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ANTIMICROBIAL POTENCY AND PHYTOCHEMICAL CONTENTS OF THREE COMMON NIGERIA MEDICINAL PLANTS' LEAF EXTRACTS

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Abstract *Morinda lucida*, *Tithonia diversifolia* and *Carica papaya* are three medicinal plants commonly used for curing several diseases in traditional medicine. However, comparative scientific information as regards their antibacterial, antifungal, and phytochemical properties remains limited. Fresh leaves of *Morinda lucida, Tithonia diversifolia,* and *Carica papaya* were harvested and extracted by soaking pulverized samples of the plants in 250 mL of water and ethanol. The phytochemical constituents were determined by subjecting the decoction to qualitative and quantitative tests using standard methods. The antimicrobial and antifungal activities were determined by solidified Mueller-Hinton agar plate and solidified Potato Dextrose agar plate respectively. Alkaloids, flavonoids, saponins, tannins, and terpenoids were shown to be the primary metabolites in the ethanolic extract of the three plant species. Cardiac glycosides are only present in *T. diversifolia.* Aqueous and ethanolic leaf extracts of the three species had significant, but varying antibacterial activity and no antifungal properties. Ethanolic extracts of *Morinda lucida* were the most active against *Escherichia coli* with a 30 mm zone of inhibition and very active against *Klebsiella pneumonia* and *Enterobacter aerogenes* with 28 mm and 24 mm zone of inhibition, respectively. The aqueous extracts of *T. diversifolia* were more active against *Staphylococcus aureus*, *Serratia marscences,* and *Enterobacter aerogenes* than ethanolic extract. The ethanolic decoction of *C. papaya* was active against *Enterococci.* This study showed that depending on the decoction solvent and the microbes under investigation, the leaf extracts of the three plant species have a tolerable but variable level of activity.

Keywords: *Morinda lucida*, *Tithonia diversifolia*, *Carica papaya,* phytochemicals, antibacterial, antifungal

I. INTRODUCTION

The use of synthetic antibiotics has continued to be the leading therapy for combating infections from arrays of microorganisms such as several fungal pathogens as well as gram-positive and gram-negative bacteria [1-2]. However, the overdependence on these antibiotics had lessened their potency and resulted in the occurrence of drug resistant strains of different kinds of bacteria and fungi [1]. The effectiveness of conventional antibiotics may be lost within a 5-year period due to the rapid development of resistance by bacteria or fungal strains [3]. Hence, the need for alternative therapeutic agents.

Medicinal plants remain the most readily available and economical form of treatment for numerous human diseases, especially in rural communities of developing countries [4]. When compared to synthetic medications, medicinal plants are more practical and affordable, have more potent pharmacological effects, and are less poisonous. Findings have reported plants as an excellent natural source of several metabolites including alkaloids, tannins, flavonoids, and phenols [5-6]. With few to no side effects, these metabolites function as therapeutic and preventative agents for maintaining a strong immune system against

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microbial infections and other illnesses [7]. They are potent sources of antiviral, antimicrobial, and anti-inflammatory agents [8- 9]. However, given that plants differed in their habit as well as growth forms, their qualitative and quantitative active biomolecules as well as potency against diseases could differ. For example, Bibi [2] reported that while *Cuscuta reflexa* inhibited all investigated bacterial strains, *Klebsiella pneumoniae* had no significant effects. Similarly, Darwish [10] noted that while *Juniperus horizontalis* displayed the highest potency against *E. coli*, *Juniperus communis* showed the highest activity against *S. aureus.* Findings had reported the quality and quantity of plant phytochemicals to be directly related to plant parts, geographical regions, and the solvents used for extraction [11]. However, there remains a limited biological knowledge of native species in most tropical countries such as Nigeria. This constitutes a breach of use and development of active compounds against varieties of microbial infections from the available abundant plant resources.

