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Phytochemical Screening, Antimicrobial and Antioxidant Activities of *Celtis integrifolia* (Lam.) Leaves

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Abstract:

Assessment of phytochemical composition, antimicrobial and antioxidant activities of the methanolic leaves extract of *C. integrifolia* (Lam.) was performed. The screening for phytoconstituents was achieved using standard methods revealed the presence of Alkaloids, Saponins, Phenols, Terpenoids, Steroids, Tannins, Flavonoids, and Glycosides while Anthraquinones was absent. Alkaloids had (19.7%), Saponins (107.78mg DE/g), Phenols (22.83mg GAE/g), Terpenoids (45.8%), Steroids (664.3µg/ml), Tannins (270.56mg TAE/g), Flavonoids (487.33mg QE/g) and Cardiac Glycosides (10.2%). Microbes used were subjected to biochemical tests for proper identification for clinical isolates of *Salmonella typhi, Staphylococcus aureus, Escherichia coli, Shigella spp.*Agar well diffusion method was used at concentrations; 500mg/ml, 250mg/ml, 125mg/ml 62.5mg/ml. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract ranges from 250-500mg/ml:*Salmonella typhi*, 125-500 mg/ml:*Staphylococcus aureus*, 250 -500mg/ml:*Escherichia coli* and 125-250 mg/ml: *Shigella spp*. 125-250 mg/ml respectively. The extract showed significant inhibition against all the test organisms, hence bactericidal in its effects. The Ferric-reducing antioxidant power (FRAP) of the leaf extract showed significant activity with 43.11mgAAE/g. The half maximal activity concentration (AC_{50}) was 10.87mg/ml and 9.43mg/ml for ascorbic acid standard. This study concludes that the leaf extract of *C. integrifolia* is related to recent findings that led to the identification of compounds having pharmacological effects such as antimicrobial, anti-inflammatory and antioxidant properties. Thus, *C. integrifolia* holds bioactive components that have extensive spectrum of pharmacological properties that can be used as a good source of drugs to manage typhoid, bleeding and dysentery.

Keywords ***—Celtis integrifolia,*** **Phytochemicals, Antimicrobial, Antioxidants**

I. INTRODUCTION

Celtis integrifolia (Lam.) commonly known as hackberry, African hackberry, or African nettle trees is a plant belonging to the *Cannabaceae* family, formerly classified under *Ulmaceae* family. It is locally called *Zuwo* in Hausa language, *Aspe* in Yoruba, *Ngezo* in Kanuri, *Gimachi* in Nupe, *Gamki* in Fulfulde and *Abun gatu* by Shuwa Arabs (Musa and Adam, 2017).

The genus *Celtis* has about 60-70 species of deciduous plants commonly found in the warm temperate regions of the Northern Hemisphere, Southern Europe, Southern and Eastern Asia, Southern and Central North America, South to Central Africa, Northern and South America (Mahre *et al.,* 2016). In Nigeria, the species *C. integrifolia* is commonly found in Northern

Nigerian savannah in places like Kano Borno, Gombe, Kaduna, Adamawa, Yobe and Bauchi state (Musa and Adam, 2017, Ezekiel, 2018, Tadzabia and Dimas, 2020). These plants have been used to support mankind sustain its well-being since the dawn of medicine. They are the most cherished bioresources of both traditional and modern drugs and have been the basis of health preservation and care (Anarado *et al.,* 2020).

PLATE 1: THE PLANT: *CELTISINTEGRIFOLIA* (LAM.)

