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PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL POTENTIAL OF ETHYL ETHER CRUDE EXTRACT OF TEPHROSIA PLATYCARPA

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Abstract: **phytochemical composition and antimicrobial potential of ethyl ether crude extract of *Tephrosia platycarpa* was carried out using standard procedures. The following phytochemicals are present: alkaloids, phenols, steroids, tannins, terpenoids, cardiac glycosides, carbohydrates, proteins, amino acids, coumarins, triterpenoids, and quinone. On the antimicrobial potential, all the organisms tested were sensitive to the extract except *Candida albicans* at the Minimum Inhibitory Concentrations of 12.5, 25, 25, and 50mg/ml for *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtillis*, and *Escherichia coli* respectively. The extract was bactericidal against *Staphilococcus aureus*, *Salmonella typhi* and *Escherichia coli* at the Minimum Bactericidal Concentrations of 25, 50 and 100mg/ml in that order. From the result of the findings, the ethyl ether crude extract of *Tephrosia platycarpa* contains secondary metabolites with antimicrobial potential.**

Keywords: **Antimicrobial, Bactericidal, Inhibition, Extract, Phytochemicals, Plants.**

1. INTRODUCTION

Plant products have been used since the wake of time for the treatment of human ailments and diseases. Nature by design endowed the plant kingdom with the greatest of these potentials (Uzor *et al.*, 2016).

Since the beginning of civilization, survival of the human race was dependent on plants not only as a source of food and oxygen, but also as a source of natural remedies (Muthu *et al.*, 2010). Plants contain numerous biologically active compounds, many of which have antimicrobial activity. This plant-based, traditional medicine system continues to play an essential role in health care with about 80% of the worlds' inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.*, 2007). An antibacterial agent is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi and protozoa. The medicinal content of plant depends on its phytochemicals. Antibiotics are one of the most important weapons in fighting bacterial and fungal infections and have greatly benefitted the health related quality of human life since their introduction (Chinyere *et al.*, 2015). The success story of chemotherapy lies in the continuous search for new drug from natural to counter the challenges posed by resistant strains of microorganisms. Medicinal plants would therefore, be the best source to obtain a variety of drugs. The investigation of certain indigenous plants for their antimicrobial properties may yield useful result. These plants emerged as compounds with potentially significant therapeutic application against human pathogens (El Astal *et al.*, 2005).

Antimicrobial compounds derived from plants might inhibit bacteria through different mechanisms and provide clinical values for the treatment of infection caused by resistant microbes. Synthetic antibiotics accumulate in the body causing liver damage and other tissue problems. Such problems are not seen, when natural antibiotics extracted from plants are used. These extracts are safe and potentially effective (Aparadh *et al.*, 2012).

Several plants species have been tested for antimicrobial properties but vast majority have not yet been adequately evaluated (Azoro, 2002). And for those already evaluated, certain solvents have not been used to test the efficacy of their extraction potential. There is no scientific data on the ethyl ether extract of *tephrosia platycarpa*. And this is what this novel study has accomplished.

2. MATERIALS AND METHODS

Collection of Plant Material

The whole plant was collected from Olangbecho village of Aiyeje Eko town, Benue state and was authenticated at the department of Biological Sciences Ahmadu Bello University Zaria.

Phytochemical Extraction

Fresh plant was washed in clean water to remove dust. It was air dried within two weeks. The dried plant was pulverized and about 80g of the powdered plant was weighed and dissolved in ethyl ether. The mixture was left for 24 hours with intermitted shaking. The mixture was filtered using Whatman no.1 filter paper. The filtrate was evaporated at 40^0C to dryness. The yield was calculated and the dried extract stored in airtight container at 4^0C until needed.

Phytochemical Screening

The screening was done by standard procedures (Raaman, 2006; Rajesh *et al.*, 2013)

Test Organisms

The test organisms were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Candida albilicans.* They were clinical isolates of bacteria and a fungus obtained from department of microbiology, Ahmadu Bello University Zaria and reconfirmed by gram staining and sub culture in appropriate selective media.

Antimicrobial Assay

Sensitivity Test

The sensitivity test of the Pure Extract was carried out by Using Agar Well Diffusion Method. The standardized broth inoculums of the bacterial and fungal isolates were streaked on sterilized Mueller-Hinton and potato dextrose agar plates respectively with the aid of a sterile swab sticks. Four wells were punched on each inoculated agar plate with a sterile cork borer. The wells were properly labeled according to different concentrations of the extract prepared which were 100, 50, 25 and 12.5mg/ml respectively. Each well was filled up with approximately 0.2ml of the extract. The inoculated plates with the extract were allowed to stay on the bench for about one hour. This is to enable the extract to diffuse on the agar. The plates were incubated aerobically at 37^oC for 24hours, while the plates of potato dextrose agar were incubated at room temperature for about 5 days.

At the end of incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameter of the zones was measured using a transparent ruler calibrated in millimeter and the result was recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using tube dilution method with Mueller-Hinton broth used as diluents. The lowest concentration of the extract showing inhibition for each organism when the extract was tested during sensitivity test was serially diluted in the test tubes containing Mueller-Hinton broth. The organisms were inoculated into each tube containing the broth and the extract. The inoculated tubes were then incubated at 37^0c for 24 hours. At the end of the incubation period, the tubes were examined /observed for the presence or absence of growth using

turbidity as a criterion, the lowest concentration in the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration (MIC). The result was also recorded.

Determination of Minimum Bactericidal Concentration (MBC)

The result from the minimum inhibitory concentration (MIC) was used to determine the minimum bactericidal concentration (MBC) of the extracts. A sterilized wire loop was dropped into the test tubes that did not show turbidity (clear) in the MIC test and a loop-full was taken and streaked on a sterile nutrient agar plates. The plates were incubated at 37^0C for 24 hours. At the end of incubation period, the plates were examined/observed for the presence or absence of growth. This is to determine whether the antimicrobial effects of the extracts are bacteriostatic or bactericidal.

