



Antimicrobial, Phytochemical Analysis and Molecular Docking (*In-silico* Approach) of *Tithonia diversifolia* (Hemsl.) A. Gray and *Jatropha gossypifolia* L on Selected Clinical and Multi-Drug Resistant Isolates

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Authors' contributions

This work was carried out in collaboration between both authors. Author AFO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OTO managed the analyses of the study, the literature searches and was in charge of direction and planning. Both authors discussed the results, read and approved the final manuscript.

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ABSTRACT

The aim of this study is to determine the zones of inhibition, phytochemical screening and molecular docking (*In-silico* Approach) of *Tithonia diversifolia* (Hemsl.) A. Gray and *Jatropha gossypifolia* L against selected clinical and multi drug resistant isolates. Crude extraction of air dried leaves were carried out by soaking the plant in ethanol and ethyl acetate, standard agar diffusion method was used for sensitivity testing, minimum inhibitory concentration and minimum bactericidal concentration values were obtained by agar dilution method. The antimicrobial activity of the leaf extracts of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L was assayed against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas*

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aeruginosa, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Candida albican* and against multi drug resistant bacteria which are *Acinetobacter baumannii*, *Enterobacter agglomerans*, *Proteus mirabilis*, *Providencia stuartii*, *Salmonella subsp 3b*. Levofloxacin and fluconazole were the standard antibiotics used. Sensitivity test revealed the highest zone of inhibition observed for *J. gossypifolia* L and *T. diversifolia* (Hemsl.) A. Gray against *Candida albican* with mean and standard deviation of 29 ± 1.414 and 19.5 ± 0.707 at 100 mg/ml respectively, while the least zone of inhibition was observed from the extracts of *J. gossypifolia* L against *Escherichia coli* with 11.75 ± 0.354 at 100 mg/ml. Both plant extracts showed antimicrobial activity against multi drug resistant isolates having zones of inhibition ranging from 0 to 15 ± 1.414 . The Minimum Inhibitory Concentration of the extracts ranges between 6.25 and 100 mg/ml as well as the Minimum Bactericidal Concentration. The qualitative and quantitative phytochemical analysis showed the presence of alkaloids, anthraquinone, cardiac glycosides, flavonoids, phlobotannins, reducing sugars saponins, steroids and tannins. Molecular docking of the phytochemicals of *T. diversifolia* (Hemsl.) A. Gray only was carried out using levofloxacin as template, which revealed the presence of compounds more effective in inhibiting DNA gyrase enzyme. Thus, the use of both plants as traditional medicine is justifiable and should be encouraged in the formulation and production of new antibiotics.

Keywords: *Tithonia diversifolia* (Hemsl.) A. gray; *Jatropha gossypifolia* L; antimicrobial activity; phytochemical screening; molecular docking; clinical isolates; multi drug resistant isolates.

1. INTRODUCTION AND LITERATURE REVIEW

Medicinal plants have provided mankind a large variety of effective drugs to mitigate or eradicate infections and suffering from diseases in spite of advancement in synthetic drugs, some of the plant-derived drugs still retained their importance and relevance [1]. Medicinal plants play a key role in maintaining human health and contribute towards well-being of human life [2]. Medicinal plants have been widely utilized as effective remedies for preventing and treating variety of disease conditions for millennia by almost every known culture [3]. Medicinal plants are important components of medicines, cosmetics, dyes, beverages [4]. In an increasing search of new antimicrobial agent to cope with the microbial resistance to antibiotics, scientists are searching from different sources including plants. Plants from different genera and species were found to have antimicrobial potentials which lead to the invention of antimicrobials or drugs [5]. Plant species contain active ingredients such as alkaloids, phenols tannins, cryogenics, glycosides, terpenoids [2]. These ingredients have been used and found effective as sweeteners, anti-infections and anti-bacterials [2]. The detection of the antimicrobial properties of a plant indicates that such plant could be a good source for the development of antimicrobial agent.

1.1 *Tithonia diversifolia* (Hemsl.) A. Gray

T. diversifolia (Hemsl.) A. Gray also known as marigold tree [6], is an herbaceous flowering plant in the Asteraceae family [7]. Native to Mexico and Central America, it has been introduced and is now naturalized in tropical parts of Asia and Africa [7]. By forming dense stands it prevents the growth of young native plants. Depending on the area, *T. diversifolia* (Hemsl.) A. Gray may be either annual or perennial. Being able to produce flowers and seeds throughout the year, coupled with the ability of seeds to be dispersed by wind, water and animals, makes it particularly easy for *T. diversifolia* (Hemsl.) A. Gray to quickly colonize new areas [8]. It contains some amounts of secondary metabolites such as alkaloids, anthocyanidins, flavonoids, saponins, steroids, tannins and terpenoids [9].

1.1.1 Ethnomedicinal uses of *Tithonia diversifolia* (Hemsl.) A. Gray

The shrub is an important medicinal plant part used alone or in the combination with other plants for the treatment of a wide variety of ailments, such as stomach pains, indigestion, sore throat, liver diseases and pain. This is because the leaf is considered to have most of the active constituents [10]. It has been reported to possess anti-plasmodial activity [11]. It also

possesses anti-inflammatory and analgesic properties [12], have resistance against bile, kidney, urinary and venereal diseases, testicular inflammation, frigidity, sterility, heavy menstruation, rheumatism and arthritis, upper respiratory tract infections ranging from cough to tuberculosis, intestinal worms and schistosomiasis, cancer chemopreventive activity [13], cytotoxic properties [14,15] and antimicrobial activity [16,17]. This plant is a weed that grows quickly and has become an option as an affordable alternative to expensive synthetic fertilizers. It has shown to increase plant yields and soil nutrients of nitrogen, phosphorus and potassium [18].

1.2 *Jatropha gossypifolia* L

Jatropha has the widest distribution, with species found in Africa, India, South America, West Indies, Central America, and the Caribbean [19]. *J. gossypifolia* L can be used for the treatment of various diseases [20] and its uses in traditional medicine are described for different parts (leaves, stems, roots, seeds, and latex) and preparations (fresh juice, infusion, decoction, and maceration, among others), by different routes (oral or topical). It is used as an antihypertensive, anti-inflammatory, analgesic, homeostatic and anti-diabetic agent has been justified [19].