Morinda lucida (benth), *Tithonia diversifolia* (Hemsl) A. Gray, and *Carica papaya* are some of the medicinal plants frequently used in Nigeria to treat a variety of diseases. The three plant species belong to two major life forms (tree and herb) and have been documented to have ethnomedicinal properties. *M. lucida* is a tropical West African rainforest tree of the family Rubiaceae. Studies have shown that *M. lucida* stems, bark, roots, and leaves are used locally as antimalarial antidiabetics, antimicrobial, and to cure jaundice [12]. The plant is reputed in Sub-Saharan Africa for its usefulness in folk medicines against fever [13]. *T. diversifolia*, on the other hand, is a perennial or annual shrub-like plant that is invasive in Africa but endemic to North and Central America [14]. In its native and most parts of its invasive regions, *T. diversifolia* is used for treating a variety of ailments. Ailments such as abscesses, muscular pain, diabetes, wounds, and skin infections can be treated using leaf extract of *T. diversifolia*. In Uganda, it is employed as a curative for infection in sexual organs [15], and in Nigeria, it is used to treat malaria.

C. papaya Linn. Is a fast-growing plant of the family Caricaceae. All the parts of *C. papaya* including leaves, stems, root fruits, flowers, and seeds are locally used to heal numerous ailments [16]. Leaf of *C. papaya* is effective against oral candidiasis, malaria, yellow fever and as a diuretic in anemics [17].

Although findings on the phytochemical and antimicrobial properties of herbal mixtures are numerous [2], so far comparative information about the antimicrobial properties of *M. lucida*, *T. diversifolia,* and *C. papaya* remains limited. This study elucidates the phytochemical and antimicrobial efficacy of the water and ethanolic decoction of leaves of *M. lucida*, *T. diversifolia,* and *C. papaya* using seven bacterial species and three fungi isolates. It is expected that the outcome of this study will guide intervention programs aimed at finding an alternative for combatting microbial infections and add to the list of plants from which antimicrobial drugs can be formulated.

II. MATERIALS AND METHODS

A. Collection and Identification of plant materials

Fresh leaves of *Morinda lucida, Tithonia diversifolia* and *Carica papaya* were harvested in March 2020 from Isale-Osun in Osogbo, Osun State, and were authenticated at the herbarium section of the Department of Plant Biology, Osun State University, Osun State, Nigeria.

B. Plant preparation, extraction and Phytochemical Screening

The leaves were air-dried for five days and pulverized into powdery form using an electric mill. The resulting powder was packed into a dry, sterile plastic container with a screw cap for subsequent work. The water and ethanol extractions of the leaf material were performed using the standard method [12]. Briefly, 50 g of the powdered samples were soaked in 250 mL of solvents (water and ethanol) in different sterilized conical flasks and plugged with wool. The mixture was placed in a shaking water bath and left to stand for 72 hrs at 37°C. This process was performed separately for each of the three species, and placed in separate labeled glass bottles. The mixtures were filtered using a muslin cloth and Whatman No.1 filter paper to obtain a homogeneous filtrate. The decoction was concentrated at 37°C using a rotary evaporator and stored at 4 °C. Decoctions were subjected to qualitative tests for the determination of the presence (+) or absence (-) of phytochemicals including alkaloids, flavonoids, cardiac glycosides, phenolics, tannins, saponins, and terpenoids.

C. Quantification of flavonoids

Aluminum colorimetric method was used for the quantification of flavonoid content [18]. Briefly, 0.2 mL of aluminum chloride (10%) and 0.2 mL of potassium acetate were added to 1 ml of the decoction. Then, 3 ml of distilled water was added and gently mixed. After 30 minutes of incubation at room temperature, the mixture was tested at an absorbance of 415 nm. The total flavonoid concentration was expressed as mg/g quercetin equivalent using a calibration curve.

D. Quantification of phenolic content

The total phenolics were quantified using Folin-Ciocalteu method. Extract (0.5 mL) was added to 5.0 ml of distilled water, and Folin-Ciocalteu reagent. Saturated sodium carbonate solution (1.0 mL) was added to the mixture after 3 min. These mixtures were shaken, allowed to stand for 1 h and then measured three times at 725 nm. The results were expressed as mg gallic acid equivalents (GAE).

E. Quantification of Alkaloids content

Alkaloids content was quantified by mixing 1 mL of test extract with 5 ml phosphate Buffer (pH 4.7) and BCG solution. A few drops of diluted sulphuric acid were added and the mixture was shaken with 4 ml of chloroform. The extracts were adjusted to volume in a 10 mL volumetric flask. Absorbance was measured at 470 nm against the blank solution.

F. Quantification of Tannins

Tannin content was determined by transferring the stock solution of the extracts (0.5 mL) into 25 mL capacity Erlenmeyer flasks. Five hundred milligrams (500 mg) of casein and 5 ml of distilled water were added to the flask. The extracts were filtered after 2 hours and the filtrates were used to quantify non-tannic phenols. Tannin content was estimated as tannic Acid Equivalent (TAE).