3. RESULTS

Percentage yield

The percentage yield is 2.5%

Phytochemicals

The phytochemical screening of the ethyl ether crude extract from the whole plant of *Tephrosia platycarpa* showed the occurrence of various secondary metabolites such as: Alkaloids, Phenols, Steroids, Tannins, Terpenoids, Cardiac glycosides, Carbohydrates, Proteins, Amino acids, Coumarins, Triterpenoids, and Quinone, (table 1).

Sensitivity

The crude extract was screened for antibacterial and antifungal activities by employing well diffusion method. The activity was recorded as diameter zone of inhibition using the crude extract concentrations ranging from 12.5 to 100mg/ml. The extract exhibited activity against *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, and *Escherichia coli* (table 2).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Next, we carried out MIC. We observed the minimum inhibitory concentration to be 12.5mg/ml on *Staphylococcus aureus*, 25mg/ml on *Salmonella typhi* and *Bacillus subtilis* each, 50mg/ml on *Escherichia coli*. The MBC were 25, 50 and 100mg/ml on *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* respectively.

Discussion

Phytochemical screening revealed that the ethyl ether crude extract contains most of the phytochemicals assayed. Twelve secondary metabolites were present out of the eighteen tested.

Alkaloids have wide range of pharmacological activities, including antimalarial, antiasthma, anticancer, analgesic antibacterial and antihyperglycemic activities (Qiu *et al.*, 2014). Phenol is used (in low concentration) as disinfectant in household cleaners and in mouthwash. Also used as surgical antiseptic, and as such functions as antimicrobial produced by some plants to protect them from pathogens (Chinyere *et al.*, 2015). Tannins are astringent in taste and help in healing of wounds and inflamed mucous membrane. They are potential metal ion chelator, proton precipitating agents and biological antioxidant (Okonkwo, 2009). Terpenoids are antifungal and antibacterial which is attributed to their membrane disruption action and inhibitory action on bacterial cell or fungus (Cichewicz and Thorpe, 1996). Cardiac glycosides are used in medicine for the treatments of congestive heart failure and cardiac arrhythmias. This they do by increasing the output force of the heart and decreasing its rate of contractions by acting on the cellular sodium-potassium ATPase pump (Patel Seema, 2016). Coumarin has clinical value as an edema modifier. It is also known, (alongside other compounds), to stimulate macrophages to degrade extracellular albumin, allowing faster resorption of edematous fluids (Casley-Smith *et al.*, 1993). Coumarin is also used as a gain medium in some dye lasers (Duarte, 2003), and as a sensitizer in older photovoltaic technologies (Loutfy, 1978). Quinone on the other hand is antimicrobial, antiparasitic and anti-cardiovascular disease (Liu, 2011).

On the side of the microorganisms, the extract was effective against all the organisms tested except the fungus *Candida albicans*. The effectiveness is greatest on the gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* in

contrast to *Escherichia coli* and *Salmonella typhi* which are gram negative. The wall of gram negative bacteria is more complex than gram positive bacteria (Kumar *et al.*, 2006). This therefore, explains why the gram negative bacteria were more resistant to antimicrobial compounds with their effective diffusion barrier. It is therefore, not surprising to note that *Escherichia coli* in our study was the most resistant microorganism among all bacteria strains tested. It is well known that *Escherichia coli* developed multidrug resistance toward different kinds of antimicrobial agents (Sader *et al.*, 2002). On the other hand, *Staphilococcus aureus* was the most susceptible bacteria of all the bacterial strains tested. Several reports suggest that *Staphylococcus aureus* is the most common pathogen to cause skin infections (Jones *et al.*,2006). Thus, the plant extract could be used in the treatment of skin infections, pneumonia, meningitis, sepsis and food poisoning, which are disease caused by *Staphylococcus aureus* (Tong *et al.*, 2015). The extract is also a potential source of drug for the treatment of typoid fever. Probably at higher concentrations, the extract could be fungicidal given the presence of terpenoids. This finding is novel in the sense that the secondary metabolites of the plant *Tephrosia platycarpa*, have never been extracted using ethyl ether before.

4. CONCLUSION

The plant, T*erphrosia platycarpa* has medicinal and pharmacological potentials, and could be used in the future to extract the active metabolites for the purpose of formulating drugs.

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Table 1: Phytochemical composition

Phytochemical	**Result**
Alkaloids	+
Anthraquinone	-
Flavonoids	-
Phenols	+
Saponin	-
Steroids	+
Tannins	+
Terpenoids	+
Cardiac glycosides	+
Reducing sugar	-
Carbohydrates	+
Proteins	+
Amino acids	+
Phlobatannins	-
Coumarin	+
Triterpenoid	+
Lignin	-
Quinone	+

Keys
+ = Present
- = absent

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Table 2: Diameter Zone Of Inhibition (mm) Of The Extract At Varying Concentrations (mg/ml)

S/N	Test of Organism	100	50	25	12.5	Control (Ciprofluxacin) (10µg)
1	*Staphylococcus aureus*	26	22	19	17	35
2	*Salmonella typhi*	19	15	13	11	38
3	*Bacillus subtilis*	18	16	14	12	32
4	*Escherichia coli*	17	-	-	-	37
5	*Candida albicans*	-	-	-	-	35

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC).

Organism	MIC	MBC
Staphylococcus aureus	12.5	25
Salmonella typhi	25	50
Bacillus subtilis	25	Bacteriostatic
Escherichia coli	50	100
Candida albicans	ND	ND

Key
ND = Not Determined