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ANALYSIS OF THE PHYTOCONSTITUENTS OF *CHROMOLAENA ODORATA LEAVES* AND ITS BIOACTIVITIES AGAINST SOME CLINICAL AND PLANT PATHOGENS

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Abstract
Medicinal plants have been a source of therapeutic agents for man for centuries now. As a result of resistance to current medicines, there has been a renaissance of interest in research for novel molecules to treat both emergent and existing illnesses. In contributing to this search, this study aimed at examining the phytoconstituents of Chromolaena odorata leaves and its bioactivities against some plant and clinical pathogens. Using the Soxhlet equipment, this powdered plant component was extracted with n-hexane. Using conventional methods, the crude extract was tested qualitatively and quantitatively for the presence or absence of different secondary metabolites. The results show the presence of tannins, alkaloids, flavonoids, saponins, steroids, triterpenoids and cardiac glycoside. The quantitative screening shows that tannins recorded the highest concentration (41.92ppm), followed by alkaloid (Epihedrine) 40.95 ppm, flavonoids such as resveratrol (40.33 ppm) and flavan-3-ol (40.33 ppm) were detected in the plant sample. The results also showed a high sensitivity of the pathogens against the extract. This present study has served as evidence for the ethnomedical claims of the use of the plant for the treatment of various ailments. Thus, it is a useful precursor for drug discovery.

Keywords: Phytoconstituents, *Chromolaena Odorata,* Leaves, Bioactivities, Clinical, Plant Pathogens

Introduction

Individuals and communities benefit greatly from medicinal plants. Plants' therapeutic properties are determined by chemical compounds that have a specific physiological impact on the human body. Tannins, alkaloids, phenolics, and flavonoids are some of the most significant bioactive components of plants (Hill, 1952). Many of these therapeutic herbs are also utilised as spices and food. For medical reasons, they are sometimes added to foods intended for pregnant or nursing moms (Okwu, 2001). Plants seldom, if ever, generate therapeutic medicines in their pure form, preferring instead to synthesise a mixture of substances. Vitamins and minerals included in them can sometimes have a synergistic effect with these medications. As a result, plant extracts are typically more medicinally efficacious than individual bloatiests (Stern*et al.,* 2003). Because medical plants have long been used to treat a variety of human ailments, most herbal medications are seen to be safer than synthetic drugs. As a result, medicinal plants have grown in prominence as an alternative source of effective drugs. Plants and natural products contain an undiscovered pool of potentially beneficial compounds that rivals that of pharmaceuticals. Natural products account for almost half of all synthesized medicines, and they play a significant part in drug research programming in the pharmaceutical industry (Sharma & Kumar, 2009).

Villagers and other rural residents with little resources can easily acquire traditional medicinal plants. To cure a variety of ailments, the herbs are consumed as decoctions, teas, and juice concoctions. It should be emphasized, however, that the volume and potency of therapeutic medicines generated by plants might vary substantially between populations or even organisms. As a result, testing is required to verify their uniformity and quality for usage (Stern *et al*., 2003).

There is a current demand for more effective and less expensive plant-based medicines with stronger antibacterial potential and fewer adverse effects. As a result, scientists devote attention to physiologically active extracts and isolates from traditional medicinal plants (Essawi & Srour, 2000). With the rise in illness caused by viruses and other harmful microorganisms, as well as medication resistance, new medicines derived from plants and other sources are needed to tackle these pathogens. Local plants have yet to be thoroughly investigated as

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potential sources of therapeutic medicines to determine their scientific effectiveness. *Chromolaenaodorata* is one of these plants, and its leaves were chosen for study because of claims of its usage in traditional medicine.

This study aimed to evaluate the bioactive constituents in the leaf of *Chromolaena odorata* and its biological activities against some clinical pathogens. Specifically, the following objectives guided the study:

(i)Qualitatively analyze the phytochemicals present or absent in the leaf extract of *Chromolaena odorata*. (ii) Quantitatively examine the secondary metabolites which could be responsible for the biological activities of the plant and (iii) Examine the biological activities of the plant extract against some clinical pathogens (Animal and Plant).