G. Quantification of Saponin

The saponin content of each plant species was analyzed by dissolving the extract in 80% methanol and then mixed with 2 Ml of Vanilin and 2 mL (72%) sulphuric acid solution. After gentle shaking, the mixture was heated at 60 °C for 10 min in a water bath. Using a reagent blank as a reference, the absorbance was measured at 544 nm. Diosgenin was utilized as a reference material, and its analogs were compared.

H. Microbial Isolates

The microorganisms used for the investigation were isolates obtained from the Microbiology Department of Osun State University, Osun State. The bacterial species used were *Pseudomonas aeruginosa*, *Staphylococcus aureus, Enterococci Enterobacter aerogenes, Serratia marscences, Escherichia coli, a*nd *Klebsiella pneumonia*. The fungi isolates used were *Fusarium oxysporum, Aspergillus versicolor,* and *Penicillium citrinium*.

I. Antibacterial Activities

Water and ethanol extracts of *M. lucida, T. diversifolia*, and *C. papaya* were applied to a solidified Mueller-Hinton agar plate using the agar well diffusion method [19]. Each plate was aseptically seeded by flooding with specific bacterial solutions (*Staphylococcus aureus, Enterobacter aerogenes, Serratia marscences, Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumonia,* and *Enterococci*) with sterile swab sticks and then allowed to settle for 10mins. Wells 8 mm in diameter and about 2 cm apart were punched in the culture media at the center of each inoculated plate and 0.1 ml of the extract was laid into each well on the inoculated media. Water and amoxycillin served as the positive and negative controls, respectively. They were also filled into each well on the inoculated plates. After 24 hours of incubation at 37°C with the inoculated plates, the zones of growth inhibition were checked. A transparent ruler was used to measure the zones of inhibition, and the result was recorded in millimeters (mm).

J. Antifungal Activity

The antifungal activities were performed on a solidified Potato Dextrose agar pate and each plate was aseptically seeded by flooding with fungal spore suspension (*Fusarium oxysporum, Aspergillus versicolor,* and *Penicillium citrinium*) with sterile swab stick and allowed to settle for 10 mins. Wells 8 mm in diameter and about 2 cm apart were punched into the culture media at the center of each inoculated plate and 0.1 ml of the extract was filtered and filled into each well on the inoculated media. Itraconazole (0.1 ml) was used for the positive control and 0.1 ml of distilled water for the negative control. They were also filled into each well of the inoculated media. After 48 hours of incubation at 37 °C, the infected plates were checked for zones where growth was inhibited.

III. RESULTS AND DISCUSSION

A. Results

i. Anti-bacterial and fungi activities of the three crude extract plant species

The rate of bacterial inhibition activities differed among the three plant species (Table 1). The crude ethanol extract of *C. papaya* inhibited five (5) of the seven tested bacterial species, while each of *M. lucida* and *T. diversifolia* inhibited 4 bacterial isolates. Ethanol extract of *M. lucida* strongly inhibited *S. marcescens, E. aerogenes, E. coli,* and *K. pneumonia,* but had no effects on other bacterial isolates. The highest antibacterial potency of ethanol decoction of *M. lucida* was observed in *E. coli* followed by *K. pneumonia*. Ethanol decoction of *T. diversifolia* strongly inhibits *S. aureus* (8 mm) and *E. coli* **(**12 mm), and faintly inhibits *E. aerogenes* and *S. marcescens* but had no effect on other bacterial isolates. Ethanol extract from *C. papaya* exerts a strong inhibitory effect on the bacterial isolates in order of *E. coli* **>** *S. aureus* **>** *Enterococci* > *E. aerogenes* (Table 1). Aqueous extracts of *M.*

lucida had no antibacterial activities on all the tested bacterial, while aqueous extract of *T. diversifolia* strongly inhibited the growth of *S. marcescens, E. aerogenes,* and *S. aureus*, but had no effect on other bacterial. The inhibitory upshot of the aqueous extract of *T. diversifolia* on these bacteria was stronger than that of the ethanol extract (Table 1).

In contrast, ethanol extract *C. papaya* leave had more antibacterial activity than the aqueous extracts. Neither the crude ethanol extract nor the aqueous extracts of the three plant species had inhibitory effects on any of the tested fungi isolates (Table 2).

