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Phytochemical Profiling and Antimicrobial Evaluation of the Whole Plant Dyschoriste Perrottetii

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Abstract: This study examined the phytochemical profile and antimicrobial efficacy of Dyschoristeperrottetii, a plant traditionally used to treat female sterility, fever, malaria, diarrhea, chickenpox, and dysentery. The plant material was identified, dried, pulverized and extracted using cold maceration method. The phytochemical screening of the crude ethyl acetate revealed that the extract showed the presence of tannis and flavonoids only. Saponins, carbohydrates, anthraquinones, cardiac glycosides, and alkaloids were found to be absent. The result of Zone of inhibition (ZI) showed inhibition which ranges from 20 -25 mm for ethyl acetate extract against the entire test organism except, Vancomycin Resistant enterococci, Campylobacterfetus, Pseudomonasaeruginosa, Klebsiella pneumonia and Escherichia coli. Drugs used as positive control had zones of inhibition of 25- 38 mm for ciprofloxacin, and 30–32 mm for fluconazole. The results of Minimum Inhibitory Concentration (MIC) revealed that all organisms had MIC of 2.5 mg/ml except Candidatropicalis with MIC of 5 mg/ml. The results of Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC) revealed that all the organisms had MBC/MFC of 5 mg/ml except MRSA, C. tropicalis and C. kruseiwith MBC/MFC of 10 mg/ml respectively. The sensitivity of MRSA, S. aureus, P. mirabilis, Candidakrusei, Helicobacter pylori and Candidatropicalis to the ethyl acetate extract implies that chemical compounds in the extract could be used to develop drugs to treat related ailments.The results indicated that the extract of D. perrottetii had potential as a source of bioactive compounds for antimicrobial drug development, particularly against multi-resistant organisms.

Key-words: Phytochemical, Dyschoriste Perrottetii, Tannins, Flavonoids, Saponins

Introduction

According to the traditional medicinal program of the World Health Organization[1], traditional medicine is defined as the sum total of all the knowledge and practices, whether they are explicable or inexplicable, that are used in the diagnosis, prevention, and elimination of physical, mental, or social imbalances. Traditional medicine relies solely on practical experience and observation that is passed down from generation to generation, either verbally or in writing[2]. The World Health Organization (WHO) estimates that almost three

quarters of the world's population relies on traditional medicines, mostly herbal remedies, for the purpose of providing medical care to their fellow citizens. Herbal remedies and plants are, in point of fact, the oldest companions of the human race. Not only did they offer sustenance and a place to settle, but they also assisted humans in the treatment of a variety of dysfunctions. Traditional medicine, which employs herbs, is widely used. The poor world makes substantial use of them because, in many locations, they provide an alternative to pharmaceutical medications that is more readily available and more reasonably priced. For instance, according to estimations provided by the World Health Organization[3], up to eighty percent of the population in Africa is dependent on them.

The continued interest in traditional medicine within the context of the health care system may be supported by two primary factors. The first problem is that there is insufficient access to allopathic drugs and western techniques of treatment. This means that the majority of people are unable to pay or receive contemporary medical care, either because it is too expensive or because there are no medical services available. Second, there is a deficiency in the availability of current medical treatments that are effective for certain conditions [3]. Plants often include a variety of phytochemicals, which are sometimes referred to as secondary metabolites. These phytochemicals can operate individually, additively, or synergistically to accomplish the goal of improving health care requirements. Indeed, medicinal plants, in contrast to pharmacological medications, typically contain many compounds that collaborate in a catalytic and synergistic manner to generate a combined effect that is greater than the sum of the individual's activities[4]. It has been known that plants are the first and only true medicines that have been used by man [5]. The use of plants for medicinal purposes is an important part of the culture and traditional practices in Africa. It is said that traditional medicines, which are also sometimes referred to as herbalism, are the most ancient method of treating diseases[6]. Most people depend directly on the traditional medicine for the primary health care [6].Today there has been an increasing incidence of multiple resistances in human pathogenic microorganism in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Scientists have been compelled to look for antimicrobial substances from alternate sources, such as medicinal plants, as a result of the development of resistance to antimicrobial agents. As a result of this, medicinal plants have garnered a lot of interest as an alternative treatment option in comparison to synthetic medications[3], [7].

