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## Phytochemical and Antibacterial Analysis of Ethanol Extract of *Triclisia macrophylla* Leaf

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### Abstract

*Triclisia macrophylla* is a climbing plant used in Nsukka in the treatment of gastrointestinal disorder. This research was designed to study the phytochemical constituents and antibacterial activity of *T. macrophylla* ethanol leaf extract. Cold maceration method was used to get the ethanol extract. Standard qualitative and quantitative phytochemical analysis methods were used for the phytochemical estimation. The antibacterial activity was determined using agar well diffusion method. The result of the phytochemical screening showed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, steroids, terpenoids, reducing sugar and saponins. The percentage yield of the estimated phytoconstituents gives 6% alkaloids, 1.2% flavonoids, 0.48% cardiac glycosides and 2% saponins respectively. The ethanol extract showed zone of inhibition ranging from 2 mm to 15.7 mm against *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Enterococcus faecalis* while *staphylococcus* and *Pseudomonas aeruginosa* where not inhibited at any concentration. The extract demonstrated highest activity against *Klebsiella pneumonia* and *Enterococcus faecalis*. Conversely, the extract showed promising antibacterial activity and might be useful alternative in controlling disease caused by pathogenic organisms. The finding validated the folkloric claims of the plant use in treating infectious diseases.

**Keywords:** phytochemicals, *Triclisia macrophylla*, ethanol, antibacterial, infectious diseases

### Introduction

Infectious diseases have been recognized as one of the major intimidations to human health throughout the world. Most of them are caused by microorganisms [1]. It is reported that bacteria are attributed to approximately up to 30% of all diseases, leading to millions of deaths every year [2]. Some of the pathogenic organisms like *E. coli* causes infection of the urinary tract, the organism normally spread from the gut to the urinary tract. *Escherichia coli* is also the commonest cause of cystitis and in a minority of patients the infection may spread up to the urinary tract to the kidneys, causing pyelonephritis [3]. *Staphylococcus aureus* infections can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person [4]. *Pseudomonas aeruginosa* is the most commonly isolated nosocomial pathogens accounting for a significant hospital related infection [5]. Efforts have been made in the use of antibiotics to combat diseases successfully but the side effects and resistance of antibiotics by organisms are discouraging its continuous usage. However the indiscriminate uses of synthetic antibiotics have gradually resulted in drug resistant bacteria resulting in global therapeutic challenge in the public health system, called antibiotic resistance [6]. To solve the challenges of bacterial infection, effective and safe antibacterial agents must be sourced for. This has led to the search of new antibacterial agents from plant extracts with the goal to discover new chemical structures which overcome the ravaging effect of infectious diseases especially in the developing countries. Medicinal Plants have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinines and coumarins [7]. These biomolecules are the source of plant-derived antimicrobial substances [8]. Some natural products are highly efficient in the treatment of bacterial infections [9].

*Triclisia macrophylla* oliv is a plant belonging to the Menispermaceae family. It is climbing shrub producing stems 10-20 metres long. *T. macrophylla* is indigenous to West and central Africa.

It is found in the lowland and submontane rainforests at elevations up to 1500 metres<sup>[10]</sup>. The leaves are hairy and alternate while the fruits which contain only one seed are green when not ripe and yellow when ripe. *T. macrophylla* have been used by different region of Africa in the treatment of diseases. In cote d'Ivoire, the root pulp is used to treat joint pains and epileptic attack. In democratic republic of congo, a decoction of the twig bark is used to treat malaria.<sup>[11]</sup> In Nigeria the plant is used in the treatment of malaria among the people of Awka ibom state<sup>[11]</sup> and is used locally in treatment of diarrhea and other gastrointestinal disorders in Enugu State. Previous researcher has documented the antiplasmodium activity of ethanol extract, n-hexane and butanol fraction of *T. macrophylla* leaf<sup>[11]</sup>.

## Materials and Methods

### Sample collection and Authentication

The leaves of *Triclisia macrophylla* were collected from the healthy plant in Nsukka, Enugu State. The plant was identified and authenticated by Mr. Alfred Ozioko, a taxonomist in international centre for Ethno medicine and Drug development #110 Aku road Nsukka, Enugu State.

### Preparation and extraction

The leaves were washed using tap water to remove dirt, air-dried under shade for 14 days and pulverized using an electric blender. A 300g of the pulverized plant material was soaked in 2L of ethanol for 72 hours with intermittent agitation. The mixture was filtered first using muslin cloth and then using whatman number 1 filter paper. The resulting filtrate was concentrated using a rotary evaporator at reduced pressure to obtain the ethanol extract. The extract was stored in a refrigerator for further analysis.

### Phytochemical analysis of the extract

Qualitative analysis:

The phytochemical screening of the ethanol extract was carried out using standard methods of Soforowa<sup>[12]</sup> and Trease and Evans<sup>[13]</sup>.