Sample Collection, Identification and Preparation

Dr. Wisuator David of the Department of Forestry, Rivers State University, Port Harcourt, identified *Chromolaena odorata* leaves collected in August 2020 around Rumuodamaya Metropolis by comparing them to existing specimens at the herbarium section of the Department of Forestry, Rivers State University, Port Harcourt (Number 918). These leaves were dried in the air before being crushed into powder.

Sample Extraction

250g of powdered material was placed in a glass container, and 1000 ml of hexane was added. The bottle was defrosted in a home microwave oven for 3 minutes before being removed and chilled. This was done ten times and then filtered. The residues were extracted again, this time using ethylacetate and then methanol adapted from Armstrong (1999). Ethylacetate was used to wash the white crystals that developed from the methanol extract.

Qualitative Phytochemical Analysis

The chemical tests were carried out on the hexanoic crude extract to confirm the presence or absence of some bioactive compounds otherwise called phytochemicals using the method described by Sabri *et al*., (2012) and Nna and Ndudee (2016).

Test for Steroid and Triterpenoids (Liebermann-Burchard Test)

A positive test for steroid and triterpenoids was indicated through coloration of the upper layer and formation of a deep red colour in the lower layer respectively. A mixture of 3mg of an extract was combined with 3 drops of acetic
anhydride boiled and cold concentrated sulphuric acid was then added from the side of the testtube, a positive t est for steroid and tritepernoidswas denoted through a coloration of the upper layer and formation of deep red c olour in the lower layer.

Test for cardiac glycosides (Keller Killiani Test)

About 3mg of extract was combined with 3 drops of diluted ferric chloride solution, mixed concentrated sulphuric acid, and concentrated glacial acetic acid, and the development of two layers was seen. A positive test for glycosides showed a lower reddish-brown layer and an upper acetic acid layer that turned blue green.

Test for Phenolics and Tannins (Ferric chloride test)

The presence of phenols was determined by adding 2 mg of crude extract diluted in 2ml of extracted solvent and treated with 4 drops of ferric chloride solution, resulting in a bluish-black colour. Gallic tannins was present as the colour turned bluish-black, and cathechic tannins werw present when the colour changed to bluish-green.

Test for Flavonoids (Alkaline test)

In 5 ml of dilute sodium hydroxide solution, 5 mg of an extract was added. The presence of flavonoids was indicated by the development of a yellow colour that faded to colourless once a few drops of weak hydrochloric acid waas added.

Test for Saponnins

The compound's capacity to cause foaming in aqueous solution and hemolyze red blood cells was utilised as a screening test (Deniz, 2009). In a test tube, distilled water (5ml) was added to an extract (5mg)

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and vigorously shaken. The presence of saponins was suggested by the formation of a considerable amount of foaming that lasted for around 30 minutes.

Test for Alkaloids

In a test tube, 3ml of the extract was combined with 1 ml of 10% HCl and heated for 20 minutes. After cooling and filtering, 1 mL of the filtrate was treated with a few drops of Mayer's reagent. The presence of alkaloids was indicated by the presence of creamy precipitated.

Quantitative Phytochemical Screening by GC-FID

BUCK M910 Gas chromatography (BUCK Scientific, USA) was used for the quantification of the phytochemicals in the extract. The gas chromatography was equipped with a flame ionization detector, a RESTEK 15m MKT-1 column (15m × 20m ×0.15um). The injection temperature was set at 280ºC and the injection velocity 30cm/s was used with 20cL splitless injection of sample. Helium (5.0pa) was used as carrier gas with a flow rate of 40ml/min. The initial oven temperature will be 200ºC, the oven was heated at the rate of 3ºC/min until a temperature of 330^{o}_{C} was achieved, while the detector was operated at 320^{0}C. The ratio of the area and mass of the internal standard to the area of the discovered phytochemicals was used to identify phytochemicals. Individual phytochemical concentrations was reported in g/mL. (Nna*et al*., 2018).

Microwave assisted extract (MAC) sequential extraction

250g of powdered material was placed in a glass container, and 1000 ml of hexane was added. The bottle was defrosted in a home microwave oven for 3 minutes before being removed and chilled. This was done ten times and then filtered. The residues were extracted again, this time using ethylacetate and then methanol adapted from Armstrong (1999). Ethylacetate was used to wash the white crystals that developed from the methanol extract.