J. gossypifolia L Linneus is a Euphorbiaceae plant popularly known worldwide as bellyache bush or black physic nut. It is widely distributed in tropical and subtropical regions of Africa and the Americas [21]. It is a pantropical species originating from South America that is cultivated in tropical countries throughout the world [22].

1.2.1 Ethnomedicinal uses of *J. gossypifolia*

J. gossypifolia L is used to treat various kinds of human diseases [23]. Root is used in treatment of diarrhea [24]. Oil used as purgative and locally applies in skin disease [19] and arthritis [25]. Latex and leaf juice are used to treat ulcer [19]. The leaf decoction is used for bathing wounds and rashes [26]. The plant parts like leaf and stem are traditionally used to cure toothache [27]. *J. gossypifolia* can be used in the treatment of arthritis [25], ulcer [19], wounds in lips and tongue [28], toothache [29], leprosy [25], eczema [30], venereal diseases [31] and as blood purifier [32]. *J. gossypifolia* is also used for commercial purpose [33].

1.3 Molecular Docking

Molecular docking is considered as the key and lock hypothesis used to detect the compatibility of ligand and protein [34]. Molecular docking is considered as the key and lock hypothesis used to find the best fit orientation of ligand and protein. This tool has emerged a reliable, cost-effective and time-saving technique in drug design by discovering lead therapeutic compounds. Bioinformatics allows (almost) accurate prediction of molecular interactions that hold together a protein and a ligand in the bound state. The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy [35]. The net predicted binding free energy (ΔG_{bind}) is revealed in terms of various parameters, hydrogen bond (ΔG_{hbond}), electrostatic (ΔG_{elec}), torsional free energy (ΔG_{tor}), dispersion and repulsion (ΔG_{vdw}), desolvation (ΔG_{desolv}), total internal energy (ΔG_{total}) and unbound system's energy (ΔG_{unb}) [36]. Therefore, good understanding of the general ethics that govern predicted binding free energy (ΔG_{bind}) provides additional clues about the nature of various kinds of interactions leading to the molecular docking [36]. Practical application of molecular docking requires data bank for the search of target with proper PDB format and a methodology to prepare ligand as a PDB file. To do this, there are various software's (Discovery studio, etc.) available from where the ligand can be made in PDB format. These tools provide the organization to ligands based upon their ability to interact with given target proteins/DNA [35].

Molecular docking of small molecules to a target includes a pre-defined sampling of possible conformation of ligand in the particular groove of target in an order to establish the optimized conformation of the complex. This can be made possible using scoring function of software. Since the infrared spectroscopy, X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy are the techniques for the investigation and establishment of three dimensional structures of any organic molecule/biomolecular targets. Hence homology modeling makes it possible to determine the tentative structure of proteins of unknown structure with high sequence homology to known structure. This provides a substitute approach for target structure establishment, which forms starting point for *in silico* drug discovery [35]. Molecular

modeling simulates the three-dimensional structural interactions between atoms and molecules [37].

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh, healthy and mature leaves of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L were collected from the surroundings of Adekunle Ajasin University and confirmed at Plant science and biotechnology department, Adekunle Ajasin University. The leaves of both plants were dried in the laboratory for over two weeks and crushed using a milling machine [20]. Both plants were selected for this study based on their ethno medicinal uses.

2.2 Extraction of Plant Material

The parts of various plants were dusted and air dried at room temperature and then grounded into coarse powder using electric miller [38]. The crushed plant materials were weighted and then soaked with ethyl acetate and ethanol. The extracts were collected by sieving the mixture using filter paper and the solvent was allowed to evaporate and the extracts were kept in the refrigerator at 4°C until use [39].

The Table below shows the Multi drug resistant bacteria and the antibiotics they are resistant to as reported by the cited authors.

Multidrug resistant organisms	Antibiotics	Reference
<i>Acinetobacter baumannii</i>	Amikacin, Ciprofloxacin, Levofloxacin, Piperacillin, Ticarcillin, Ceftazidime, Ceftriaxone, Cefotaxime, Cefepime, Trimethoprim/Sulfamethoxazole, Gentamicin, Tetracycline, Tobramycin, Ampicillin/Sulbactam, Aztreonam, Meropenem, Imipenem	[40]
<i>Entamoeba agglomerans</i>	Cefotaxime, Moxifloxacin, Cotrimoxazole, Ticarcillin, Carbenicillin, Ampicillin, Piperacillin and Mezlocillin	[41]
<i>Proteus mirabilis</i>	Amoxicillin, Cefoxitin, Chloromphenicol, Nalidixic Acid, Ampicillin, Amikacin, Gentamicin, Nitrofurantoin Ciprofloxacin	[42]
<i>Providencia stuartii</i>	Gentamicin, Tobramycin, Aminopenicillins and First-Generation Cephalosporins Carbapenem	[43]
<i>Salmonella subsp 3b</i>	Ampicillin, Cephalothin, Kanamycin, Nalidixic Acid, Neomycin, Streptomycin, Tetracycline	[44]

2.3 Standardization of Plant Extracts

The extracts were standardized by adding 1 g of each extract to 7.5 ml of distilled water and 2.5 ml of dimethyl sulfoxide making it 100 mg/ml. The concentration was reduced by adding 5 ml of distilled water into three sterile bijou bottles labelled A, B and C. 5 ml from the 100 mg/ml bijou bottle was taken and dispensed into the bijou bottle A making it 50 mg/ml. Same process was repeated to get a concentration of 25 mg/ml and 12.5 mg/ml [20].

2.4 Test Organism and Source of the Test Organisms

The test organisms include some clinical isolates which are *Bacillus subtilis*, *Candida albican*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Streptococcus pyogenes* and multidrug resistant isolates which are *Acinetobacter baumannii*, *Enterobacter agglomerans*, *Proteus mirabilis*, *Providencia stuartii*, *Salmonella sub sp 3b*. The multi drug resistant isolates were selected based on literature review.