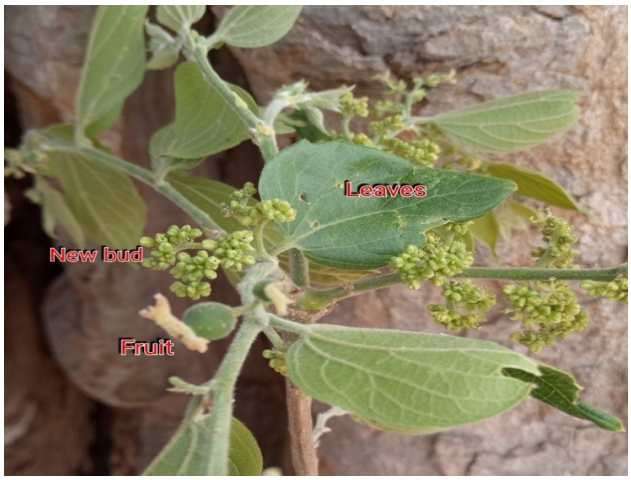

PLATE 2: THE PLANT: LEAVES, NEW BUD AND FRUIT.

Majority of the species of the genus *Celtis* have been found to possess medicinal values. *C. africana*, *C. australis* and *C. occidentalis* have been reported to possess phytocompounds that exhibit numerous important pharmacological activities such as antioxidant and cytotoxic properties (Ezekiel, 2018). *C. integrifolia* is implicated in the management of epilepsy (Manchishi, 2018), mental disorder, weakness, as pain killer, treatment of Chicken pox, Measles, Gout, Ecbolic, treatment of Diarrhea, Sore throat (Mahre *et al.,* 2016), Cancer, wound healing, bleeding, Spices and Aphrodisiac in Northern Nigeria.

With new discovery in science of the adverse effects of synthetic drugs, people are clamouring for the days when medication was comprised solely of natural products, which have low toxicity and no known side effects. Hence there is upsurge in the use of herbal remedies. There is therefore the need for a thorough scientific evaluation to validate or disprove the supposedly therapeutic effects of some of these medicinal plants. The aim of this study was to evaluate the phytochemical, antimicrobial and antioxidant activities of *Celtis integrifolia* in Ndala-diyo (Jalam district) Dambam L.G.A of Bauchi State, Nigeria.

II. MATERIALS AND METHODS

Sample Collection and Authentication

The leaves of *Celtis integrifolia* plant was collected from Ndala-diyo (Jalam district) Dambam L.G.A of Bauchi State, located at latitude 11°33'43.75"North, Longitude 10°50'14.84"East Nigeria (Fig.1). The plant material was identified and authenticated by a specialist, given voucher number GUH 192 and deposited at the herbarium of Department of Botany, Gombe State University Gombe, Nigeria. The Fresh leaves of the plant were collected and air dried. The dried material was pulverized to powder.