Antimicrobial Screening

Test organisms

Using animal and plant diseases, the antibacterial activity of the isolated compounds from the plants under research were evaluated. The animal pathogens were collected from the ABU Teaching Hospital Zaria's Department of Medical Microbiology. I.A.R, Ahmadu Bello University, Zaria. *Methicillinresist staphaureus*was one of the animal pathogens (bacteria) utilized in the antimicrobial test. Plant pathogens (fungi) utilized were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus nigre, Coniophora puteana, Fibrophoria vaillentii, Fomitopis pinicola, Fusarium oxysporum, Fusarium proliforatum, Rhizopus sp, Sclerotium rofusum, Sclerotium rofusum*. All microorganisms were tested for purity and kept in agar slants (Tor-Anyiin*et al*., 2016).

Cultivation and standardization of test organisms

The test organisms were removed from the agar slant and subcultured into test tubes containing sterile nutritional agar for bacteria and sabouraud dextrose medium (20 mL) for fungi. The test tubes were incubated at 37^{0}C for 48 hours. To attain a density of 106 cfu/mL for bacteria, broth cultures were standardised using sterile normal saline. A sporulated test fungal spore was collected in sterile normal with 0.05 percent, Tween80 and standardised to 106 spores/mL.

Antimicrobial profile (Sensitivity test)

To produce a concentration of 20 g/mL, 0.002 mg of a DMSO was weighed and dissolved in 10 mL of DMSO. For screening, the diffusion technique was utilized. Mueller Hinton agar and Sabouraud dextrose agar were employed as the microorganisms' growth media. The media were made according to the manufacturer's instructions, sterilised for 15 minutes at 121°C, put onto sterile petri dishes, covered, and allowed to cool and harden. Mueller Hinton agar received 0.1 mL of standard test bacteria inoculum, whereas subouraud dextrose agar received 0.1 mL of test fungus. A sterile swab was used to distribute the inoculum evenly throughout the surface of the medium. A well was cut in the centre of each infected medium using a standard sterile cork borer with a diameter of 6 mm. After that, around 0.1 mL of a 20 g/mL solution of DMSO was added to the well on the infected medium and allowed to diffuse into the agar. After incubation at 37 °C for 24 hours for bacteria and 30

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°C for 1-7 days for fungi, the plates containing the medium were examined for zones of growth inhibition. With a clear ruler, the observed zones were measured and the results were recorded in millimetres.

Determination of minimum inhibitory concentration (MIC)

A broth dilution technique was used to determine a compound's minimum inhibitory c oncentration. Mueller Hinton broth and Sabouraud dextrose broth were made; 10 mL of each broth was poured into test tubes and sterilized at 121 °C for 15 minutes, after which the broth was allowed to cool. A turbid solution was produced using Mc-turbidity Farland's standard scale number 0.5. The test microbe was injected and cultured at 37 °C for 6 hours after 10 mL of normal saline was poured into sterile test tubes. The test microorganism was diluted in normal saline until the turbidity matched Mc-scale farland's when compared visually. The test microorganism has a concentration of 1.5 108cfu/mL at this stage. The chemical was serially diluted twice in sterile broth to generate concentrations of 20 g/mL, 10 g/mL, 5 g/mL, 2.5 g/mL, and 1.25 g/mL. 0.002 mg of DMSO was dissolved in 10 mL of sterile broth to produce the starting concentration. After obtaining various concentrations of a chemical in sterile broth 0.1 mL of a test microbe in normal saline was inoculated into the various concentrations and incubated at 37 °C for 24 hours for bacteria and 30 °C for 1-7 days for fungus. Following that, the turbidity of the broth test tubes was examined (growth). The minimal inhibitory concentration was determined by taking the lowest concentration of a chemical in sterile broth that produced no turbidity (MIC).