The test organisms were collected from the Department of Microbiology laboratory stock culture at Adekunle Ajasin University.



Fig. 1.1 *Tithonia diversifolia* (Hemsl.) A. Gray
Source: tamil.net



Fig. 1.2 *Jatropha gossypifolia* L
Source: jircas.go.jp

2.5 Standardization of Test Organisms

Pure culture of the test organisms was transferred into 5 ml of nutrient broth and incubated for 24 hours. 0.1 ml of the overnight culture was transferred into 9.9 ml of distilled water in a test tube using a sterile needle and syringe and then mixed by shaking it. The liquid contains approximately 10^6 cfu/ml of bacterial suspension [20].

2.6 Antibacterial and Antifungal Screening

1 ml of the liquid mixture was dispensed into a sterile petri dish and 20 ml of prepared Mueller Hinton agar was poured into the petri dish. It was gently swirled and allowed to set. A sterile cork borer of 6 mm diameter was used to bore hole in the agar plate. Drops of the prepared extracts were dispensed into the holes till it was filled, having concentrations between 100 mg/ml to 12.5 mg/ml. Levofloxacin and fluconazole were used as the control experiment for bacteria and fungi respectively. After an hour the plates were incubated at 37°C for 24 hours and 24°C for 48 hours for the bacterial isolates and for the fungal isolate respectively. The diameter of the zones of inhibition were measured in millimeter (mm) and recorded accordingly [20].

2.7 Minimum Inhibitory Concentration (MIC)

The MIC of the extracts against the test organisms was determined using the broth dilution method. Aliquots of 1 ml of stock extract at the concentration of 100 mg/ml was added to 1 ml of fresh nutrient broth and serially diluted to obtain extract concentrations 50 mg/ml, 25 mg/ml, and 12.5 mg/ml in three different test tubes [20].

2.8 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

In determining the MBC/MFC, the solution in the test tubes used in carrying out the MIC was used. 1 ml of the solution from a test tube that shows no growth after incubation and was dispensed in Nutrient broth and Sabouraud Dextrose broth for bacteria and the fungus respectively and then incubated for 24 hours and 72 hours. After incubation, the MBC/MFC values were recorded by selecting the lowest concentration of the extract that has a bactericidal/fungicidal effect [20].

2.9 Phytochemical Screening

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts as well as powder specimens using the standard procedures as described by the authors cited.

2.10 Test for Alkanol

2.10.1 TLC method

The powdered test samples were wet with a half diluted NH_4OH and lixiviated with EtO Ac for 24 hr at room temperature. Separate the organic phase from the acidified filtrate and basify with NH_4OH (pH 11-12). Then extract it with chloroform (3X), condense by evaporation and use for chromatography. Separate the alkaloid spots by using mixture of chloroform and methanol solvent (15:1) [45]. Spray the spots with Dragendorff's reagent. An orange spot show is a positive result [46].

2.10.2 Test for anthraquinone

Borntrager's test: Heat about 50 mg of extract with 1 ml 10% ferric chloride solution and 1ml of concentrated hydrochloric acid. Cool the extract and filter. Shake the filtrate with equal amount of diethyl ether. Further extract the ether extract with strong ammonia. Pink or deep red coloration of aqueous layer [47,48].

2.10.3 Test for cardiac glycosides

TLC method - Extract the powdered test samples with 70% EtOH on rotary shaker (180thaws/min) for 10hr. Add 70% lead acetate to the filtrate and centrifuge at 5000rpm/10 min. Further centrifuge the supernatant by adding 6.3% Na₂CO₃ at 10000 rpm/10min. Dry the retained supernatant and redissolved in chloroform and use for chromatography. Separate the glycosides using EtOAc-MeOH-H₂O (80:10:10) solvent mixture. Under ultraviolet (UV254 nm) light, the color and the values of hRf spots can be recorded [45,46,48].

2.10.4 Test for flavonoid

TLC method - Extract 1 g powdered test samples with 10 ml methanol on water bath (60°C/ 5 min). Condense the filtrate by evaporation, and add a mixture of water and EtOAc (10:1 mL), and mix thoroughly. Retain the EtOAc phase and use for chromatography. Separate the flavonoid spots using chloroform and methanol (19:1) solvent mixture. Under ultraviolet (UV254 nm) light, the color and the values of hRf spots can be recorded [45,46,47,49].

2.10.5 Test for phenol

Phenol test Spot the extract on a filter paper. Add a drop of phosphomolybdic acid reagent and expose to ammonia vapors. Blue coloration of the spot, shows is a positive result [47].

2.10.6 Test for reducing sugars

Fehling A and Fehling B were separately mixed with 1 ml of the plant filtrate. The appearance of green colour with Fehling A and a brown colour with Fehling B shows that reducing sugars were present [20].

2.10.7 Test for saponin

TLC method- Extract two grams of powdered test samples with 10 ml 70% EtOH by refluxing for 10

min. Condense the filtrate, enrich with saturated n-BuOH, and mix thoroughly. Retain the butanol, condense and use for chromatography. Separate the saponins using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. Expose the chromatogram to the iodine vapors. By the exposure of chromatogram to iodine vapours, the colour (yellow) and the values of the hRf spots were recorded [45].

2.10.8 Test for steroid

TLC method Extract two grams of powdered test samples with 10 ml methanol in water bath (80°C/15 min). Use the condensed filtrate for chromatography. The sterols can be separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. After the plates have been sprayed with anisaldehyde-sulphuric acid reagent and heating (100°C/6 min) under visible light, the color and the values of hRf spots can be recorded. The color (Greenish black to Pinkish black) and the values of hRf spots can be recorded under visible light [45, 46].

2.10.9 Test for tannin

Braemer's test 10% alcoholic ferric chloride was added to 2-3 ml of methanolic extract (1:1) Dark blue or greenish grey coloration of the solution [47,50].

