materials. Such identification largely relies on the presence, absence, and specific forms of certain cell types and inclusions [14]. These findings can be utilized as diagnostic tools for the proper identification

and standardization of *C. integrifolia*, helping to prevent adulteration, especially when the plant material is used in powdered form for medicinal purposes. Physicochemical parameters such as moisture content (loss on drying), total ash content, water-soluble ash, acid-insoluble ash, and extractable matter content are essential for standardization and quality control. These parameters ensure the purity, stability, and phytochemical integrity of plant-based drugs [15]. In this study, the moisture content of *C. integrifolia* powdered leaf was found to be 6.59%. According to general pharmacopoeial guidelines, the moisture content in crude drugs should not exceed 14% [16]; thus, the observed value falls within the acceptable range. Moisture content determination is critical in preventing drug degradation during storage, as lower moisture levels enhance product stability and reduce the likelihood of microbial growth. Excess moisture is considered an adulterant because it increases weight and promotes the proliferation of mold and bacteria [11]. Ash values serve as crucial indicators of the purity and quality of crude drugs, providing insight into the presence of impurities such as carbonates, oxalates, and silicates. In this study, the water-soluble ash content was found to be 17.5%, primarily consisting of silica, particularly from sand, suggesting potential contamination with earthy material. The acid-insoluble ash value was determined to be 1.5%, while the total ash content was 21.93%, representing both physiological and non-physiological ash. Non-physiological ash consists of inorganic residues that remain after incineration of the plant material and serves as a useful parameter for detecting adulteration in herbal drugs [17].

Analysis of the alcohol and water extractive values revealed that the water extractive value was higher than the acid extractive value for *C. integrifolia*. Water, due to its high polarity, is capable of extracting a greater range of phytochemical constituents compared to alcohol, which has lower polarity. However, this does not fully explain why water is the preferred solvent among traditional medicine practitioners. Despite alcohol's lower extraction capacity, it is sometimes preferred in natural product research because of its preservative properties, its ability to inhibit microbial growth, and its ease of evaporation and handling [18]. Qualitative phytochemical screening of the methanol leaf extract of *C. integrifolia* confirmed the presence of carbohydrates, flavonoids, steroids, cardiac glycosides, triterpenes, tannins, alkaloids, and saponins. The medicinal value of plants is largely attributed to their phytochemical constituents, which exert various physiological effects on the human body [19]. The presence of alkaloids, flavonoids, tannins, saponins, and glycosides in *C. integrifolia* is consistent with previous findings [20]. Flavonoids are well known for their potent antioxidant properties. They are one of the most abundant polyphenolic compounds in the human diet and are widely distributed in plants [21]. Additionally, flavonoids exhibit significant anti-inflammatory activity due to their ability to scavenge hydroxyl radicals, superoxide anions, and lipid peroxyl radicals [22]. Alkaloids, which have been utilized by humans for centuries, are a diverse group of nitrogen-containing compounds found in approximately 20% of plant species. These compounds form the basis of several pharmacologically important drugs, including analgesics (e.g., morphine, codeine), anticancer agents (e.g., vincristine, vinblastine), gout suppressants (e.g., colchicine), muscle relaxants (e.g., tubocurarine), antiarrhythmics (e.g., ajmalicine), antibiotics (e.g., sanguinarine), antimalarial agents (e.g., quinine), sedatives (e.g., scopolamine), and stimulants (e.g., caffeine) [23, 24]. Tannins, on the other hand, have long been used in wound treatment, particularly for varicose ulcers and hemorrhoids [25]. The antioxidant activity of *C. integrifolia* methanol leaf extract was evaluated using DPPH radical scavenging activity. While the antioxidant potential of the leaf extract was lower than that of standard gallic acid at 517 nm, the inhibition concentration at 1000 µg/mL was found to be 93.61 µg/mL for the methanol leaf extract compared to 98.75 µg/mL for gallic acid. The IC50 value (the concentration required to scavenge 50% of DPPH radicals) for *C. integrifolia* was determined to be 74.192 µg/mL, whereas that of gallic acid was 0.009 µg/mL. These results indicate that the antioxidant activity of *C. integrifolia* increases with higher extract concentrations, effectively inhibiting free radical activity. According to [26], antioxidant activities are classified into five categories: highly active (<50 µg/mL), active (50–100 µg/mL), moderate (101–250 µg/mL), weak (250–500 µg/mL), and inactive (>500 µg/mL). Based on these classifications, *C. integrifolia* can be categorized as an active antioxidant agent. Additionally, [27] reported that IC50 values between 200 and 1000 µg/mL indicate lower antioxidant activity, yet such compounds may still have potential antioxidant benefits. The relatively high polyphenolic content in *C. integrifolia* likely contributes to its significant antioxidant activity. When consumed, plant-based products rich in such bioactive compounds may confer similar protective effects against oxidative stress-related diseases [28]. The search for natural alternatives to synthetic antioxidants has driven significant interest in medicinal plants that can prevent, reverse, or mitigate oxidative stress-induced diseases [29]. Based on the findings of this study, the alternate hypothesis is accepted, and the null hypothesis is rejected, confirming the antioxidant potential of *C. integrifolia*.

