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PHYTOCHEMICAL EXTRACTION AND MEDICINAL POTENTIAL OF TEPHROSIA PLATYCARPA

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Abstract: **the phytochemical extraction and medicinal potential of both the aqueous and chloroform crude extracts of *Tephrosia platycarpa* were carried out using standard procedures. The following phytochemicals are present: alkaloids, glycosides, carbohydrates, proteins, phytosterols, tannins, phenols, flavonoids, coumarines, saponins, quinone, cardiac glycosides, terpenoids, phlobatannins, steroids, cholesterol, triterpenoids, and sugar. On the medicinal potential, the aqueous extract was bactericidal against *Staphylococcus aureus* and *Salmonella typhi* at the Minimum Bactericidal Concentrations (MBC) of 50mg/ml each. While the chloroform extract was bactericidal against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* at MBCs of 25mg/ml, 50mg/ml and 50mg/ml respectively. From the result of the findings, the plant, *Tephrosia platycarpa* has medicinal potential.**

Keywords: **Phytochemicals, Plant, Extracts, Medicinal, Inhibition, Bactericidal.**

1. INTRODUCTION

The world is fertile with natural and medicinal plants. Medicinal plants are now more focused than ever because they have the capacity of producing many benefits to society, indeed to mankind, especially in the line of medicine and pharmacological. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological effects on human body (Akinmoladun *et al.*, 2007). Plants are important source of drugs, especially in traditional medicine (Bako *et al.*, 2005). The active principle of many drugs found in plants is phytochemicals (Oluwakayode *et al.*, 2016).

Phytochemicals are nutritive or non – nutritive metabolites found in plants that have protective or disease preventive properties. They occur in medicinal plants, vegetables and fruits and work with nutrients and fibers to act against diseases or more specifically to protect against diseases (Agte *et al.*, 2000).The use of plants as source of remedies for the treatment of diseases dates back to prehistory and people of all continents have this tradition (Ibrahim *et al.*, 2014). Herbal medicine is still the mainstay of about 75 – 80% of the world population. Over 60% of the world human population, 80% in developing countries depends directly on plants for their medicinal purposes (Dhillion *et al.*, 2002). Over 25% of the prescribed medicines in industrialized countries are derived directly or indirectly from plants (Hemal *et al.*, 2008). Most of the diseases are caused by microorganisms. The substances that interfere with the growth and metabolism of microbes are called antimicrobial agents. Some phytochemicals are antimicrobial agents. Antimicrobial agents that inhibit the growth of microorganisms are called microbiostatic. Those that kill are microbiocidal. Microbial growth is inhibited through interference of active metabolite with cell membranes, enzymes activity or genetic mechanisms of microorganisms (Branen and Davidson, 1983).

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Some antimicrobial agents are chemotherapeutic with a chemical used for the treatment of infectious diseases or diseases caused by the proliferation of malignant cells. These substances are prepared in the chemical laboratory or obtained from microorganisms, and some plants and animals in general. Chemotherapeutic agents must have selective toxicity for host cells and high toxicity for the parasite (EPC, 2004).

Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulteration and side effects (Teklit *et al.,* 2016). The ethnopharmacologists, botanists, biochemists, microbiologists and natural – product chemists world over today are constantly still in search of medicinal efficacy of plants and their phytochemicals, since the reported data so far available on plants are comparatively meager before the vast number of plant population. The drugs which are already in use to treat infectious diseases is of concern because, drug safety remains an enormous global issue. It was estimated that 2.22 million hospitalized patients had serious Adverse Drug Reactions (ADR) and 106000 died in a single year in USA (Bishnu *et al.,* 2011). This herbal and natural products have been used in folk medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, this coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair *et al.,* 2005). Through a number of medical and clinical researches, drugs extracted and synthesized through derivatization from medicinal plants were found to have reduced toxicity and side effect (Assareh *et al.,* 2010). Aside from these, there has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious disease and to lesser extent through mutation of genetic material or by acquiring pieces of DNA that code for the resistance properties from other bacteria (Roberts, 2017).