Determination of minimum bactericidal/ fungicidal concentration (MBC/MFC)

The minimal bactericidal/fungicidal concentration (MBC/MFC) was determined in order to see if the test micro organisms were killed or if their development was slowed. Mueller agar and sabouraud dextrose agar were mad e, sterilized at 121 °C for 15 minutes, and then put into sterile petri dishes to cool and solidify. The contents of the MIC were then sub-cultured onto prepared medium in successive dilutions. Fungi were subcultured on Sabouraud dextrose agar, whereas bacteria were subcultured on Mueller agar. Bacteria were incubated at 37°C for 24 hours and fungi were incubated at 30°C for 1-7 days. The plates of the medium were then examined for colony growths. MBC/MFC was plates lacking colony development that had the lowest concentration of the chemical. After 24 hours, the results were recorded (Usman *et al*., 2007).

RESULTS

The results of the phytochemical screening of the extract of *Chromolaena odorata*

Table 1: Qualitative Phytochemical

Class of compound	Test /Reagents	Observation/Inference
Tannins	Feric chloride (5% $FeCl_3$)	Dark green precipitate .Presence of tannins
Alkaloids	Mayer's	Pale yellow precipitate. Alkaloid present
Flavonoid	Shinoda	Cloudy solution. Flavonoid present
Saponins	Foam test	Foam seen. Presence of saponins
Triterpenoids	Liebermann-Burchard	Brown-ring colouration. Presence of triterpenoids/steroids.
Cardiac glycoside	Keller-killiani	No reaction indicating absence of Cardiac glycoside.

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Table 2: Quantitative phytochemical screening of *Chromolaena odorata*

Parameters	Concentration ($\mu g/g$)	Class of compound
Proanthocyanin	0063	Flavonoid
Naringin	0.173	Flavonoid
Lunamarin	34.73	Alkaloid
Quinine	4.61	Alkaloid
Anthocyanin	9.48	Flavonoid
Ribalindine	10.87	Alkaloid
Naringenin	13.12	Flavonoid
Spartein	14.89	Alkaloid
Sapogenin	16.96	Saponin
Phenol	19.44	-
Flavonones	22.88	Flavonoid
Steroids	25.71	Steroid
Epicatechin	28.61	Flavonoid
Kaenpferol	30.28	Flavonoid
Phytate	32.73	-
Flavones	34.83	Flavonoid
Oxalate	37.89	Dicarbohylicaid
Catechin	38.53	Flavonoid
Rutin	39.32	Flavonoid
Resveratrol	40.33	Flavonoid
Epihedrine	40.95	Alkaloid
Flavan-3-ol	40.33	Flavonoid
Tannin	41.93	Tannin

Table 3: Zone of inhibition and antibacterial activities of *Chromolaena odorata*

Test organisms	Zone of inhibition and activity (mm)	Sparfloxacin	Fluconazole
Methicillin rest staphaureus	S(24)	S(30)	R(0)
Staphylococcus aureus	R(0)	S(31)	R(0)
Vanomylin resist enterococcin	S(23)	S(27)	R(0)
Streptococcus pneumoniea	S(20)	S(29)	R(0)
Bacillus, Subtilis	S(21)	R(0)	R(0)
Escherichia coli	R(0)	R(0)	R(0)
Klebsiella pneumoniea	S(23)	S(32)	R(0)
Salmonella typhi	R(0)	S(25)	R(0)
Proteus mirabilis	R(0)	S(31)	R(0)
Candida krusei	S(20)	R(0)	S(32)
Candida tropicalis	R(0)	R(0)	S(35)
Candida albicans	S(27)	R(0)	S(34)

Table 4: Minimum Bactecidal/Fungicidal concentration of the extract of *Chromolaenaodorata*

Organism	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.63 mg/ml
Methicillin rest staphaureus	-	0*	+	++	+++
Staphylococcus aureus	-	0*	+	++	+++
Vanomylin resist enterococcin	0*	+	++	+++	+++
Streptococcus pneumoniea	0*	+	++	+++	+++
Bacillus, Subtilis					
Escherichia coli	-	0*	+	++	+++
Klebsiella pneumoniea					
Salmonella typhi					
Proteus mirabilis	-	-	0*	+	++
Candida krusei	0*	+	++	+++	+++
Candida tropicalis	-	+	++	+++	+++
Candida albicans	-	+	+	++	+++

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-	=	No colony growth
0*	=	MBC/MFC
+	=	Scanty colonies growth
++	=	Moderate colonies growth
+++	=	Heavy colonies growth

Table 5:Minimum inhibitory concentration of the extract against the test microorganism