## 5. CONCLUSION

This study establishes the pharmacognostic profile and antioxidant potential of *C. integrifolia* leaves, which will help in setting a suitable plant profile for the proper identification, quality control, and compilation of a suitable monograph on the plant. The antioxidant activity assessment revealed moderate to high free radical scavenging potential,

classifying *C. integrifolia* as an active natural antioxidant. These findings validate its folkloric applications and underscore the need for further research to isolate and characterize the specific bioactive constituents responsible for the antioxidant effects.

**Declarations**

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**Conflict of Interest**

Authors have declared that no conflict of interest exists.

**Contribution of the Authors**

This work was carried out in collaboration with all other authors. SMZ and AAA designed the study and performed the experimental procedures and SMZ and AN wrote the first draft of the manuscript. Authors JMZ and AN supervised the lab experiment. All authors read and approved of the final manuscript.

**6. REFERENCE**

[1] Ayeni EA, Nuhu A. *Tetracarpidium conophorum* (African walnut) Hutch. & Dalziel: Ethnomedicinal uses and its therapeutic activities. *J Med Plants Econ Dev.* 2018;2(1):1-10. https://doi.org/10.4102/jomped.v2i1.47

[2] Chikezie PC, Ojiako OA, Nwufo KC. Overview of anti-diabetic medicinal plants: The Nigerian research experience. *J Diabetes Metab.* 2015;6:546. doi:10.4172/2055-6156.1000546

[3] Nuhu A, Danmalam UH, Ilyas N, Zakariya AM, Abdulhamid Z, Abubakar AZ. Pharmacognostic evaluation of the leaves and stem-bark of *Commiphora africana* (A. Rich) Engl. (Burseraceae). *Niger J Nat Prod Med.* 2016;20(1):56-60. http://dx.doi.org/10.4314/njnpm.v20i1.10

[4] Imam KI, Ahmed A, Ilyas N, Nuhu A. Pharmacognostic studies and development of quality control parameters for leaves of *Albizia chevalieri* Harms (Fabaceae). *FUDMA J Sci.* 2019;3(4):251-7.

[5] Burkill HM. *The Useful Plants of West Tropical Africa*. 2nd ed. Royal Botanic Gardens; 2004. p. 98-157.

[6] Sattarian A, Van Den Berg RG, Van Der Maesen LJG. Pollen morphology of *Celtis* (Celtidaceae). *Feddes Repertorium.* 2005;177(1-2):34-40.

[7] Herber PND, Penton EH, Burns JM, Janzen DH, Winnie H. Ten species in one: DNA barcoding reveals cryptic species in the semitropical skipper butterfly *Astraptes fulgerator*. *Proc Natl Acad Sci U S A.* 2004;101(41):14812-7.

[8] Mahre MB, Umaru B, Ojo NA, Saidu AS, Yahi D, Ibrahim RM, Mshelbwala PP. Acute toxicity, phytochemistry, and anti-diarrheal effects of *Celtis integrifolia* Lam. aqueous leaf extract in Wistar albino rats. *Br J Pharm Res.* 2016;14(5):1-7.