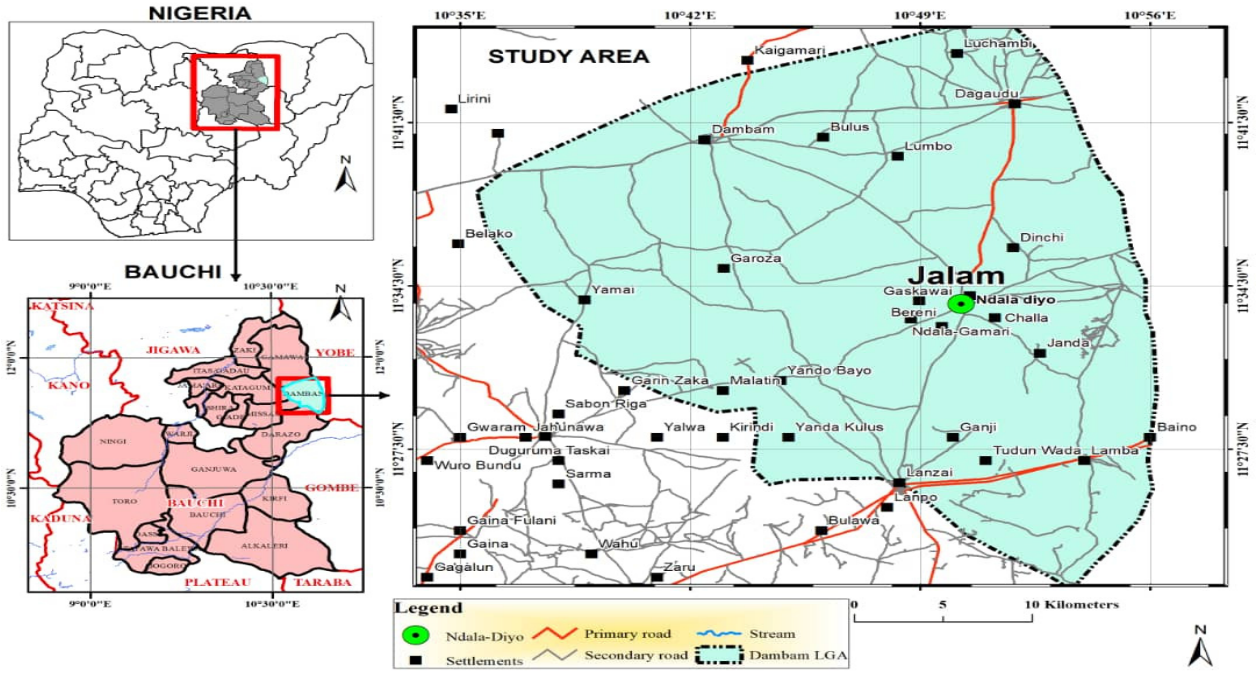

Figure 1: Map Showing Study Site of Plant Collection. **Source: Field work (2020).**

Extraction of Phytochemicals from *Celtisintegrifolia*(Lam.) Leaves

About Sixty gram (120g) of the powdered sample of the plant was weighed on an electric balance and transferred into a labelled conical flasks containing 800ml of methanol. The mixture was placed on a rotary shaker and agitated for three days and then filtered. The filtrate was then transferred into another flask, and heated on a water bath to recover the extract (Bhojwani and Dantu, 2013). A measurement of 3 grams of the methanol extract was analysed for the presence of alkaloids, anthraquinones, saponins, phenols, terpenoids, steroids, tannins, flavonoids and glycosides according to standard methods (Zak *et al.,* 1954; Ferguson, 1956; Harborne, 1973; Hiai *et al.,* 1976;Mallick and Singh, 1980;Sofowora, 1993; Evans, 2002; Gini and Jeya 2013; Banu and Cathrine, 2015; Salman *et al.,* 2015; Twinkle and Salalkar, 2015; Madhu *et al.*, 2016; Ogungbenro*et al.,* 2018). The constituents were qualitatively and quantitatively determined.

Determination of Antimicrobial Activity:Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Leaves Extract

Four different microbes were used, three of which are gram negative and one gram positive strain; *Salmonella typhi, Escherichia coli, Shigella spp.* and *Staphylococcus aureus.*The test organisms were aseptically sub cultured on nutrient agar and thereafter double disc synergy test (DDST) method was performed to identify beta lactamase producing organisms as described by Cheesbrough, (2006).The test organisms were standardized until turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard ((Clinical & Laboratory Standards Institute [CLSI], 2012).A 500 mg/ml concentration of the extracts were constituted by dissolving 0.5 g in 1 ml each of 20% v/v dimethyl sulfoxide (DMSO) and 2-fold serial dilutions were made. Using a sterile cork borer, four wells of 6mm in diameter were bored in the inoculated Muller Hinton agarusing a micropipette

(200=50µl) of each concentration. The plates were left on the bench for 30 minutes to allow the extracts to diffuse into the agar and incubated at 37°C for 24 hours. Each test was carried out in triplicate and the mean inhibition zone diameter were recorded to the nearest whole millimetre (Table 3) according to standard method of Esimone *et al.,* (2003).To measure the Minimum inhibitory concentration (MIC), various concentrations of the stock at 500mg/ml, 250mg/ml, 125mg/ml 62.5mg/ml were prepared against a positive control Augmentin 30µg (Esimone *et al.,* 2003). The minimum bactericidal concentration (MBC) was determined by sub culturing the last test dilution that showed visible growth (turbidity) and all others in which there was no growth on a fresh extract solid medium. The least concentration of extracts that showed no single or with lowest bacterial colonies was taken as the minimum bactericidal concentration MBC according to standard method of Bergen *et al.,*(2010) (Table 4).