Organism	10 mg/ml	5 mg/ml	2 mg/ml	1.25 mg/ml	0.63 mg/ml
Methicillin rest staphaureus	-	-	0*	+	++
Staphylococcus aureus	-	-	0*	+	++
Vanomylin resist enterococcin	-	-	0*	+	++
Streptococcus pneumoniea	-	-	0*	+	++
Bacillus, Subtilis	-	-	-	-	-
Escherichia coli	-	-	0*	+	++
Klebsiella pneumoniea	-	-	-	-	-
Salmonella typhi	-	-	-	+	++
Proteus mirabilis	-	-	-	0*	+
Candida krusei	-	-	0*	+	++
Candida tropicalis	-	-	-	+	++
Candida albicans	-	-	-	++	+++

Key:

-	=	No turbidity (no growth)
0*	=	MIC
+	=	Turbid (light growth)
++	=	Moderate turbidity
+++	=	High turbidity

Discussion

Qualitative Phytochemical Screening

The preliminary qualitative screening of the *chromolaena odorata*. Leaf showed the presence of some phytochemicals such as tannins, alkaloids, saponins, flavonoids, steroids and triterpenoids but cardiac glycoside was absent as shown in (Table1). These phytochemicals are known for their activities including antimicrobial, antihypertensive, antioxidant, anti-inflammatory and analgesic etc. the presence of these constituents is responsible for the biological activity displayed by the extracts of *chromolaenaodorata.* Flavonoids in plants are a diverse group of biologically active compounds that have long been used in traditional medicine for their anti-inflammatory and anti-fibrotic properties, particularly in the treatment of chronic inflammatory and allergic diseases, breast cancer, and coronary artery disease (Vishnu *et al*., 2013). Flavonoids have also been discovered to be a potent antibacterial agent against a range of microorganisms in vitro, with their action attributed to their capacity to combine with bacterial extracellular and soluble proteins (Rajani et al.,2013). Because of their significant physiological significance, terpenes and steroids in general have gotten a lot of research (Sabriet al., 2012). Several authors have demonstrated the anti-malarial effects of terpenoids and steroids (Forghe &Nna, 2020, Sabri et al., 2012). Saponins have been found to have anti-inflammatory properties (Tor-Anyiinet al., 2016). Saponins are used as emulsifying agents in medicine while tannins have been found to possess oxidative inhibiting properties (Nnaet al., 2019). Alkaloids have been reported for their antimalaria property and pain relief (Nna, et al., 2018).

Quantitative Phytochemical Analysis

Some components of the extract were further quantified by a Gas chromatography flame ionization detector (GC-FID) and results are reported in Table 2. The result showed a significant quantity of flavonoids such as resveratrol (40.33 $\mu g/g$), flavan-3-ol (40.33 $\mu g/g$), rutin (39.32 $\mu g/g$), catechin (38.53 $\mu g/g$), kaempferol (30.28 $\mu g/g$), epicatechin (28.61 $\mu g/g$). Others identified and quantified flavonoids include flavanones (22.88 $\mu g/g$), naringenin (13.12 $\mu g/g$), anthocyanin (9.48 $\mu g/g$), naringin (6.173 $\mu g/g$), and proanthocyanin (0.063 $\mu g/g$).