The Plant Family Acanthaceae

The Acanthaceae family is a dicotyledonous family of flowering plants that has roughly 221 genera and 4000 species [8]. The majority of these plants are tropical herbs, shrubs, or twining vines, and some of them are epiphytes. In places that are classified as temperate, just a few species may be found. It is important to note that Indonesia, Malaysia, Africa, Brazil, and Central America are the four primary

distribution centers. The representative of this family may be found in almost every environment, including dense and open forests, scrubland, wet fields and valleys, seacoast and marine regions, and swamps. It is also possible to find it in wetlands. Simple, opposite, decussated leaves that are decussated and have whole edges (or sometimes toothed lobed or spiny margins) and no stipules are the characteristics of plants belonging to this family[8]. In addition to being zygomorphic to almost actinomorphic, the flowers are grouped in an inflorescence that can be a spike, raceme, or cyme. Stamen number two or four are organised in pairs and inserted on the corolla, and the ovary is either superior or bicarpellated with axile placentation, the calyx typically has four or five lobes, and the corolla is tubular, two-lipped, and five-loped, the fruit is a two-celled capsule that dehisces and has a somewhat explosive quality[9]. In the majority of species, the seeds are linked to a short stalk that is hooked and that causes them to be expelled from the capsule. The Acanthaceae family of plants is a significant one for indigenous people, particularly in terms of their usage for medical purposes. The leaves and other components of the plant species are the ones that are most frequently utilised for the treatment of a variety of maladies, including but not limited to diarrhoea, diabetes, fever, dysentery, labour pains, cough, and typhoid[10].

Dyschoriste Perrottettii (Nees) and its Traditional Uses

The plant known as Dyschoristeperrottetii is a shrub that stands around half a meter tall and has branches and a square woody stem that roots at lower nodes[11]. It is virtually absent in artistic regions, while it is abundantly distributed in tropical regions and commonly found in temperate regions, it is commonly known as fiddahakukuwa, momodil, and bidi-diyan among the Hausas and Fulani communities in Nigeria. The plant is utilized in traditional medicine for the purpose of making labor easier, as well as for the treatment of yellow fever and measles[12]. Additionally, the seeds of the plant are utilized for the removal of foreign material from the. Traditional medicine in Burkina Faso involves the use of a decoction made from the aerial portions of this plant for the purpose of treating and preventing malaria in children. According to the findings of [13], it was used to treat fever, malaria, and diarrhea. Oral administration of an infusion made from the leaves is used to treat both primary and secondary infertility[14], It has been shown that the leaves may be used as an infusion (both internally and externally) to treat eye infections, wounds, and skin problems[15]. The Zaypeople of Ethiopia utilize a decoction made from the whole plant to cure tooth pain. It is widely acknowledged that dyschoristelitoralis, when combined with ginger, is an extremely effective treatment for coughs of any kind. Rheumatism can be treated using medicinal plants. The leaves are dried, and then manufactured into cigarettes, which are then smoked to cure asthma. Additionally, the juice extract is utilized for the treatment of diarrhea and dysentery. So far, pharmacological actions that have been documented include insecticidal activity, antifungal

activity, antibacterial activity, anti-inflammatory activity, antipyretic activity, and anti-inflammatory activity[16].

Medicinal Uses of Dyschoriste Perrotettii

The usage of extract from Dyschoristeperrotettii for therapeutic purposes has been demonstrated to be effective in a variety of ways, including the following:

Antimicrobial

The fungus DyschoristePerrotettii has shown a wide variety of antimicrobial properties against strains of bacteria and fungi that were isolated from farm animals and were resistant to a number of different medications. The fact that this Dyschoristeperrotettii extract has the ability to inhibit the growth of certain bacteria species, such as agrobacterium, tumefaciens, Bacillus sp, Escherichia coli, proteussp, Pseudomonas sp, and Salmonella sp, which have been linked with crown gall or gastrointestinal tract and wound infections, is a clue that it is being used in ethno medical applications[17].

Antioxidants

The plant known as DyschoristePerrotettii possesses a variety of antioxidant activities. There is a possibility that the antioxidant capacity of the extract will vary depending on the harvesting time of the plant as well as the characteristics of the host tree; the seed of the plant is utilized for the elimination of foreign material in the eyes[18].

Phytochemicals

Plant substances that are not only non-nutritive but also possess protective and preventative effects are known as phytochemicals. There are over a thousand identified phytochemicals that have the potential to protect humans from illnesses, according to research that was conducted not too[10]. A variety of plants have been utilized for medicinal purposes in traditional medicine for a considerable amount of time. A few of them appear to be effective. Although there has not been adequate scientific evidence to establish their usefulness, phytochemical screening is necessary in order to know the components of a medical plant and to determine what it may be used for[10]. This is because it is necessary to know what the components of a medicinal plant Researchers have been able to successfully separate the bioactive components of big botanicals, and the therapeutic components of these plants have been removed and analyzed using pharmacological methods. Many plant components are now synthesized in the laboratory for use in the preparation of pharmaceuticals. For instance, vinceristine, which is an anti-tumor drug, digitals, which is a heat regulatory, and aphadin, which is a bronchrolator used in respiratory congestion, were all initially discovered through research on plants [19]. According to the World Health Organization's (2000) estimation, just twenty percent of the plant flora has been

investigated, while sixty percent of medicines have their roots in plants[20]. Ancient wisdom, when combined with scientific principles, has the potential to emerge as a strong agent that may give the human race with effective treatments for the elimination of any and all ailments[21].