Antioxidant Assay(Ferric reducing anti-oxidant power (FRAP) assay

Different concentrations of the sample (leavesextract) in various fractions (10-50 µg/mL) were prepared. 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solutions were added. The mixture was vortex and incubated at 50°C for 20 min, then mixed with 2.5 mL of 10% trichloroacetic acid. The solutions were then centrifuged at 3,000 rpm for 10 min. About 0.5 mL of 0.1% ferric chloride 2.5 mL of the supernatant and mixed well. The absorbance was measured at 700nm after 10min against the blank with reference to standard using UV-Spectrophotometer (CE 7400)as described by Vijayalakshmi and Ruckmani, (2016).Results were expressed as mg Ascorbic acid equivalents per gram of dry extract.

111. RESULTS.

Table 1: Qualitative phytochemical test of *Celtis integrifolia* leaf extract.

Phytochemical Compounds	Methanolic Extract
Tannins	++
Steroids	++
Phenols	++
Alkaloids	++
Glycosides	++
Flavonoids	++
Saponins	++
Anthraquinones	--
Terpenoids	++

***Key**: (++) =Present, (--) Absent.*

Table 2: Quantitative phytochemical test of *Celtis integrifolia* leaf extract.

Phytochemical Compound	Concentration
Alkaloids (%)	19.7
Cardiac glycosides (%)	10.2
Terpenoids (%)	45.8
Saponins (mg DE/g)	107.78
Phenols (mg GAE/g)	22.83
Flavonoids (mg QE/g)	487.33
Tannins (mg TAE/g)	270.56
Steroids (µg/ml)	664.3

Table 3: Zone of inhibition (mm) of Methanolic leaf extract of *Celtisintegrifolia* against the tested organisms.

Conc. mg/ml		Test organisms							
		Salmonella typhi		***Staph. Aureus***		***E. coli***		***Shigella spp.***	
		Z. D	**S/R**	**Z. D**	**S/R**	**Z. D**	**S/R**	**Z. D**	**S/R**
A1	**500**	23.0±0.58	S	20.0±1.33	S	18.0±0.88	S	19.0±1.53	S
A2	**250**	18.0±0.88	S	15.0±1.45	S	16.0±0.67	S	12.0±1.00	S
A3	**125**	15.0±0.88	S	9.00±0.58	S	12.0±1.00	S	10.0±0.58	S
A4	**62.5**	10.0±0.33	S	6.00±0.00	R	6.00±0.00	R	6.0±0.00	R
AUG	**30µg**	10.0±0.33	S	11.0±0.58	S	10.0±0.33	S	11.0±0.33	S

Key: AUG 30µg = *Augmentin 30µg (Control), S=Sensitive, R=Resistant, Z. D= Zone diameter (mm)* ***Note****: Diameter of zone of inhibition is 6 mm; any zone of inhibition greater than 6 mm implies activity; n=3, values are given in Standard error of mean (SEM).*

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Methanol leaf extract of *Celtisintegrifolia* against the tested organisms.

Test organisms	Concentrations (mg/ml)	
	MIC	**MBC**
Salmonella typhi	250	500
Staph. aureus	125	500
Escherichia coli	250	500
Shigella spp.	125	250

Table 5: Showing Half maximal activity concentration (AC_{50}) of Ascorbic acid (Standard) and leaf extract of *Celtisintegrifolia*.