Table 1: Inhibition Zone (mm) for each bacterial isolate by the three medicinal plant species

Isolates	*M. lucida*				*T. diversifolia*				*C. papaya*			
	Itraconazole (+ve CT)	Water (-ve CT)	Ethanol extract	Aqueous extract	Itraconazole (+ve CT)	Water (-ve CT)	Ethanol extract	Aqueous extract	Itraconazole (+ve CT)	Water (-ve CT)	Ethanol extract	Aqueous extract
S. marcescens	12	-	10	-	12	-	5	11	12	-	4	-
E. aerogenes	21	-	24	-	21	-	2	21	21	-	9	2
S. aureus (G+)	31	-	-	-	31	-	8	16	31	-	17	4
E. coli (G-)	29	-	30	-	29	-	12	-	29	-	21	12
P. aeruginosa (G-)	55	-	-	-	55	-	-	-	55	-	-	-
Enterococci	70	-	-	-	70	-	-	-	70	-	15	-
K. pneumonia	40	-	28	-	40	-	-	-	40	-	-	-

CT: Control; +ve: Positive; -ve: Negative

Table 2: Inhibition zone (mm) for each fungal isolate by the three medicinal plant species

Isolates	***M. lucida***				***T. diversifolia***				***C. papaya***			
	Itraconazole (+ve CT)	Water (-ve CT)	Ethanol extract	Aqueous extract	Itraconazole (+ve CT)	Water (-ve CT)	Ethanol extract	Aqueous extract	Itraconazole (+ve CT)	Water (-ve CT)	Ethanol extract	Aqueous extract
A. versicolor	**25**	-	-	-	**25**	-	-	-	**25**	-	-	-
P. citrinium	**20**	-	-	-	**20**	-	-	-	**20**	-	-	-
F. oxysporum	-	-	-	-	-	-	-	-	-	-	-	-

CT: Control; +ve: Positive; -ve: Negative

ii. **Variation in phytochemical content of the three plant species**

Alkaloids, flavonoid saponins, tannins, and terpenoids were all detected in the crude extract of the leaves of the three species (Table 3). Cardiac Glycosides were detected in *T. diversifolia* and absent in other species. Phenolic acid was detected in *C. papaya* and absent in other species**.** The quantitative determination of the crude decoction of the plant species showed varying quantities of phytochemical constituents among them (Figure 1). Alkaloids and saponins contents of *M. lucida* and *T. diversifolia* were significantly higher than that of *C. papaya* (Figure 1A and B). In contrast, *C. papaya* had the highest tannin and phenolic contents, while *M. lucida* had the least (Figures 1C and D). Compared to *M. lucida* and *C. papaya*, *T. diversifolia's* crude extract had a significantly higher flavonoid concentration. (Figure 1E).

Table 3: Qualitative analysis for phytochemical constituents present in *Morinda lucida, Tithonia divesifolia,* and *Carica papaya.*

Phytochemicals	*Morinda lucida*	*Tithonia diversifolia*	*Carica papaya*
Alkaloids	+++	+++	+++
Saponins	+++	+++	+++
Tannins	+++	+++	+++
Phenol	---	---	+++
Flavonoids	+++	+++	+++
Terpenoid	+++	+++	+++
Cardiac Glycosides	---	+++	---

+: Present; -: Absent

B. Discussion

The efficiency of medicinal plants to prevent or slow down the growth of any bacterium is what determines how effective they are as antimicrobial agents [2]. The genetic diversity of microorganisms however gives them the capacity to swiftly elude the action of antimicrobial agents by becoming resilient to them. In this study, ethanol decoction of *M. lucida* and *C. papaya* exhibited appreciable repressive activities against the majority of the tested bacteria strains but had no effect on the fungi. The observed inhibitory effects of ethanol extract are consistence with the finding on several plant species and pointed to the possibility of getting good antimicrobial properties from these plants through ethanol [7, 20]. Compared to the water extract, the ethanol extract of *M. lucida* showed a maximum inhibition of 30 mm against *Escherichia coli* and a minimum inhibition of 10 mm against *Serratia marcescens*. While *M. lucida* had no inhibitory effect on *Staphylococcus aureus,* it inhibits *Enterobacter aerogenes* and *Klebsiella pneumonia* by 24 and 28 mm respectively.