2.10.10 Estimation of cardiac glucosides (Borntrager's Test)

To 2 ml of filtrate hydrolysate, 3 ml of ethyl acetate was added and shaken, ethyl acetate layer was separated and 10% ammonia solution was added to it. Formation of pink color indicated the presence of anthroquinone glycosides [51].

2.10.11 Estimation of phlobatannins

0.5 g of the plant extracts were disintegrated in distilled water and filtered. The filtrates were boiled in 2% HCl. The appearance of red precipitate reveals the presence of phlobatannins [20].

2.11 Quantitative Method of Analysis of Medicinal Plant

2.11.1 Estimation of saponins

100 ml of 20% ethanol and aqueous were added to 20 g of grinded plant samples in a conical flask

and heat was applied using a water bath at a temperature of 55°C for 4 hours and stirred continuously after which the mixture was filtered. The residue was re-extracted using 200 ml of 20% ethanol. The combined extracts were reduced to about 40 ml at 90°C over a water bath. 20 ml of diethyl ether was added to the concentrate that was transferred into a 250 ml separatory funnel and vigorously shaken. The ether layer was disposed while the aqueous layer was recovered. The process of purifying it was repeated three times and 60 ml of n-butanol was added. Using 10 ml of 5% aqueous sodium chloride, the combined n-butanol extracts were washed twice and the solution that remains was heated using a water bath. The samples were dried in the oven to a constant weight after evaporation. The content of the saponin was calculated as percentage of the starting material [20].

2.11.2 Estimation of total flavonoid concentration

The concentration of flavonoids in the extract was estimated spectrophotometrically according to the procedure of Sun et al. [52]. The extract (0.1 g) was dissolved in 20 ml of 70% (v/v) ethanol to give a final concentration of 0.5 mg/ml. To clean dry test tubes (in triplicate) were pipetted 0.5 ml of working solution of sample and diluted with 4.5 ml distilled water. To each test tube was added 0.3 ml of 5% (w/v) NaNO₂, 0.3 ml of 10% AlI₃ and 4 ml of 4% (w/v) NaOH. The reaction mixtures were incubated at room temperature for 15 minutes. The absorbance was read at 500nm against reagent blank. The standard calibration curve was prepared by pipetting 0.2, 0.4, 0.6, 0.8, 1.0 ml of 1 mg/ml rutin into clean dry test tubes. The volumes were made up to 5 ml with distilled water. To each of the tubes were added 0.3 ml of 5% (w/v) NaNO₂, 0.3 ml of 5% (w/v) AlCl₃ and 4 ml of 4% (w/v) NaOH. At room temperature for 15 minutes, the reaction mixtures were incubated [53]. Absorbance was taken at 500nm and was plotted against the concentration to give the standard calibration curve. The concentrations of the flavonoids in the extract was extrapolated from standard calibration curve and expressed as milligram rutin equivalent per g of extract (mg RE/g extract) [54,55].

2.11.3 Detection of alkaloid content

200 ml of 10% acetic acid in ethanol was added into a 250 ml beaker containing 5 g of the plant

sample. It was covered and allowed to stand for 4 hours. It was filtered concentrated using a water bath to about one quarter of the original volume. Drops of concentrated ammonium hydroxide was added to the extracts until the precipitation was completed. The precipitate was collected after the whole solution has settled, washed with dilute ammonium hydroxide and then filtered. The residue which is the alkaloid was dried and weighed to a constant mass [20].

2.11.4 Estimation of total phenolic concentration

Estimation of total phenolic content was carried out using Folin-Ciocalteu's phenol reagent reaction as reported by Singleton, (1999). The assay involved pipetting 0.2, 0.4, 0.6, 0.8, 1.0 ml of gallic acid solution (1.0 mg/l) in triplicate in clean dried test tubes. The volumes were made up to 1.0 ml with distilled water. To each of the test tube was added 1.5 ml of 10% (w/v) NaHCO₃ solution to give a total volume of 4.0 ml. The reaction mixtures were further incubated for additional one and half hours. The estimation of phenol in ethanolic extract of *S. mombin* involved pipetting 0.5 ml each of 5 mg/ml ethanolic extract into clean dry test tubes in triplicate. The volumes were adjusted to 1.0 ml with distilled water. To each of the tubes was added 1.5 ml of Folin-Ciocalteu's phenol reagent (1:10). At room temperature for 5 minutes, the reaction mixture was incubated [53]. To the reaction mixture was added 5 ml of 10% (w/v) NaHCO₃ solution. The reaction mixture was incubated for one and half hour. The absorbance was read at 725nm against the blank containing all reagents except the standard gallic acid. The absorbance at 725nm was plotted against the concentration to produce the standard curve. The concentrations of the phenolics in the extract was extrapolated from standard curve and expressed as milligram tannic acid equivalent per g of extract (mg TAE/g extract) [56].

2.12 Molecular Docking

In this study, *in-silico* approach was employed to study the interaction of plant phytochemicals from selected plants with three proteins; the *Staphylococcus aureus* topoisomerase iv, *Salmonella typhi* topoisomerase iv, and the yeast *Candida albicans* 14 α demethylase. It is a computational screening technique known as Virtual high throughput screening (vHTS) used to screen a pool of compounds libraries to explore the attractive force of the target receptor with

library of compounds [57]. Computer-aided docking is a tool to understand the binding between a ligand and a target protein/receptor [58].

2.13 Protein Generation and Preparation

The 3-dimensional crystallized structures of *Staphylococcus aureus* topoisomerase iv and yeast *Candida albicans* 14 α demethylase were downloaded from the Protein Data Bank (PDB) repository (www.rcsb.org) with the PDB ID of 4URN and 5FSA with crystallographic resolutions of 2.30Å and 2.86Å respectively. The 3D structure of the *Salmonella typhi* topoisomerase IV (not found on the PDB repository) was retrieved by modelling the FASTA sequence of the protein gotten from the NCBI database (www.ncbi.nlm.nih.gov/protein/) using the swiss model server. The downloaded proteins were viewed with Schrodinger Maestro11.1. Proteins were prepared using Protein Preparation Wizard tool of the Schrodinger suite. The missing side-chains within the protein residues and the missing loops were filled using Prime (Schrodinger). The Co-crystallized molecules of water, cofactors and ions were removed, hydrogen atoms were added and the formal charges with bond in orderly manner were assigned to the structures [59]. The grid coordinate was generated around the co-crystallized ligand of the proteins with a grid box of 20Å×20Å×20Å [60].