Concentration (mg/ml)	
Half Maximal Activity Concentration (AC_{50})	
Ascorbic Acid	**Leaf extract**
9.43	10.87

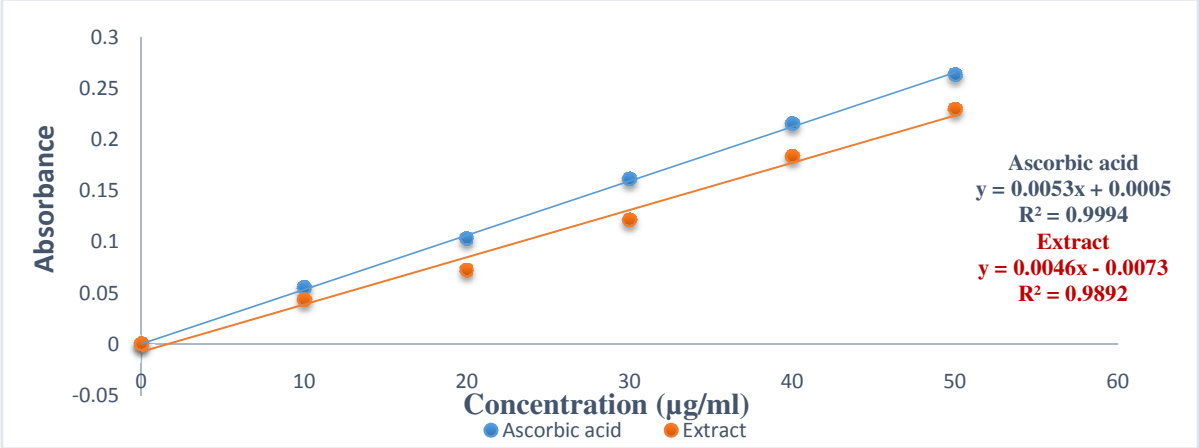

Figure 2: Showing (Antioxidant assay) Standard Graph of Ascorbic acid (Vitamin C) and methanolic leaf extract of *Celtis integrifolia*.

IV. DISCUSSION

Phytochemical analysis

The present study undertaken revealed the presence of a number of bioactive compounds which can be used as a lead compound for synthesizing drugs for various ailments(Bernardini *et al.,* 2018; Ogunnupebi *et al.,* 2020).The results of the phytochemical analysis revealed that the methanol extracts were positive for Phytocompounds like Alkaloids, Saponins, Phenols, Terpenoids, Steroids, Tannins, Flavonoids and Glycosides. However, Anthraquinones was not detected (Table 1). This is in line with the works of Mahre *et al.,* (2016) in the aqueous leaf extract of *Celtis integrifolia* who also confirmed the presence of tannins, saponins, flavonoid, phenol, Cardiac glycosides, steroids, while Alkaloids and Anthraquinones were not detected. The quantitative analysis revealed astonishing results with alkaloids (Table 1), having (19.7%), Phenols 22.83mg GAE/g, Flavonoids 487.33 mg QE/g, Saponins 107.78mg DE/ml, Terpenoids 45.8%, Steroids (664.3µg/ml), Tannins (270.56mg TAE/g), Flavonoids (487.33mg QE/g) and C. Glycosides (10.2%) as shown in (Table 2).These active phytochemical components are known for their medicinal and biological activities as well as their physiological actions; as such they determine the therapeutic potentials of all medicinal plants (Samatha *et al.,* 2017; Usman *et al.,* 2018; Steven *et al.,* 2019; Abba and Abubakar, 2019; Amir *et al.,* 2020; Anarado *et al.,* 2020).

Antimicrobial activity

The antimicrobial activities of the methanol leaf extract of *C. integrifolia* against test isolates; *Salmonella typhi, Escherichia coli, Shigella spp.* and *Staphylococcus aureus* exhibited a strong antimicrobial potential.Anincrease in the zones of inhibition was observed with increasing concentrations from 62.5 mg/ml to 500mg/ml (Table 3). The highest inhibition zone was found against *Salmonella typhi* (23.0±0.58mm), followed by *Staphylococcus aureus* (20.0±1.33mm), *Shigella spp.* (19.0±1.53mm) *and Escherichia coli* (18.0±0.88mm). At a concentration of 250mg/ml (Table 3), the highest inhibition zone was found against *Salmonella typhi* (18.0±0.88mm), followed by *Staphylococcus aureus* (15.0±1.45mm), *Escherichia coli* (16.0±0.67mm) and *Shigella spp.* (12.0±1.00mm). At a concentration of 125mg/ml (Table 3), the highest inhibition zone was found against *Salmonella typhi* (15.0±0.88mm), followed by *Escherichia coli* (12.0±1.00mm), *Shigella spp.* (10.0±0.58mm) and *Staphylococcus aureus* (9.00±0.58mm). At a concentration of 62.5mg/ml (Table 3), the highest inhibition zone was found against *Salmonella typhi* (10.0±0.33mm), followed by *Staphylococcus aureus* (6.00±0.00mm), *Shigella*