Bioactive Compounds in Medicinal Plants

These secondary metabolites in plants are known as bioactive chemicals, and they are responsible for producing pharmacological or toxicological effects in both humans and animals. In addition to alkaloids, saponins, tannin, steroids, phenol, flavonoids, glycosides, and other bioactive chemicals, medicinal plants also contain a variety of other essential bioactive components[22].

Alkaloids

Plants may be utilized to make preparations for the purpose of causing physiological changes such as pain relief and tranquillization. Alkaloids are an important chemical molecule that serves as a rich reservoir for the development of new drugs through the process of drug discovery. The presence of alkaloid can also bestow some emetic effects on the plant, and as a result, it might potentially be used locally as a purgative agent and stimulant. In addition, it provides security for the plant by preventing attacks by animals or insects[23]. Some alkaloids that were extracted from natural herbs have been shown to have anti-metastasis and anti-proliferation properties.There is a wide variety of compounds that belong to the alkaloids category. These compounds have a ring structure and some nitrogen[24]. The majority of the time, the nitrogen atom is situated inside the middle of the heterocyclic ring structure.

Steroids

A steroid is a type of chemical substance that is distinguished by the presence of a particular arrangement of four cycloalkane rings that are connected to one another throughout the structure. The dietary lipid cholesterol, the steroid sitosterol, the sex hormone oestradiol and testosterone, and the anti-inflammatory medication dexamethasone are all examples of steroids. Steroids are made up of seventeen carbon atoms that are bound together to create the shape of four fudes rings[25].

Tannins

Tannins have astringent properties, which means that they speed up the healing process of wounds and mucous membranes that have been inflamed. Tannins have garnered a lot of attention in the fields of nutrition, health, and medicine, primarily because of their physiological activity, which includes antioxidant, antimicrobial, and anti-inflammatory properties. Tannins contribute to the property of astringency, which is the speeding up of the healing process of wounds and mucous membranes that have been inflamed. Tannins are complex

moieties that are generated by the majority of plants and have been employed as tanning agents for a long time. Tannins contain astringent, anti-inflammatory, anti-diarrheal, antioxidant, and antibacterial effects [26].

Antibacterial Property of Dyschoriste Perrottetii

The antibacterial properties of D.Perrottetii have been demonstrated against a wide range of harmful bacteria, including Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Salmonella typhi, among others[18],discovered that the aqueous extract of D. Perrottetii was effective against Salmonella typhi. Therefore, D. Perrottetii displayed an outstanding property against microorganisms (bacteria), which has the potential to be utilized as an antibacterial agent against a variety of diseases[18].

Anti-Plasmodial Property of Dyschoriste Perrottetii

The disease known as malaria is among the most significant threats to public health. It may be found in places that are classified as tropical or subtropical, such as a portion of the Americas, Asia, and Africa. South Africans frequently make advantage of the medicinal plants that it produces[27]. Traditional health care services are effective in treating a variety of illnesses, including malaria. Through the use of the parasite lactate dehydrogenase (PIDU) assay, the plant extract of D.Perrottetii was examined to see whether or not it exhibited any in vitro action against Plasmodium falciparum strain Div. Extracts were prepared using a variety of solvents, including aqueous extraction (infusion in water), percolating in methanol, and others. Anti-plasmodial properties of D. Perrottetiiwere reported in vitro and in vivo, either on their own or in a conventional combination[27].

Anti-Diarrhea Property of Dyschoriste Perrottetii

Diarrhoea is frequent loose of liquid bowel movement, the spacing of 'diarrhea" is an appropriation of the Greek meaning lowing through. Diarrhea is defined as the passage of abnormal liquid or unformed stool at an increased frequency[28]. Aqueous extract of the plant showed anti-diarrheal effect in rat and is being used in Nigeria for the management of diarrhea Crude extract of leaves and stem of D. Perrottetiiwas evaluated in perfuse isolated rabbit Jejunum and castor oil induced diarrhea mode in rats[29].

Hepatoprotective Effect of Dyschoriste Perrottetii

Natural product still represents a vital source for biologically active drugs with unique mechanism of action; many traditional plants are used for the treatment of liver problems. Study of these led to the discovery of active compounds yet to be developed to successful drugs. Evaluation of the hepatoprotective effect of D. Perrottetii from Saudi folk medicine against experimentally induced liver injury in Wistar albino rat showed that ethanol extract of the leaves were hepatoprotective[30].

Shigellocidal Property of Dyschoriste Perrottetii

D.Perrottetii was investigated for activities against multi drug resistance Shigella species isolated from patients with bacillary dysentery in Lagos [31]. Extract of the

plant possessed Shigellocidal potential. The result suggests that aqueous extract of the plant as decoctions and concoctions could be useful in the treatment of shigellosis and should be clinically evaluated specially in Nigerian Region

Materials and Methods

Sample collection and handling

The whole plant D.perrottetiiwas collected in fresh condition at Mando,in Kaduna on 20th may,2017.The fresh plant D.perrottetiiNeeswere collected, identified and authenticated at the Herbarium section of the Department of Biological Science,Ahmadu Bellow University, Zaria, where avoucherNo 1186 has been deposited for reference purpose. The fresh sample collected were properly washed and dried at room temperature for one week and the leaves are grounded to fine powder using mortar and pestle, it was sieved to remove coarse plant materials, which was then weighed using electronic weighing balance and stored in a clean dry container until needed for use.