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Flavonoid have been known to exhibit antifungal, antibacterial, anti-oxidant and anti-inflammatory as well as anti-cancerous. Naringin is rapidly transformed to naringenin by the action of enzymes such as ∝-rhamondase and β-glucosidase in the body (Vishnu et al., 2013). Naringin or naringenin exhibit various pharmacological and therapeutic properties such as antimicrobial, antimutagenic, anti-inflammatory, free radical, scavenging, anti-cancer, cholesterol lowering effects (Vishnu, et al, 2013). Anthocyanins play an important role in visual acuity, heart disease, treatment of cancer, age-related neurodegenerative disorders and in angiogenesis is (Vishnu et al., 2013). Resveratrol has been reported for its usefulness in the treatment of high cholesterol, cancer, heart diseases, antioxidant property and thus, serve as promising supplement for lowering blood pressure (Nnaet al., 2019). Proanthocyanins protect the heart and cardiovascular system. They are antioxidant, anti-inflammatory, antioxidant and anticancer activities. It prevents urinary treat infection in women and also works with vitamin C to lower the risk of breast cancer (Nnaet al., 2020). It is used in the treatment of varicose veins. Resveratrol was shown to have antibacterial activity against gram-positive bacteria, and time-kill tests revealed that its effects were attributable to a bacteriostatic effect (Franck *et al.,* 2004). Resveratrol has antiproliferative and androgen-lowering effects on the interstitial cells of the ovary, in addition to its cardioprotective, antioxidant, anticancer, neuroprotective, anti-inflammatory, anti-dyslipidemia, and antidiabetic properties (Okwu & Okwu , 2004). The following alkaloids were also identified and quantified in the study. Lunamarin (34.73 $\mu g/g$), quinine (4.61 $\mu g/g$), ribalindine (10.87 $\mu g/g$), and spartein (14.89 $\mu g/g$)). Alkaloids are known to exhibit many physiological properties in the body. Quinine has been reported severally for its antimalarial activities as drug candidate for malaria drug, though discontinued due to its side effects (Tor-Anyiin*et al.,* 2016). This account for the use of the plant for the treatment of malaria by the Ikwerre ethnic group. Lunamarine has been reported to have radical scavenging and anti-amoebic activities (Feghe and Nna, 2020). Ribalimdine and lunamarine and spartein have been reported to have potential health benefits as well (Franck *et al,* 2004).

The plant under investigation is used to treat open wounds. This practice could be traceable to the presence of tannins and saponins (Okwu & Okwu, 2004). Saponins are a group of compounds that are known for their cardiotonic effect (Finar, 2005). Sapogenins serve as a precursor for the synthesis of drugs or analogues of drug that are used in the treatment of heart related problems (Deniz, 2009). Tannins have been described as antioxidants, metal chelators, and regulators of damaging pre-oxidative enzymes and the lipid peroxidation process. More recently, tannins have been shown to have antiseptic, anticarcinogenic, anti-inflammatory, anticancer, and antidiabetic effects (Rajani et al., 2013). Tannins are also sued medically to treat diarrhea, inflammation and burn (Rajani et al., 2013). Phytate (32.73 $\mu g/g$) was another secondary metabolite quantified in the sample. Phytate has been reported to have antilipidemic and anti-inflammatory activities (Rajani et al., 2013). Phytates are however crowning to act as a nutrient in the body. They serve as metal chelate (Rajani et al., 2013). Tannins play important role in the control of gastritis, esophagitis, enteritis, and imitating bowel disorders. Tannins can heal burns, stop bleeding as well as infection while they continue to heal the wound internally (Praveen & Kumud, 2012). This could be attributed to the use of the leaf of the plant in dressing sharp cut wounds and stop of blood in a fresh wound traditionally. Oxalate (36.89) is a dicarboxylic acid that was quantified in the sample. It has been reported to be an antibacterial, anticancer agent and also to treat cardiovascular diseases. Steroids were quantified in a significant amount (25.71 $\mu g/g$). Steroids have a role in a variety of physiological processes, including stress, immunological response, glucose metabolism, protein catabolism, blood electrolyte levels, and inflammation and behaviour control. Anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, anthelminthic, cytotoxic, and cardiotonic action are only a few of the medical, pharmaceutical, and agrochemical properties of plant steroids. The hypocholesterolemic and anti-inflammatory properties of steroidal substances have also been documented. In the sample, kaempferol (30.28 g/g) was found. It has been shown to lower the risk of chronic illnesses, including cancer. It boosts the antioxidant defences of the human body against free radicals. Apoptosis, angiogenesis, inflammation, and metastasis are all influenced by it. Rutin (39.32 g/g) was also found in the sample as a significant flavonoid. It is frequently used to relieve arthritis pain and to help patients with arthritis reduce oxidative stress. This may be due to rutin's powerful anti-inflammatory and antioxidant capabilities. Rutin has also been shown to enhance knee function in certain patients suffering from arthritis. This is evidence of the plant's historic usage in the treatment of paralysis and arthritis. Phenol (19.44 g/g) has long been used to disinfect and alleviate itching on the skin. It is also used as an oral analgesic or anesthetic in products such as chloroacetic to treat pharyngitis. It is also an antiseptic agent. However, other pharmacological properties including antioxidant, antiviral, anticancer, and anti-inflammatory activities have been reported (Nnaet al., 2018).