spp. (6.00±0.00mm) *and Escherichia coli* (6.00±0.00mm).The MICs and MBCs of the leaf extract ranges from250-500mg/ml for *Salmonella typhi*, 125-500 mg/mlfor *Staphylococcus aureus,* 250 -500mg/mlfor *Escherichia coli* and 125-250 mg/mlfor *Shigella spp.* 125-250 mg/ml respectively (Table 4). This suggest that the plant can be used as a good source of drugs to cure inflammatory diarrhea, abdominal cramp, typhoid, fever, pneumonia, boils, styes, meningitis, haemorrhagic colitis, wound healing and bleeding.

FRAP Antioxidant assay

The FRAP examination for antioxidant activity showed vital potential. The reducing ability and antioxidant potentials of the methanol extracts of the leaves of *C. integrifolia* was estimated from its ability to reduce the Fe^{3+}-2,4,6-tripyridyl-s-triazine (TPTZ-Fe (III)) complex to Fe^{2+}-2,4,6-tripyridyl-s-triazine (TPTZ-Fe (II)) complex, having 43.11mgAAE/g.**Figure 2** revealed an increasing trend in total antioxidant capacity with increasing the extracts concentrations. The calculated half maximal activity concentration (AC_{50})was10.87mg/ml and 9.43mg/ml for ascorbic acid standard (Table 5). The activity of the extract on FRAP implies that the extract contains bioactive constituents that stimulates antioxidant properties and possibly could be the reason why the plant is used by the locals to cure epilepsy (Manchishi, 2018), as pain killer (analgesic), Measles, Gout, diarrhea (Mahre *et al.,* 2016), Cancer, wound healing, and bleeding (Abah *et al*., 2018; Ezekiel, 2018).

V. CONCLUSION

The leaf extracts of *C.integrifolia* possesses phytochemicals such as Alkaloids, Saponins, Phenols, Terpenoids, Steroids, Tannins, Flavonoids and Glycosides in varying quantities. Most of the phytochemicals have shown valuable therapeutic activities such Antifungal, antimicrobial, anticonstipative, anti-inflammatory, antiplasmodial, hepatoprotective, anticancer, and antioxidants activities. The antimicrobial activities of the methanol leaf extract of *C.integrifolia* against test isolates; *Salmonella typhi, Escherichia coli, Shigella spp.* and *Staphylococcus aureus* revealed a strong antimicrobial potential. An increase in the zones of inhibition was observed with increase at different concentrations. This suggest that the plant can be used as a good source of drugs to cure inflammatory diarrhea, abdominal cramp, typhoid, fever, pneumonia, haemorrhagic colitis, wound healing and bleeding. The bioactivity assay exhibited moderate level of antioxidant properties using Ferric-reducing antioxidant power (FRAP). These bioactivities exhibited by the leaf extract is in line with numerous research on plant extracts that could rise to novel therapeutic/pharmacological agents.

RECOMMENDATION

Further research are recommended to focus on:

1. Isolation, purification and molecular characterization of the bioactive compounds responsible for the activity of this plant.
2. Evaluate the antibacterial and antifungal ability with callus extract of the plant.
3. Utilization of solvents other than methanol should also be given preference in future studies, as it could lead to the separation of some new therapeutic compounds that could be active against microbes which could result in development of new antimicrobial drugs with lesser side effects.
4. The metabolic isolate responsible for the antioxidant and other pharmacological properties exhibited by the plant parts (stem bark and root) and the development of the metabolites into drugs.

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