Microbial resistance to antibiotic is one of the most serious public health problems, especially in developing countries where infectious diseases still represent a major cause of human mortality (WHO, 2014). Thus, knowledge about the therapeutic potential of plants is of great scientific and medical interest, as an effective alternative to the battle against microorganisms (dos Santos *et al.,* 2015)

According to research, there are 422,127 plant species growing on earth; among them about 35,000 to 70,000 plants are used as medicinal plants (Yolwin and Merlyn, 2012). The rest remain unexplored for their potentials, depriving the world of unique and rare nutritional or medicinal effective metabolites that might be synthesized by these plant species.

Terphrosia platycarpa falls under this category. Scientific facts have been published for other species under the genus Terphrosia, examples: *Terphrosia vogelli* (Teryila, 2015), *Tephrosia apollinae* (Cheruth *et al.,* 2012), *Terphrosia purpurea* (Suriyalhana *et al.,* 2014) etc. But there is no scientific data on the nutritional or medicinal potentials of *Terphrosia 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 in two places and dissolved in distilled water and chloroform respectively. The mixtures were left for 24 hours with intermitted shaking. The mixtures were filtered separately using Whatman no.1 filter paper. The filtrates were evaporated at 40^0C to dryness. The yields were calculated and the dried extracts stored in airtight container at 4^0C until needed.

Phytochemical Screening

The screening was done by standard procedures (Raaman, 2016; Rajesh *et al.,* 2013)

Test Organisms

The test organisms were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Candida albilican.* 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 Extracts 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^{o}C 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^{0}c 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^{0}C 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 AND DISCUSSION

Percentage Yields

The percentage yields for aqueous and chloroform extracts are 4.73% and 3.50% respectively.

Phytochemicals

The phytochemical screening of both the aqueous and chloroform extracts from the whole plant of *Tephrosia platycarpa* showed the occurrence of various secondary metabolites such as alkaloids, glycosides, carbohydrates, proteins, phytosterols, tannins, phenols, flavonoids, coumarines, saponins, quinone, cardiac glycosides, terpenoids, phlobatannins, steroids, cholesterol, triterpenoids, and sugar (Table 1).

Sensitivity

The successive *Tephrosia platycarpa* crude extracts viz: aqueous and chloroform were screened for antibacterial and antifungal activities by employing well diffusion method. The activity was recorded as the diameter zone of inhibition using the crude extract concentration ranging from 12.5 to 100mg/ml. Aqueous extract exhibited activity against *Staphylococcus aureus* and *Salmonella typhi* (Table 2). The chloroform extract exhibited significant antibacterial activity against *Staphilococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Bacillus subtilis* (Table 3). These results suggest the antibacterial activity was found to be stronger with non-polar fractions compared to polar fractions.

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

Next, we carried out MIC for each of the extracts. We observed minimum inhibitory concentration for aqueous extract to be 25mg/ml on *Staphilococcus aureus* and *Salmonella typhi* each (table 4). For the Chloroform extract, the lowest MIC was 12.5mg/ml on *Staphylococcus aureus* and 25mg/ml each on *Salmonella typhi*, *Bacillus subtilis* and *Escherichia coli* respectively (Table 5). The MBC of the aqueous extract was 50mg/ml on *Staphilococcus aureus* and *Salmonella typhi* each (Table 4). While the MBC for Chloroform extract was 25mg/ml on *Staphylococcus aureus*, 50mg/ml on *Salmonella typhi* and *Escherichia coli* each (Table 5).

Discussion

It could be deduced that the chloroform extract had a better extraction potential than that of distilled water.