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Antimicrobial Study

The sensitivity test and inhibition zones of plant extract were measured and compared to those of standard drugs such as sparfloxacin and fluconazole for bacteria and fungi respectively (Table 3). The susceptible bacteria used for the study were *methicillin resistant staph aureus, staphycococus aureus, vacomycin resistant enterococein, streptococcuspneunoniea, bacillus subtilis, Escherichia coli, klebsiella pneumoniea, salmonella typhi, protens mirabilis, candida krusei, candida tropicalis*, and *candida albicans*. The zone of inhibition of the microbes ranges from 20-27mm with candida albicans the most sensitive (27mm) followed by *methicillin resistant staph aureus* (24mm) and the least were *streptococcus pneumonia* and *candidate krusei* (20mm) respectively. However, organisms such as *staphylococcus aureus*, *Escherichia coli*, *salmonelletyphi,protens mirabilis* and candida tropicalis were resistant to the study sample extracts. *Methicillin resist staph aureus* (MRSA) has been reported to be responsible for skin infections and pneumonia (lung infection). VRE causes a variety of diseases, including bloodstream infection (sepsis), urine infection, abscesses, pneumonia, and heart infection (endocarditis or meningitis). Middle ear infections, sepsis (blood infection) in youngsters, and pneumonia in immunocompromised people and the elderly are all caused by Streptococcus pneumonia. It can also lead to meningitis (inflammation of the brain and spinal cords coverings) or sinus infections. *Streptococcus pneumonia* is gram-positive anaerobic bacteria. *Bacillus subtilis* are responsible for the following infections, bacteremia, endocarditis, pneumonia and septicemia. However, diseases such as antitax, abscesses, ear infections, meningitis, ophthalmitis, osteomyelitis, and peritonitis. It is a gram-negative bacterium. *Klebsiella pneumoniea* is a bacterium that has been linked to a variety of illnesses, including UTI, pneumonia, intra-abdominal infection, bloodstream infection (BSI), meningitis, and pyogenic liver abscess. It's a rod-shaped gram-negative, non-motile, lactose-fermenting, facultative anaerobic bacteria. The symptoms of pneumonia include fever, chills, coughing, yellow or bloody mulus, shortness of breath and chest pains. *Candida krusei*, *candida tropicalis* and *candida albicans* are fungi. *Candidate krusei* has been implication in endocarditis, ocular infection as well as fungemia. It is also responsible for pediatric arthritis and urinary infection. The results showed that all test bacteria were sensitive to sparfloxacin the control drug except bacillus subtilis and *Escherichia coli* were resistant. Again, *vanomycin resist enterococein*, *bacillus subtilis*, *Escherichia coli* and *protens mirabilis* were sensitive to ciprofloxacin. All the tested fungi were sensitive to fluconazole (Table 3). The extract also showed high turbidity against some test organisms used in the study (Table 4 and 5). This, indicates the activity of the extract against the pathogens invariably accounting for its uses as a precursor for antimicrobial drugs.

Conclusion

The qualitative phytochemical screening of the plant extract under investigation reveals the presence of tannins, alkaloids, flavonoid, saponins, steroids, and triterpenoids. However, cardiae glycoside was absent. These metabolites play a significant role in human and plant health. The n-hexane leaf extract of *chromoleana odorata* had shown potential antimicrobial activities. This means that the plant could be an effective candidate for the treatment of the diseases caused by those pathogens. This study justifies the use of the leaf of *chromoleana odorata* in traditional medicines to treat arthritis, skill infections, pneumonia, urinary tract infection, septicemia, meningitis, and intra-abdominal infection among others.

The quantification of the phytochemicals using GC-FID further revealed the presence of some metabolites including quinine, lunamarin, spartein, oxalate catechin, resveratrol and soon whose medicinal potencies are high and well established. These phytochemicals account for the use of the plant in ethnomedical practices. Thus, drugs prepared from *chromoleana odorata* could be an active ingredient to combat certain diseases and also help to build the body system.

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