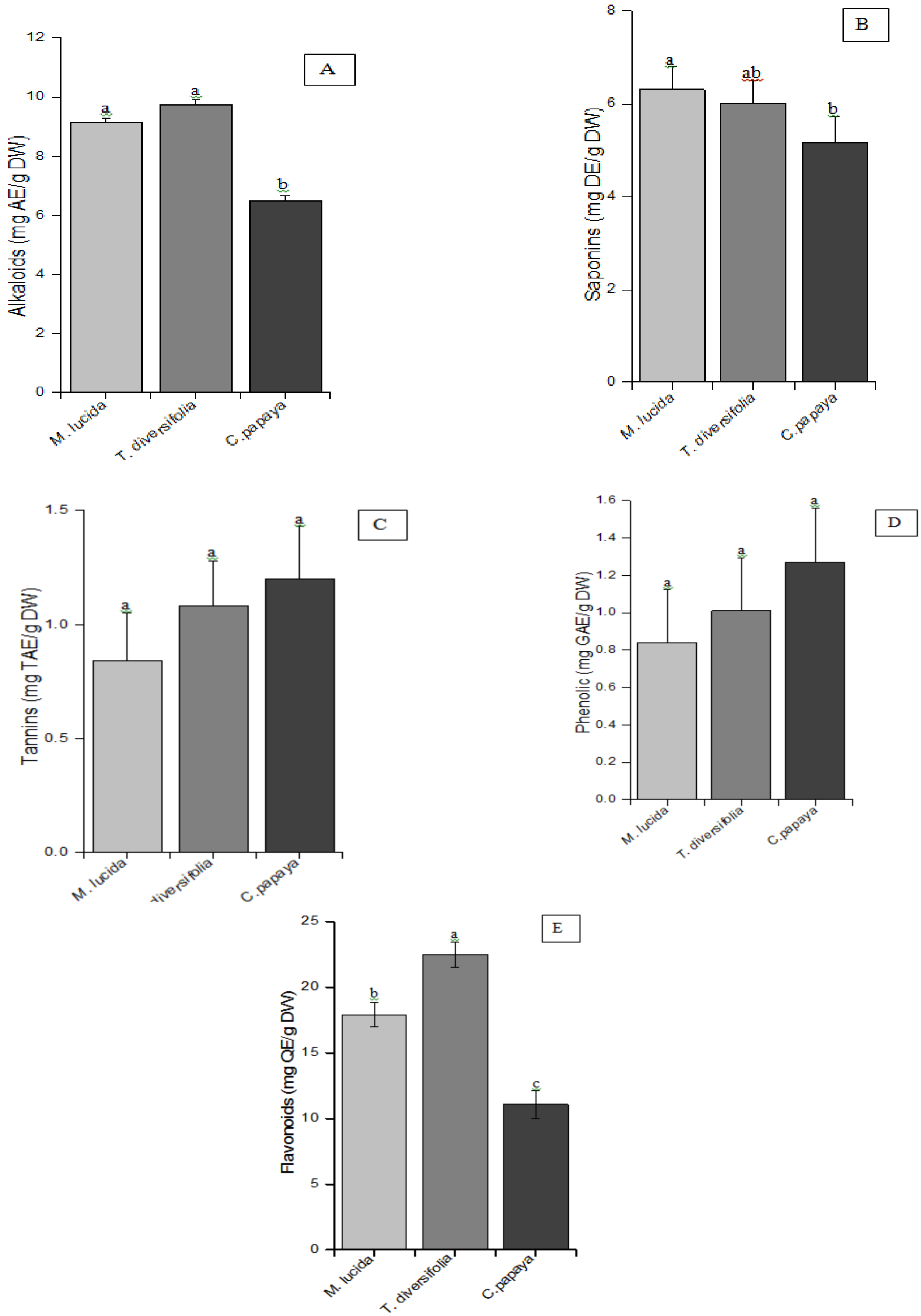

Figure 1: Quantitatively analyzed phytochemical constituent of the three medicinal plant species (A) Alkaloids, (B) Saponins, (C) Tannins, (D) Phenolic (E) Flavonoids. The different lower-case letter indicates significant differences among the three plant species at p<0.05

The variation in the inhibitory zone among the bacteria strains might be due to their genetic variability which patterned their response to the active phytochemical constituent of leaf extract [21]. The leaf extract of *M. lucida* had previously been found to include phytochemicals such as saponins, alkaloids, and anthraquinones [12]. In this investigation, the content of flavonoids present in the extract of *M. lucida* was quite higher than those of Alkaloids, Saponins, and Phenolic acid. Phytochemicals present in *M. lucida***,** especially flavonoids**,** might be reason for the antimicrobial activity displayed by the extracts. Moreover, findings noted that flavonoids give defense against cancers, viruses, bacteria, hepatotoxins, free radicals, inflammation, allergies, ulcers, and platelet aggregation [22].