Phytochemical screening revealed that both aqueous and chloroform whole plant extracts of *Tephrosia platycarpa* contained almost all the phytochemicals assayed. The phytochemicals were present in varied quantities as judged by the degree of colour changes during the test. Tannins are astringent in taste and help in healing of wounds and inflamed mucous membrane (Njoku and Akumefula, 2007). Tannins are potential metal ion chelator, proton precipitating agents and biological antioxidant (Okonkwo, 2009). Flavonoids are mostly known for their antioxidant activity and act as transformers which modify the body's reactions to carcinogens, viruses and allergens. They possess anti-cancerous, anti-inflammatory, anti-microbial and anti-allergic activity (Balch and Balch, 2000) and may therefore be useful in therapeutic roles. 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). Many alkaloids for example are known to have effect on the central nervous system and act as antipyretic such as morphine, a painkiller. Glycoside is a major bioactive component that offers anti-secretory and antiulcer effects (Bandyopadhay *et al.*, 2002). Similarly, saponins which are a special class of glycosides possess pharmacological and medicinal activities such as control of blood cholesterol levels, enhancement of bone health, cancer treatment, and building up of immune system (Xu *et al.*, 1996), as well as industrial uses, as in the manufacturing of fire extinguisher foam, toothpaste, shampoos, liquid soaps and cosmetics and to increase the foaming qualities of beer and soft drinks (Francis *et al.*, 2002). Plants containing saponins are used to heal wounds because saponins have the ability to precipitate and coagulate Red Blood Cells (RBCs) (Sood *et al.*, 2012). Sterols have been used in medicine to treat variety of conditions ranging from endocrine hormonal alteration to coronary insufficiency (Clifford *et al.*, 1973). Phenols are known to inhibit the mutagenity of cell DNA and neutralize free radicals (Heinnonen *et al.*, 1998). They also function as antimicrobial compounds produced by some plants to protect them from pathogens (Chinyere *et al.*, 2015)

On the side of the microorganisms, the extracts are effective against the microbes. The aqueous extract is bactericidal against *Staphylococcus aureus*, and *Salmonella typhi* while the chloroform extract is bactericidal against *Staphylococcus aureus*, *Escherichia coli and Salmonella typhi.* That shows that, *Terphrosia platycarpa* is a potential source of drugs for the treatment of the diseases caused by these bacteria such as Typhoid fever, Urinary Tract Infection (UTI). The plant is a potential source of drugs for the treatment of pneumonia, meningitis, sepsis and food poisoning which are diseases caused by *Staphylococcus aureus* (*Tong et al.*, 2015).

Probably at higher concentrations, the extract could be fungicidal given the presence of terpenoids in the aqueous extract. This finding is novel in the sense that the potentials inherent in this plant species, *Tephrosia platycarpa,* have never been explored or reported 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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APPENDIX – A

List of tables:

Table 1: Result of Phytochemical Screening of Crude Extracts of *Tephrosia platycarpa*

Phytochemical	Aqueous extract	Chloroform extract
Alkaloids	+	+
Glycosides	-	+
Carbohydrates	++	+
Proteins	-	+
Amino acids	-	-
Phytosterol	++	ND
Tannins	++	+
Phenols	++	++
Flavonoids	++	-
Coumarines	++	+

Saponins	+++	-
Quinone	+	+
Cardiac glycosides	+	+
Terpenoids	++	-
Phlobatannins	+	-
Steroids/phytosteroids	+++	ND
Cholesterol	+	ND
Anthocyanins	-	ND
Anthraquinone	-	-
Triterpenoids	ND	+
Sugar	ND	+

KEYS
+++ = Highly present
++ = Moderately present
+ = Mildly present
ND = Not determined

Table 2: Diameter Zone of Inhibition (mm) at Varying Concentrations (mg/ml) of the Aqueous Extract of *Tephrosia platycarpa.*

Test organisms	100	50	25	12.5	(control) ciprofloxacin 10µg/ml
Staphylococcus aureus	20	17	15	14	35
Escherichia coli	-	-	-	-	37
Bacillus subtilis	-	-	-	-	32
Salmonella typhi	18	16	14	12	38
Candida albicans	-	-	-	-	35

Table 3: Diameter Zone of Inhibition (mm) at Varying Concentrations (mg/ml) of the Chloroform Extract of *Tephrosia Platicarpa*

Test organisms	100	50	25	12.5	(control) ciprofloxacin 10µg/ml
Staphylococcus aureus	21	18	16	14	35
Salmonella typhi	19	16	-	-	38
Bacillus subtilis	22	17	14	12	32
Escherichia Coli	19	15	-	-	37
Candida albicans	-	-	-	-	35

Table 4: Result of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Aqueous Extract of Tephrosia platycarpa.

Test organisms	MIC	MBC
Staphylococcus aureus	25	50
Salmonella typhi	25	50

Table 5: Result of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Chloroform Extract of Tephrosia Platycarpa.

Test organisms	MIC	MBC
Staphylococcus aureus	12.5	25
Salmonella typhi	25	50
Bacillus subtilis	25	-
Escherichia coli	25	50