Extraction of thewhole plant D. perrottetii

Maceration method was employed; 250g of the plant sample was placed in a stoppered container with the solvent and allowed to stand at room temperature for 3 - 4 days with frequent agitation until the soluble matter has dissolved. The mixture then was strained, the marc (the damp solid material) was pressed, and the combined liquids were clarified by filtration or decantation after standing

Phytochemical Screening of the Crude Extracts

Chemical test were carried out on the plant extract to identify the secondary metabolites such as Alkaloids, Saponins, Tannins, Steroids, Flavonoids, Anthraquinone glycoside, carbohydrate [32] the test were expressed as absent and present

Preliminary Phytochemical Screening

About 5g of the ethyl acetate extract was used for the preliminary phytochemical screening according to the procedure summarized below

Test for Carbohydrate

Molish Test: about 3 drops of molish reagent were added to 2ml of aqueous ethyl acetate extract in 1ml of concentrated H_2SO_4without mixing, occurrence of a purple ring in the interface indicate a positive test[33].

Test for Alkaloid:

Wagner's test: addition of 3 drops of Wagner's reagent to 2ml of the acidified ethyl acetate gave a brown presence of alkaloids [34]

Test for Flavonoid

Ferric Chloride test: small quantity of ethyl acetate extract was boiled with water and filter to 2ml of the filtrate, 2 drop of ferric chloride (freshly prepared) solution

was added, a green blue or violet colouration indicate the presence of phenolic hyrloxy[34]

Test for Tannin

Small quantity of ethyl acetate extract was boiled with water and filtered. 2 drops of ferric chloride were added to the filtrate. A blue black, green precipitate indicate the presence of tannins[34]

Test for Anthraquinones

Free anthraquinones: about 0.5g of ethyl acetate extract was shaken with 10ml organic solution (Benzene), after filtration, 5ml of ammonia solution (NH_4OH) was added and shaking a pink colored observed in the aqueous layer (lower layer) indicates a positive test [34]

Test for Saponins

Frothing Test: about 0.5g of ethyl acetate extract was shaking with water in a test with frothing which persisted for 15minutes or when warm on water bath indicate the presence of saponins[34].

Test for Cardiac Glycosides

Salkowski test: about 0.2 gram of ethyl acetate extract was dissolved in 1ml of concentrated H2SO4 was added down the texture tube to form 2 layers a red yellow colour indicate the presence of sterol or methylated sterols[34].

The Microbial Screening

Collection of test organisms

The antimicrobial activities of ethyl acetate from leaves and stem extract was determine using some pathogenic microbes, the microbes were obtained from the department of medical microbiology Ahmadubello university teaching hospital Zaria.

Antimicrobial Screening

0.1 g of the extract was weighed and dissolves in 10mls of Dimethylsulphoxide (DMSO) to obtain a concentration of 10mg/ml. diffusion method was used for screening the extract. Mueller Hinton agar was the medium used as the growth medium for the microbes. The medium was prepared according to the manufacturer's instructions sterilized at 121°c for 15 minutes, poured into sterilized petri dishes and were allowed to cool and solidify. The sterilized medium was seeded with 0.1 ml of the standard inoculums of the test microbes. The inoculum was spread evenly over the surface of the medium by the use of a sterilized swab. By the use of a standard sterile cork borer of 6mm in diameters, a well was cut at the center of each inoculated medium. 0.1 ml of solution of extract of concentration 10mg/ml was then introduced in to the well of the inoculated mediums. Incubation was made at 37°c for 24hrs after which the plates of the media was observed for the zone of Inhibition of growth, the zone was measured with a transparent ruler and the result recorded in millimeter.

Determination of the minimum inhibition concentration (MIC) of the extract

The minimum inhibition concentration of the extract was determined using the both dilution method. Mueller Hinton broth prepared, 10mls of the broth was dispensed into test tube and was sterilized at 121°c for 15 minutes, the broth was allowed to cool. McFarland's turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared, 10mls was dispensed into sterile test tube and the test microbe was inoculated and incubated at 37°c for 6hours. Dilution of the test microbe was done in normal saline until the turbidity marched that of McFarland's scale by visual comparison at this point the test microbe has a concentration of 1.5 x 10^8cfu/ml. Two-fold serial dilution of the extract was done in the sterile broth to obtain the concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.63 mg/ml. Having obtained the different concentration of the extract in the sterile broth, a 0.1 ml of the test microbes in the normal saline was then inoculated into the different concentration, incubation was made at 37°c for 24 hours, after which the test tubes of the broth were observed for turbidity (growth) the lowest concentration of the extract in the sterile broth which shows no turbidity was recorded as the minimum inhibition concentration.