[9] Sa'id AI, Abba HM. Phytochemical screening, antimicrobial, and antioxidant activities of *Celtis integrifolia* (Lam.) leaves. *Int J Sci Res Eng Dev.* 2021;4(2):13-21.

[10] Evans WC. *Trease and Evans Pharmacognosy*. 16th ed. W.B. Saunders; 2009. p. 191-393.

[11] World Health Organization (WHO). *Quality Control Methods for Medicinal Plants*. WHO; 2011. p. 9-31.

[12] Brand-Williams W, Cuvelier ME, Berset WT. Use of free radicals method to evaluate antioxidant activity. *Food Sci Technol.* 1995;28:25-30.

[13] Graca J, Cabral V, Santos S, Lamos B, Sena O, Mohinas M. Partial depolymerization of genetically modified potato tuber periderm reveals intermolecular linkages in suberin polyester. *Phytochemistry.* 2015;117:209-19.

[14] Eggeling WJ, Willian J, Ivan RD. *The Indigenous Tree of the Uganda Protectorate*. Government Printer Entebbe, Uganda and Crown Agents of the Colonies, MB London SW; 2000. p. 130-2.

[15] Bharat G, Parabia MH. Pharmacognostic evaluation of bark and seeds of *Mimusops elengi* L. *Int J Pharm Pharm Sci.* 2010;2:110-3.

[16] British Herbal Pharmacopoeia. *British Herbal Medicine Association*. 1st ed. Dorset: Bournemouth; 1990. p. 1-2.

[17] World Health Organization (WHO). *Quality Assurance of Pharmaceuticals: A Compendium of Guidelines and Related Materials, Good Manufacturing Practices and Inspection*. WHO; 1996. p. 40.

[18] Ajazuddin N, Shailendra S. Evaluation of physicochemical and phytochemical properties of Safoof-E-Sana, Unani polyherbal formulation. *J Pharmacogn Res.* 2010;2(5):318-22.

[19] Amin MM, Sawhney SS, Jassal MMS. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *J Pharmacol.* 2013;2:1-5.

[20] Musa A, Auwal BA. Anticonvulsant activity of methanol leaf extract of *Celtis integrifolia* Lam (Ulmaceae) in chicks and mice. *Afr J Pharm Ther.* 2017;6(2):117-22.

[21] Spencer H, Jason M, Rodolt M, Sophie R, Emma F, Toner M, Marica C. Water carbonate chemistry. *Int Soc Cult Hist.* 2008;454:96-9.

[22] Okwu DE. Phytochemicals and vitamin content of indigenous spices of Southeastern Nigeria. *J Sustain Agric Environ.* 2004;6(1):30-7.

[23] Kam PC, Liew A. Traditional Chinese herbal medicine and anesthesia. *Anaesthesia.* 2002;57(11):1083-9.

[24] Ramawat KG. Secondary metabolites in nature. In: Ramawat KG, Merillon JM, editors. *Biotechnology: Secondary Metabolites.* Enfield, CT: Science Publishers; 2007. p. 21.

[25] Njoku PC, Akumufula MI. Phytochemical and nutrient evaluation of *Spondias mombin* leaves. *Pak J Nutr.* 2007;6(6):613-5.

[26] Jun M, Fu HY, Hong J, Wan X, Yang CS. Comparison of antioxidant activities of isoflavones from kadzu root (*Pueraria lobata* Ohwi). *J Food Sci.* 2003;68:2117-22.

[27] Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl for estimating antioxidant activity. *Songklanakarin J Sci Technol.* 2004;26:211-9.

[28] Ojiewo C, Tenkouano A, Hughes JA, Keatinge JDH. Diversifying diets: using indigenous vegetables to improve profitability, nutrition, and health in Africa. *Routledge.* 2013;291-302.

[29] Moriasi G, Anthony I, Mathew PN. In vitro antioxidant activities of the aqueous and methanolic stem bark extracts of *Piliostigma thonningii*. *J Evid Based Integr Med.* 2020;25:1-9.