2.14 Ligand Generation and Preparation

A list of phytochemical constituents of *Tithonia* was obtained from various literatures [61,62,63,64,65]. The 2D structure of the ligands was retrieved from the NCBI Pubchem database. The respective 3D conformers of ligands were generated using the LigPrep under Schrodinger-Maestro tools. It also applies sophisticated rules to correct Lewis structures and to eliminate mistakes in ligands in order to reduce downstream computational errors [38]. Moreover, it optionally expands tautomeric and ionization states, ring conformations, and stereoisomers to produce broad chemical and structural diversity from a single input structure.

2.15 Ligand Docking

This was carried out with the use of GLIDE (Grid-based Ligand Docking with Energetics). Glide searches for favorable interactions between one

or more ligand molecules and a receptor molecule, usually a protein [66]. Glide was run in rigid or flexible docking modes, and automatically generated conformations for each input ligand. The selection of the best pose was done on the interaction energy between the ligand and the protein [67], as well as on the interactions the ligand shows with experimentally proved important residues (Schrodinger). Standard precision (SP) of docking of ligand was performed in Glide of Schrodinger-Maestro11.1 followed by the extra-precision (XP) mode which was used to combine a powerful sampling protocol with a custom scoring function designed to identify ligand poses that would be expected to have unfavorable energies, based on well-known principles of physical chemistry [59].

3. RESULTS

Ethyl acetate and ethanol extracts of *T. diversifolia* (Hemsl.) A. Gray and *Jatropha gossypifolia* L demonstrated varied ranges of antibacterial and antifungal activities against the tested microorganisms at concentrations ranging from 100 mg/ml to 12.5 mg/ml. Levofloxacin was used as control for bacteria, while fluconazole was used as control for fungi. The minimum inhibitory concentration and minimum bactericidal concentration values were also recorded.

4. DISCUSSION

The purpose of this work was to investigate the antimicrobial properties present in *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L leaf extracts using ethyl acetate and ethanol as the extracting solvents, to know the docking scores of the phytochemicals present in the plant and to scientifically verify their use for medicinal purposes.

In this study, leaves of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L were extracted using ethyl acetate and ethanol, and were tested for antimicrobial properties against some clinical isolates such as *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* and multi drug resistant isolates which are *Acinetobacter baumannii*, *Enterobacter agglomerans*, *Proteus mirabilis*, *Providencia stuartii*, *Salmonella subsp 3b*.

Table 1. Zones of inhibition of ethyl acetate leaf extracts of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypiifolia* L against selected clinical isolates

Test Organisms	Leaf extracts of <i>T. diversifolia</i> (Hemsl.) A. Gray						Leaf extracts of <i>J. gossypiifolia</i> L						Control	
	100	50	25	12.5	MIC	MBC	100	50	25	12.5	MIC	MBC	L	F
<i>B. subtilis</i>	-	-	-	-	-	-	-	-	-	-	-	-	24	-
<i>E. coli</i>	13.5±0.707	11±1.414	8.5±2.121	-	50.0	100.0	11.75±0.354	9.5±0.707	-	-	-	-	20	-
<i>K. pneumonia</i>	15.5±0.707	13.5±0.707	11.75±0.354	-	50.0	100.0	19.75±0.354	15.5±0.707	11.5±2.121	-	25.0	50.0	16	-
<i>P. mirabilis</i>	-	-	-	-	-	-	-	-	-	-	-	-	25	-
<i>P. aeruginosa</i>	-	-	-	-	-	-	16.5±0.707	13±0.0	10.75±0.354	-	50.0	100.0	27	-
<i>S. typhi</i>	15.5±0.707	12.75±0.354	11.65±0.495	9.5±0.707	50.0	100.0	15.75±0.356	11.75±1.601	9.5±0.707	-	100.0	-	26	-
<i>S. dysenteriae</i>	-	-	-	-	-	-	-	-	-	-	-	-	23	-
<i>S. aureus</i>	15.5±0.707	13±1.414	11.5±0.707	9±1.414	50.0	100.0	18.5±0.707	13±2.828	-	-	50.0	100.0	19	-
<i>S. pyogenes</i>	-	-	-	-	-	-	11.5±2.121	-	-	-	100.0	-	17	-
<i>C. albican</i>	26±1.414	19.5±0.353	15.75±0.353	12.5±0.707	25.0	50.0	29±1.414	26.5±0.707	21±2.828	14.5±0.707	12.5	25.0	-	20

The tests were performed in duplicates and the results expressed as mean and standard deviation

Key: - No inhibition, L Levofloxacin, F Fluconazole

Table 2. Zones of inhibition of ethanol leaf extracts of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypiifolia* L against selected clinical isolates

Test Organisms	Leaf extracts of <i>T. diversifolia</i> (Hemsl.) A. Gray						Leaf extracts of <i>J. gossypiifolia</i> L						Control	
	100	50	25	12.5	MIC	MBC	100	50	25	12.5	MIC	MBC	L	F
<i>B. subtilis</i>	-	-	-	-	-	-	-	-	-	-	-	-	24	-
<i>E. coli</i>	14.5±0.707	12.75±0.354	11±0.0	-	50.0	100.0	13.5±0.707	10.5±0.707	-	-	100.0	-	20	-
<i>K. pneumonia</i>	-	-	-	-	-	-	17.5±0.707	14±0.0	12.5±0.707	9.5±0.707	50.0	100.0	16	-
<i>P. mirabilis</i>	-	-	-	-	-	-	-	-	-	-	-	-	25	-
<i>P. aeruginosa</i>	-	-	-	-	-	-	14±0.0	12.5±0.707	-	-	50.0	100.0	27	-
<i>S. typhi</i>	15±0.0	13.5±0.707	11.75±0.354	9±1.414	25.0	50.0	17.5±0.707	13.5±0.707	11.5±0.707	10±0.0	50.0	100.0	26	-
<i>S. dysenteriae</i>	18±0.0	14.5±0.707	13.5±0.707	11.5±0.707	50.0	100.0	-	-	-	-	-	-	23	-
<i>S. aureus</i>	18±0.0	14.75±0.354	12.5±0.707	9.5±0.707	25.0	50.0	21.5±0.707	16±1.414	12.5±0.707	-	25.0	50.0	19	-
<i>S. pyogenes</i>	-	-	-	-	-	-	-	-	-	-	-	-	17	-
<i>C. albican</i>	21.5±0.707	17±0.0	12.5±0.707	10.5±0.707	25.0	50.0	24.5±0.707	21.5±0.707	17±1.414	14±1.414	12.5	25.0	-	20