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

Minimum bactericidal were carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121°c for 15mins poured into sterile Petri dishes and were allowed to cool and solidify. The contents of the MIC in the serial dilution were then sub-cultured onto the prepared medium; incubation was made at 37°c for 24hrs, after which the plates of the medium were observed for colony growth, minimum bactericidal and fungicidal concentration were the plates with the lowest concentration of the extract without colony growth.

Results and Findings

Phytochemical screening

The result of the phytochemical screening of the extracts using standard procedure to check for the presence of Carbohydrate, alkaloids, saponins, tannins, steroids, flavonoids and cardiac glycoside were reported. The result is as shown in table 1

Table 1: Results of phytochemical screening of the whole plant D. perrottetii

Secondary Metabolites	ethyl acetate extract
Carbohydrate	
a. Fehlings test	–
Saponins k	–
Tannins	+
Anthraquinones	–
Alkaloids	

a. Wagner's test	+
Flavoniods	
a. Ferric chloride test	–
Cardiac glycosides	
a. Salkowski's test	–

Key: + = Present, - = Absent

The phytochemical screening results of the whole plant **D. perrottetii** using an ethyl acetate extract reveal the presence or absence of several secondary metabolites. The analysis shows that tannins and alkaloids are present, with a positive result for tannins and Wagner's test for alkaloids. However, carbohydrates (as tested by Fehling's test), saponins, anthraquinones, flavonoids (as tested by ferric chloride), and cardiac glycosides (as tested by Salkowski's method) are absent, as indicated by negative results in the screening. This suggests that the ethyl acetate extract of **D. perrottetii** contains only a limited range of secondary metabolites, specifically tannins and alkaloids, which could contribute to the plant's biological or medicinal properties.

Table 2: Results of antimicrobial activities of Dyschoristeperrottetii extract and control

Test organisms	**E.A.E**	**Ciprofloxacin**	**Fluconazole**
MRSA	S	R	R
VRE	R	S	R
S.aureus	S	R	R
E.coli	R	S	R
K. pneumonia	R	R	R
P. mirabilis	S	S	R
P. aeruginosa	R	R	R
H. pylori	S	S	R
C.fetus	R	S	R
C.tropicalis	S	R	S
C.krusei	S	R	S

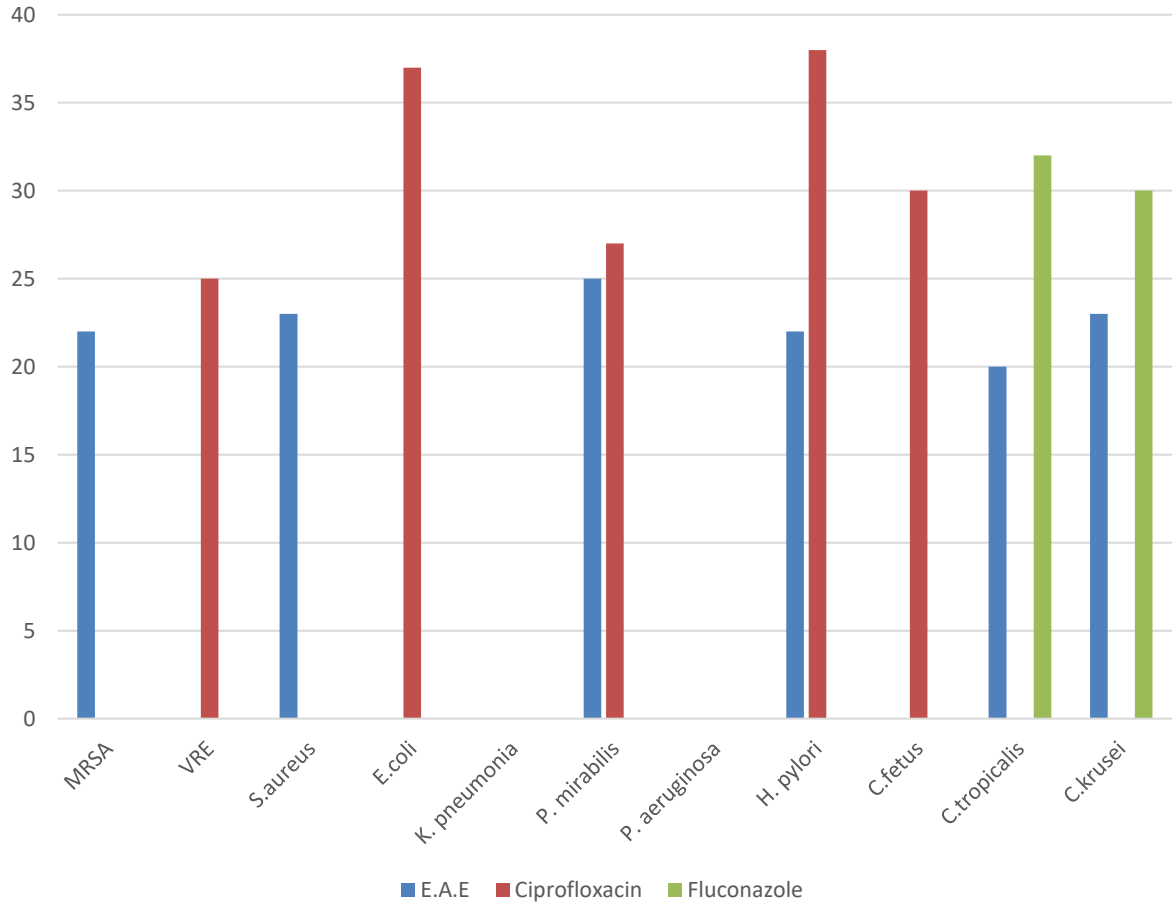
Key:E.A.E = Ethyl acetate extract, MRSA = Methicillin resistant staphylococcus aureus, VRE = Vancomycin resistant enterococci, S= staphylococcus, P=Pseudomonas, E= Escherichia, K= Klebsiella, P= proteus, H= Helicobacter, C= campylobacter, C= candida