Unlike in *M. lucida,* aqueous extract *T. diversifolia* leaves showed maximum inhibitory zone on three bacteria strains i.e., *Serratia marcescens*, *Enterobacter aerogenes,* and *Staphylococcus aureus* than ethanol extract (Table 1). The minimum inhibitory concentration range of 2 mm and 5 mm were noted in *Enterobacter aerogenes* and *Serratia marcescens* indicating very limited antimicrobial activities of the ethanol decoction of *T. diversifolia* leaves. While *P. aeruginosa*, *Enterococci,* and *Klebsiella pneumonia* were found to be resistant to both water and ethanol decoction of *T. diversifolia leaves*. Similar results were also reported by [23], where methanol extracts of the leaf part showed very little antimicrobial activity against some bacterial strains. Previous finding had found *Fusarium solani*, *Aspergillus parasiticus, Saccharomyces cerevisiae***,** *Candida albicans***,** *Macrophomina***,** and *Yersinia aldovae* to be inhibited by petroleum ether extract of *T. diversifolia* leaves [24]**.** However, the investigated fungal strains did not exhibit any inhibition when treated with aqueous or ethanol extract**.** This sturdily suggests that the components existing in extracting agents could be responsible for plant activeness in combating bacteria and fungi infections. Consistent with Umar [25], alkaloids, saponins, tannins, phenolic acid, and flavonoids were found in the leaf **decoction of** *T. diversifolia.* However, compared to other phytochemicals in the leaf extract of *T. diversifolia,* flavonoids, and alkaloids were more prominent.

Several researchers had noted the antibacterial capability of *C. papaya* [26-27]. According to Peter [26], methanolic extract of *C. papaya* seeds had a 70% inhibitory efficacy against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E. coli.* In this study, ethanol decoction of *C. papaya* showed maximum inhibitory zone of 21 mm, 17 mm, and 15 mm against *Escherichia coli, Staphylococcus aureus,* and *Enterococci,* and moderate activity against *Enterobacter aerogenes* but failed to show any inhibition against *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* Although the maximum inhibition reported for ethanol extract of *C. papaya* in this study is lower than the inhibition activities reported by Peter [26] yet it indicated that the components present have a significant level of antimicrobial properties. The papaya ethanol extract contained secondary metabolites like alkaloids, flavonoids, saponins, tannins, phenolic acid, and terpenoids, just like the other plant species under investigation. However, the amount of flavonoids present was significantly higher than those of the other metabolites. The existence of these metabolites emphasizes the significance of *C. papaya* in the treatment of numerous diseases given that they are known to be anti-allergic, anticancer, anti-diabetic, antibacterial, and anti-inflammatory**.** Although rural dwellers are less informed about the presence of these metabolites in *C. papaya*, they are well aware of its health benefits. Hence, the reason for using it in various ethnomedicinal preparations.

IV. CONCLUSION

The ethanol leaf extracts of *M. lucida, T. diversifolia,* and *C. papaya* contained important bioactive components with efficient antibacterial activity against the tested organisms. The outcomes showed the activeness of the ethanol decoction of these plants as bacteria strain dependent. While ethanol extract of *C. papaya* actively inhibited *Staphylococcus aureus*, extract of *T. diversifolia* showed moderate effects and *M. lucida* was not effective. In a contrary scenario, *M. lucida* actively inhibits *Klebsiella pneumonia,* whereas *T. diversifolia* and *C. papaya* showed no activities. However, the zones of inhibition for the ethanol extract of the leaves of these plants plausibly reflect the pharmacological importance. Further studies however are needed to ascertain the effectiveness of their combination as antimicrobial agents. Also, the antimicrobial agents found in these plants should be isolated and purified in order to determine their full potential in treating many illnesses plaguing the population.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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