The tests were performed in duplicates and the results expressed as mean and standard deviation

Key: - No inhibition, L Levofloxacin, F Fluconazole

Table 3. Zones of inhibition of ethyl acetate leaf extracts of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypiifolia* L against multiple resistant isolates

Test organisms	Leaf extract of <i>T. diversifolia</i> (Hemsl.) A.Gray				Leaf extract of <i>J. gossypiifolia</i> L			
	100	50	25	12.5	100	50	25	12.5
<i>Acinetobacter baumannii</i>	11.5±0.707	-	-	-	9.5±0.707	-	-	-
<i>Enterobacter agglomerans</i>	12±0.0	-	-	-	14.5±0.707	11.25±0.354	-	-
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-
<i>Providencia stuartii</i>	14.5±0.707	12.5±0.707	11.5±0.707	-	14.5±0.707	12±0.0	10±0.0	-
<i>Salmonella subsp 3b</i>	14.5±0.707	12±0.0	-	-	15±1.414	11.5±0.707	9.5±0.707	-

The tests were performed in duplicates and the results expressed as mean and standard deviation

Table 4. Zones of inhibition of ethanol leaf extracts of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypiifolia* L against multiple resistant isolates

Test organisms	Leaf extract of <i>T. diversifolia</i> (Hemsl.) A. Gray				Leaf extract of <i>J. gossypiifolia</i> L			
	100	50	25	12.5	100	50	25	12.5
<i>Acinetobacter baumannii</i>	12.5±0.707	10±0.0	-	-	9.5±0.707	-	-	-
<i>Enterobacter agglomerans</i>	14±1.414	9.5±0.707	-	-	14±1.414	10.75±1.061	-	-
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-
<i>Providencia stuartii</i>	9.5±0.707	-	-	-	14.5±0.707	11.75±0.354	9.5±0.707	-
<i>Salmonella subsp 3b</i>	-	-	-	-	14.5±2.121	12±0.0	10±0.0	-

The tests were performed in duplicates and the results expressed as mean and standard deviation

Table 5. Qualitative phytochemical analysis of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypiifolia* L using different solvents

Phytochemicals	Leaf extract of <i>T. diversifolia</i> (Hemsl.) A. Gray				Leaf extract of <i>J. gossypiifolia</i> L			
	Methanol	Acetone	Dichloromethane	Ethyl acetate	Methanol	Acetone	Dichloromethane	Ethyl acetate
Alkaloid	+ve	-ve	-ve	+ve	+ve	±ve	+ve	-ve
Anthraquinone	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve
Cardiac glycoside	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Flavonoids	+ve	+ve	ND	+ve	-ve	+ve	+ve	+ve
Phenol	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Reducing sugars	-ve	-ve	-ve	+ve	+ve	±ve	+ve	+ve
Saponin	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Steroids	-ve	+ve	+ve	±ve	-ve	+ve	+ve	±ve
Tannins	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Key: N.D: Not detected

Table 6. Quatitative phytochemical analysis of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypiifolia* L using different solvents

Phytochemicals	Leaf extract of <i>T. diversifolia</i> (Hemsl.) A. Gray				Leaf extract of <i>J. gossypiifolia</i> L			
	Methanol	Acetone	Dichloromethane	Ethyl acetate	Methanol	Acetone	Dichloromethane	Ethyl acetate
Alkaloid	2.20	4.80	1.34	1.34	2.20	4.89	9.82	9.82
Alkaloid	2.20	4.80	1.34	1.34	2.20	4.89	9.82	9.82
Cardiac glycoside	6.49	3.21	3.51	3.51	2.32	3.89	2.49	9.49
Flavonoids	2.34	2.82	9.42	9.42	ND	2.89	4.78	4.78
Phenol	2.37	4.02	9.62	9.62	2.37	4.01	2.21	4.21
Phlobotannins	2.10	2.70	2.57	10.57	2.10	2.89	4.77	4.77
Phlobotannins	2.10	2.70	2.57	10.57	2.10	2.89	4.77	4.77
Saponin	2.62	4.00	1.22	10.22	2.25	4.89	6.53	6.53
Tannins	2.64	5.04	7.56	7.56	2.30	5.01	3.10	6.10

Table 7. Docking scores of the phytochemicals present in *T. diversifolia* (Hemsl.) A. Gray plant against some clinical isolates

<i>Salmonella typhi</i>		<i>Staphylococcus aureus</i>		<i>Candida albican</i>	
Phytochemicals	Docking scores	Phytochemicals	Docking scores	Phytochemicals	Docking scores
Levofloxacin-1	-7.372	p-Hydroxynorephedrine	-4.876	Erucic acid	-10.215
Phenylephrine-1	-4.9	DI-Phenylephrine	-4.872	Coligand5fsa	-9.835
Fenchol-1	-4.722	Metaraminol	-4.317	Cadalene	-7.482
Cavacrol-1	-4.402	Phenylephrine	-4.141	Metaraminol	-7.101
6-Camphenone-1	-4.22	Cavacrol	-4.052	Cavacrol	-6.962
Sabinene hydrate-1	-3.714	Thymolmethylether	-4.02	Thymol	-6.641
p-Hydroxynorephedrine-1	-3.228	Thymol	-3.953	Sabinene hydrate	-6.303
Metaraminol-1	-2.595	Levofloxacin.sdf	-3.436	Levofloxacin-3	-1.557
DI-Phenylephrine-1	-2.05	Cadalene	-3.267	Verbenone	-0.5
Amphetamine-1	-1.619	Trans-Verbenol	-3.197		

The crude plants extract tested in this study showed antimicrobial activities against all the test bacterial and fungal isolates. However, differences were observed between their antimicrobial activities. These differences could be as a result of disparity in their chemical composition and amount of the bioactive compounds extracted by the solvent [20]. These compounds usually accumulate in different parts of the plants and such secondary metabolites have been found to produce many effects including antibacterial and antiviral properties [68].