*S=susceptible (shows activity), R=resistance (No activity)

The table above shows the sensitivity test of the extract against the test organisms, the result was compared with standard commercial antibioticsciprofloxacin and fluconazole. The antimicrobial activities of Dyschoristeperrottetii ethyl acetate extract (E.A.E) were compared to ciprofloxacin and fluconazole across a range of bacterial and fungal organisms. The results show that E.A.E is effective against MRSA, S. aureus, P. mirabilis, H. pylori, C. tropicalis, and C. krusei, indicating susceptibility to these organisms. However, it shows no activity against VRE, E. coli, K. pneumoniae, P. aeruginosa, and C. fetus, where resistance was observed.

Ciprofloxacin demonstrated effectiveness against VRE, E. coli, P. mirabilis, H. pylori, and C. fetus, but was not effective against MRSA, S. aureus, or the fungal species. Fluconazole, as expected, was primarily active against the fungal organisms C. tropicalis and C. krusei, while showing resistance to the bacterial species. Overall, the ethyl acetate extract exhibited selective antimicrobial activity, particularly against certain Gram-positive bacteria and fungi, making it a potential candidate for further investigation in treating infections caused by these organisms.

Figure1: Results of Zone of inhibition of the ethyl acetate extracts



Key: E.A.E = Ethyl acetate extract, MRSA = Methicillin resistant staphylococcus aureus, VRE = Vancomycin resistant enterococci, S= staphylococcus, P=Pseudomonas, E= Escherichia, K= Klebsiella, P= proteus, H= Helicobacter, C= campylobacter, C= candida.

Table 3: Results of Minimum inhibitory concentration of the various extracts

Test organisms	**10mg/ml**	**5mg/ml**	**2.5mg/ml**	**1.25mg/ml**	**0.63mg/ml**
MRSA	–	–	0X	+	++
VRE					
S.aureus	–	–	0X	+	++
E.coli					
K. pneumonia					

P. mirabilis	–	–	0X	+	++
P. aeruginosa					
H. pylori	–	–	0X	+	++
C.fetus					
C.tropicalis	–	0X	+	++	+++
C.krusei	–	–	0X	+	++

Key: - = No turbidity (no growth), ox = Minimum Inhibitory Concentration, + =Turbidity (light growth), ++ =Moderate turbidity, +++ = Heavy colonies growth, MRSA = Methicillin resistant staphylococcus aureus, VRE = Vancomycin resistant enterococci, P=Pseudomonas, E= Escherichia, K= Klebsiella, P= proteus, Helicobacter, C= campylobacter C= candida

The table shows the Minimum inhibitory concentration of the ethyl acetate extract of the D.perrottettii against the listed microbes.

The table presents the minimum inhibitory concentration (MIC) results for various extracts tested against different microorganisms. The MIC, which indicates the lowest concentration of an extract that inhibits visible growth, is represented by "0X," while varying levels of growth are indicated by turbidity (+, ++, +++).

For MRSA and Staphylococcus aureus, the extracts show no growth at concentrations of 10 mg/ml and 5 mg/ml, with inhibition occurring at 2.5 mg/ml, making this the MIC. At lower concentrations of 1.25 mg/ml and 0.63 mg/ml, light and moderate growths are observed, indicating reduced effectiveness at these concentrations. A similar pattern is seen with Proteus mirabilis and Helicobacter pylori, where the MIC is also 2.5 mg/ml, with no growth at higher concentrations and some growth at lower ones.

In the case of Candida tropicalis, the extract inhibits growth at 5 mg/ml, with the MIC at this concentration. At lower concentrations, growth is evident, ranging from light to heavy. Candida krusei follows a similar trend, but its MIC is 2.5 mg/ml, with light and moderate growth at lower concentrations.

In summary, the extracts show effective inhibition of growth for MRSA, S. aureus, P. mirabilis, and H. pylori at 2.5 mg/ml, while the fungal species C. tropicalis and C. krusei exhibit MICs at 5 mg/ml and 2.5 mg/ml, respectively. At concentrations below the MICs, the extracts are less effective, allowing for varying degrees of microbial growth. These results highlight the antimicrobial potential of the extracts, particularly against Gram-positive bacteria and certain fungal species.

Table 4: Minimum bactericidal/ fungicidal concentration of the extracts

Test organisms	**10mg/ml**	**5mg/ml**	**2.5mg/ml**	**1.25mg/ml**	**0.63mg/ml**
MRSA	0X	+	++	+++	++++
VRE	–	–	–	–	0X
S.aureus	-	0X	+	++	+++
E.coli	–	–	–	0X	+

K. pneumonia	–	–	–	–	–
P. mirabilis	–	0X	+	++	+++
P. aeruginosa	–	–	–	–	0X
H. pylori	–	0X	+	++	+++
C.fetus	–	–	0X	+	++
C.tropicalis	0X	+	++	+++	++++
C.krusei	0X	+	++	+++	++++

Key:– = No turbidity (no growth), ox = Minimum Inhibitory Concentration, + =Turbidity (light growth), ++ =Moderate turbidity, +++ = Heavy colonies growth, ++++ = very heavy colonies growth, MRSA = Methicillin resistant staphylococcus aureus, VRE = Vancomycin resistant enterococci, S= staphylococcus, P=Pseudomonas, E= Escherichia, K= Klebsiella, P= proteus, Helicobacter, C= campylobacter C= candida

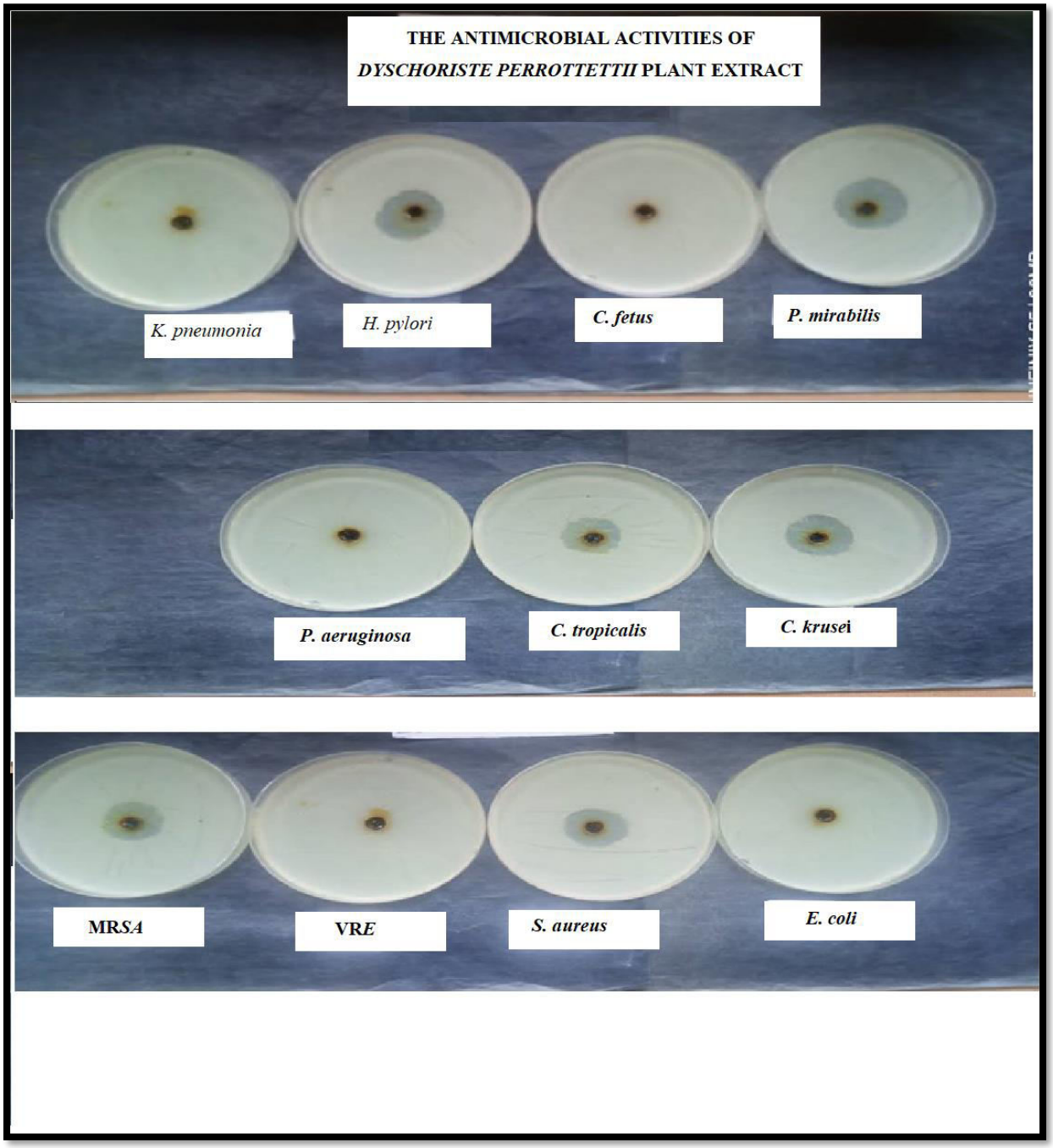
The table shows the Minimum Bactericidal/Fungicidal concentration of the ethyl acetate extract of D. perrotettii against various test organisms. The MBC/MFC represents the lowest concentration of the extract required to kill the microorganism, as indicated by the absence of turbidity (0X). The levels of growth at different concentrations are indicated by turbidity (+, ++, +++, ++++), reflecting the intensity of microbial growth.