The results recorded from this study indicated that ethyl acetate and ethanol extracts of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L had static or cidal effects on the test organisms having zones of inhibition ranging from mean and standard deviation of 0 to 29±1.414.

Table 1 shows the zones of inhibition of bacterial and fungal growth at different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml) of ethyl acetate extract of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L leaf. The antibacterial and antifungal activities were expressed as the zone of inhibition in mean and standard deviation produced by the plant extracts. The ethyl acetate extract of the leaf of *T. diversifolia* (Hemsl.) A. Gray inhibited some of the bacteria and fungi tested with a measurable zone of inhibition.

The extract had higher inhibitory activity on *Staphylococcus aureus* (Gram-positive bacteria),

Klebsiella pneumonia (Gram-negative bacteria) and *Candida albican* (fungi) with zones of inhibition of 15.5±0.707, 15.5±0.707 and 26±1.414 at 100 mg/ml. *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* were resistant to the extract. The plant extract also inhibited the growth of *E. coli* and *Salmonella typhi* with zones of inhibition of 13.5±0.707 and 15.5±0.707 at 100 mg/ml.

For *J. gossypifolia* L, the extract had higher inhibitory activity on *Staphylococcus aureus* (Gram-positive bacteria), *Klebsiella pneumonia* (Gram-negative bacteria) and *Candida albican* (fungi) with zones of inhibition of 18.5±0.707, 19.75±0.354 and 29±1.414 at 100 mg/ml respectively. *Bacillus subtilis*, *Proteus mirabilis*, *Shigella dysenteriae* and *Streptococcus pyogenes* were resistant to the extract. The plant extract also inhibited the growth of *E. coli* and *Salmonella typhi* with zones of inhibition of 11.75±0.354 and 15.75±0.356 at 100 mg/ml. The table also shows the MIC and MBC values. Both extracts had MIC values ranging from 12.5-100 mg/ml, and the MBC values ranged from 25-100 mg/ml.

Table 2 shows the zones of inhibition of bacterial and fungal growth at different concentration (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml) of ethanol extract of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L leaf. *Candida albican* was the most susceptible organism to the extracts and exhibited the maximum zones of inhibition diameter of 21.5±0.707 and 24.5±0.707 at 100

mg/ml respectively for both extracts. *Shigella dysenteriae* and *Staphylococcus aureus* was observed to have the same value of zone of inhibition of 18 ± 0.0 at 100 mg/ml. *Pseudomonas aeruginosa* was susceptible to *J. gossypifolia* L extract having zone of inhibition of 14 ± 0.0 at 100 mg/ml but was resistant to the extract of *T. diversifolia* (Hemsl.) A. Gray. Both plant extracts had no inhibitory activity on *Bacillus subtilis* (Gram-positive bacteria), *Proteus mirabilis* (Gram-negative bacteria) and *Streptococcus pyogenes* (Gram-positive bacteria). *Klebsiella pneumoniae* was susceptible to the extract of *J. gossypifolia* L having zone of inhibition of 17.5 ± 0.707 at 100mg/ml while it was resistant to *T. diversifolia* (Hemsl.) A. Gray extract. *Staphylococcus aureus* was the most susceptible Gram positive organism having a zone of inhibition of 21.5 ± 0.707 at 100 mg/ml and 12.5 ± 0.707 at 25 mg/ml. The table also shows the MIC and MBC values of the extracts on the isolates. Both extracts had MIC values ranging from 12.5-100 mg/ml, and the MBC values ranged from 25-100 mg/ml.

Table 3 shows the zones of inhibition of multi drug resistant bacteria at different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml) of ethyl acetate leaf extract of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L. The leaf extract of both plants inhibited all test organisms except *Proteus mirabilis*. Both plants had higher inhibitory effect on *Salmonella subsp 3b* having zones of inhibition of 14.5 ± 0.707 and 15 ± 1.414 at 100 mg/ml respectively. *Enterobacter agglomerans* and *Providencia stuartii* were also inhibited by both plant extracts, having zones of inhibition of 12 ± 0.0 and 14.5 ± 0.707 at 100 mg/ml respectively. The least susceptible organism was *Acinetobacter baumannii* having zone of inhibition of 9.5 ± 0.707 at 100 mg/ml for *J. gossypifolia* L and 11.5 ± 0.707 at 100 mg/ml for *T. diversifolia* (Hemsl.) A. Gray.

Table 4 shows the zones of inhibition of multi drug resistant bacteria at different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml) of ethanol extract of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L. The leaf extract of *T. diversifolia* (Hemsl.) A. Gray had lower inhibitory effect on *Providencia stuartii* having zone of inhibition of 9.5 ± 0.707 at 100 mg/ml than extract of *J. gossypifolia* L having zone of inhibition of 14.5 ± 0.707 at 100 mg/ml. It was observed that extract of *T. diversifolia* (Hemsl.) A. Gray was not active against *Salmonella subsp 3b* while that of *J. gossypifolia* L had zones of inhibition ranging

from 14.5 ± 2.121 to 10 ± 0.0 . *T. diversifolia* (Hemsl.) A. Gray had inhibitory effect on *Acinetobacter baumannii* with zones of inhibition of 12.5 ± 0.707 and 10 ± 0.0 at 100 mg/ml and 50 mg/ml respectively, while the ethanol extract of *J. gossypifolia* L had zones of inhibition of 9.5 ± 0.707 . *Enterobacter agglomerans* was susceptible to both extracts at different concentration having zones of inhibition ranging from 14 ± 1.414 to 9.5 ± 0.707 . *Proteus mirabilis* was the only organism that was resistant to the both plant's extracts, showing no inhibition.

The antimicrobial activity was more effective on the Gram positive bacteria (*Staphylococcus aureus*) than the Gram negative bacteria (*Salmonella typhi*). Gram negative bacteria has a complex cell wall which makes the cell wall impermeable to antimicrobial substances, unlike the Gram positive bacteria that has no complex cell wall. This serves as a reason why Gram positive bacteria are more susceptible to antimicrobials than Gram negative bacteria [20]. Gram negative bacteria are known to be resistance to certain antibiotics and common example is *E. coli*. Although Gram negative bacteria are resistance to certain antibiotics, some plant extracts have been proven to have antimicrobial activity against Gram negative bacteria and also Gram positive bacteria [20]. In the review of Ogunfolakan *et al.*, [69], it was recorded that *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L had antimicrobial activity against both Gram positive and Gram negative bacteria.

Bacillus subtilis, a Gram positive bacteria was resistant to the plant extracts (Table 1). It has endospore that serves as a contributing factor for its resistance [20]. *Salmonella typhi*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the only Gram negative bacteria that were inhibited by the leaf extracts of both plants. *Pseudomonas aeruginosa* was resistant to the extract of *T. diversifolia* (Hemsl.) A. Gray but was susceptible to the extract of *J. gossypifolia* L. It is to be noted that anthraquinone was present in the methanol, acetone, dichloromethane and ethyl acetate extracts of *J. gossypifolia* L and was absent in the extracts of *T. diversifolia* (Hemsl.) A. Gray (Tables 5 and 6). This component is the explainable reason for the difference in their ability to be able to inhibit or not inhibit *Pseudomonas aeruginosa* because phytochemicals have been investigated and found responsible for various activities of plants such as cytoprotective, immunodulatory and antimicrobial potential [20].

Candida albican was the most susceptible test organism (fungus). The review of Packialakshmi and Archana [70] on the bioautography of *J. gossypifolia* shows that *Candida albican* was more susceptible to the plant extracts among all other clinical isolates.

T. diversifolia (Hemsl.) A.Gray and *J. gossypifolia* L were further studied for MIC and MBC. The MIC and MBC of both plant extracts on the test organisms used ranges from 12.5 to 100 mg/ml in concentration (Tables 1 and 2). The highest MIC value recorded was against *Salmonella typhi* exhibited by the ethyl acetate extract of *J. gossypifolia* L was 100 mg/ml and the least MIC recorded was against *Candida albican* exhibited by ethyl acetate and ethanol extracts of *J. gossypifolia* L was 12.5 mg/ml.

Among the multi drug resistant isolates, *Proteus mirabilis* was resistant to the plant extracts. *Proteus mirabilis* can be naturally resistant to antibiotics, such as benzylpenicillin, oxacillin, tetracycline, and macrolides. *Proteus spp* can acquire resistance to ampicillin through plasmid mediated beta-lactamases, and chromosomal beta-lactamase expression has now been reported. In the last decade there have also been numerous reports of production of extended-spectrum beta-lactamases (ESBLs) by *Proteus sp.* The ESBLs can confer resistance to third generation cephalosporins [71]. *P. mirabilis* synthesise a lot of virulence factors, e.g. urease. The activity of this enzyme leads to biofilm encrustation, hence its resistance to both plant extracts in this study. Encrusted biofilms are more resistant to antimicrobial agents, host defenses and environmental stress conditions [72].

A total of 9 compounds were tested for in both plants using different solvents. Some compounds tested positive (+ve) while some tested negative (-ve). The qualitative phytochemical screening of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L leaf using acetone, dichloromethane, ethyl acetate and methanol shows the presence of active medicinal constituents in which some possess antibacterial activity as recorded by Ogundare, 2007.

Molecular docking of the phytochemicals present in *T. diversifolia* (Hemsl.) A. Gray was carried out against 3 isolates; a Gram negative bacterium, a Gram positive bacterium and a fungus which are *Salmonella typhi* (Gram negative bacterium), *Staphylococcus aureus* (Gram positive bacterium) and *Candida albican* (fungus).

The leaf of *T. diversifolia* (Hemsl.) A. Gray contains some phytochemicals which were detected during the molecular docking analysis such as (p-Hydroxynorephedrine, verbenol, Phenylephrine-1, DI-Phenylephrine-1, Metaraminol Cavacrol, thymol) the above mentioned phytochemicals belongs to the phenol group. These phytochemicals had docking scores which are higher than the docking score of levofloxacin, which means that these phytochemicals are more active than levofloxacin against the clinical isolates. Plant polyphenols interact with each other to improve the antibacterial activity in which Phenols and phenolic acids could play a positive role in the treatment of infections caused by the resistant bacteria since they have the abilities to link with and disable some bacterial enzymes essential for bacterial cell wall synthesis [73,74].

However, an in vivo antimicrobial as well as the toxicological analysis of the plant extracts which were not carried out in this study need to be investigated to know the safety dose of the plants in case of oral administration to humans. The in vivo test will reveal whether or not the plants' antimicrobial activity will reduce, increase, or give same results as the in vitro test). Double synergy of both plant extracts can be effective to fight against infections caused by microorganisms.

5. CONCLUSION

Leaf extracts of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L showed broad spectrum antibacterial and antifungal activities in this study, although different solvent extracts showed differential effectiveness against the tested microbial species. The leaf extracts of *T. diversifolia* (Hemsl.) A. Gray and *J. gossypifolia* L have a promising antimicrobial activities against the microorganisms studied, further purification should therefore be done to extract the active ingredients which may be used for the development of drugs that will be beneficial to man. Phytochemical analysis is responsible for the identification of components which are responsible for antimicrobial activity of plant, thus these plant species can be a good source of medicine against various diseases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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