For MRSA, the extract completely kills the organism at 10 mg/ml (0X), but light to very heavy growth is observed at lower concentrations, showing reduced effectiveness. In the case of Vancomycin-resistant Enterococci (VRE), the extract shows no growth across most concentrations, with the MBC observed at 0.63 mg/ml. Staphylococcus aureus is killed at 5 mg/ml (0X), but some growth is evident at lower concentrations.

For Escherichia coli, the extract is effective at 1.25 mg/ml (0X), with light growth at lower concentrations, while Klebsiellapneumoniae shows no growth at any concentration, indicating the extract does not kill this organism. Proteus mirabilis shows complete killing at 5 mg/ml, with growth increasing at lower concentrations, similar to the pattern observed in Helicobacter pylori, which is also killed at 5 mg/ml.

Campylobacter fetus has an MBC of 2.5 mg/ml, with increasing growth at lower concentrations. For the fungal organisms Candida tropicalis and Candida krusei, the MFC is 10 mg/ml, with heavy and very heavy growth observed at lower concentrations.

In summary, the extract exhibits bactericidal and fungicidal activity against MRSA, S. aureus, VRE, P. mirabilis, H. pylori, E. coli, and C. fetus, with MBC/MFC values ranging from 10 mg/ml to 0.63 mg/ml depending on the organism. The extract is particularly effective against VRE, showing no growth at most concentrations, but it is less effective against organisms like K. pneumoniae and fungi, which require higher concentrations to achieve killing.



Discussion

The plant material was identified, dried, pulverized and extracted using cold maceration method. The plants have been used traditionally as medicine in the treatment of female sterility and other ailments such as fever, malaria, diarrhea, chicken pox and dysentery. The crude extracts from of D.perrottetti were subjected to phytochemical screening and the results (Table 1) revealed the ethyl acetate extract showed the presence of Tannin and Alkaloid only. Saponins, carbohydrates, anthrax quinones, cardia cglycosides, and flavonoid were found to be absent. In general, the accumulation and concentration of secondary metabolites are responsible for antimicrobial activity and this varies according to plant extracts depending on their polarity (Essawi and Srours, 2000). These

Antimicrobial screening showed that all the extracts of Dyschoristeperrottetti exhibited moderate to good antibacterial activities. The result of Zone of Inhibition (ZI) (table 2) showed inhibition which ranges from 20 -25 mm for ethyl acetate extract against the entire test organism except, Vancomycin Resistant enterococci. C.fetus, P. aeruginosa, K. pneumonia and E. coli.Drugs used as positive control had zones of inhibition of 25-38 mm for ciprofloxacin, and fluconazole 30–32 mm. The results of MIC Table 3 revealed that all organisms had MIC of 2.5 mg/ml except C. tropicalis with MIC of 5 mg/ml. The results of MBC/MFC revealed that all the organisms had MBC/MFC of 5mg/ml except MRSA, C. tropicalis and C. krusei with MBC/MFC of 10 mg/ml respectively.The sensitivity of MRSA, S. aureus, P. mirabilis, C. krusei,H. pylori C. tropicalisto the ethyl acetate extract implies that chemical compounds in the extract could be used to develop drugs to treat related ailments (Ramanathan et al., 2013). Therefore, the extract could serve in one way or the other as source of compounds that may be effective in the management of the ailments associated with the causative agents.

Conclusion

This study demonstrated that Dyschoristeperrottetii has considerable potential for developing new antibacterial therapies to treat diseases associated with the tested microorganisms. The results validate the plant's ethnomedicinal use by traditional medicine practitioners for treating various ailments. Phytochemical and antimicrobial evaluations of D. perrottetii supported its traditional role in combating infections, with tannins and alkaloids in the ethyl acetate extract likely contributing to its observed antimicrobial effects. The extract showed moderate to good efficacy against several pathogens, suggesting it could be a valuable source of alternative antimicrobial agents. These findings underscore the need for further research to isolate and characterize the active compounds, which could lead to effective treatments for infections caused by resistant organisms. This work highlights the importance of medicinal plants as sources of new therapeutic agents in the fight against increasing antimicrobial resistance.

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