### Test for Alkaloids

1g of extract was boiled with 5 ml of 2% HCL on a steam bath for 10 minutes and the mixture was filtered and cooled. 2 ml of the filtrate was treated with 2 drops of Meyer's reagent, a creamy white precipitate was observed. To confirm this result, 2 ml portion of the filtrate was treated with Dragendoff's reagent which gave a red precipitate to indicate the presence of alkaloids.

### Test for Flavonoids

1g of extract was introduced into a test-tube containing 10 ml ethyl acetate solution and boiling for 1 min, the mixture was filtered. 1 ml of dilute ammonia solution was added to 3 ml filtrate. The formation of a yellow coloration indicated

the presence of flavonoids.

### Test for Saponins

To 1 ml of the filtrate was diluted with 4ml of distilled water in a test tube. The mixture was shaken and formation of stable frothing on standing was observed which indicated positive for saponins.

### Test for terpenoids

To 3 ml of the extract was mixed in 2 ml of chloroform and 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added into the test-tube in slant position to form a layer. A reddish brown coloration of the interface was formed to show positive results for terpenoids.

### Test for Cardiac Glycosides

To 3ml of aqueous extract was treated with 2 ml of glacial acetic acid and one drop of FeCl<sub>3</sub> solution followed by 1 ml of concentrated sulphuric acid. A brown ring of the interface indicated the presence of cardiac glycosides.

### Test for steroids

To 2 ml of methanol extract was treated with 2 ml of acetic acid and 19 ml of acetic anhydride at 0°C followed by 2 ml of concentrated sulphuric acid. A blue or green coloration indicated the presence of steroids

### Test for Tannins

To 3ml of methanol extract was added 10% FeCl<sub>3</sub>. Blue coloration indicated the presence of tannins.

### Test for Reducing Sugar

To 1 ml of a mixture of Fehling's solution A and B were added to 2g of the powdered plant materials in the test tube. The mixtures were heated in a boiling water-bath for five minutes. The colour changed from deep blue to brick red, indicating the presence of reducing sugar.

### Quantitative determination of phytochemicals

The quantitative determination was done using standard methods.

Alkaloids determination<sup>[14]</sup>.

200 ml of 20% acetic acid in ethanol was added to a 250 ml beaker containing 5 g of the sample which was covered and allowed to stand for 4 hours at 25 °C. This was filtered and the filtrate was concentrated using a water bath to one quarter of the original volume. Concentrated NH<sub>4</sub>OH was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate which was collected and washed with dilute NH<sub>4</sub>OH was filtered with pre-weighed filter paper. The residue on the filter paper is the alkaloids, which was dried in the oven at 80 °C. the alkaloids content was calculated and expressed as a percentage of the weight of the sample analyzed.

$$\% \text{ weight of alkaloid} = \frac{\text{weight of filter paper with residue} - \text{Weight of filter paper}}{\text{Weight of sample analyzed}} \times 100$$

Flavonoids determination<sup>[15]</sup>.

10 g of the plant sample was extracted repeatedly with 100 ml of 80 °C aqueous methanol at room temperature. The

whole solution was filtered. The filtrate was transferred into a crucible, evaporated to dryness over water bath and weighed to a constant weight<sup>[12]</sup>.

$$\% \text{ flavonoids} = \frac{(\text{Weight of crucible} + \text{residue}) - (\text{Weight of crucible})}{\text{Weight of sample analyzed}} \times 100$$

#### Saponin determination [16].

The method of Obadoni and Ochuko (2001) [16] was used. Out of the grinded samples, 20g of each was put into a conical flask and 100ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55 °C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90 °C. The concentrate was transferred into a 250ml separatory funnel and 20ml of

diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of N-butanol was added. The combined N-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was then calculated as percentage.

$$\% \text{ saponin} = \frac{(\text{Weight of filter paper} + \text{residue}) - (\text{Weight of filter paper})}{\text{Weight of sample analyzed}} \times 100$$

#### Cardiac glycosides determination [17].

1 ml of 2% solution of 3,5 Dinitro salicylic acid (DNS) in methanol and 1 ml of 5% aqueous NaOH was added to 1 ml of the extract. It was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was

filtered. The weight of the filter paper was weighed before filtration. The filter paper with absorbed residue was dried in an oven at 50 °C till dryness and weighed of the filter paper with residue was noted. Wang and Filled method was used for this determination.

$$\% \text{ cardiac glycoside} = \frac{(\text{Weight of filter paper} + \text{residue}) - (\text{Weight of filter paper})}{\text{Weight of sample analyzed}} \times 100$$

#### Sources of organism

The Organisms used were clinical isolates of gram positive and gram negative bacterial *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*. The organisms were obtained from the Department of Pharmaceutical Microbiology and biotechnology Laboratory, Enugu State University of Science and Technology, Enugu.

#### Preparation of media and reagents

Nutrient broth (13 g/L) and Nutrient agar (28 g/L) were prepared following manufacturer's specifications by dispersing the required quantity of the powder in distilled water. It was homogenized and then 20 ml each of the solutions were dispensed into McCartney bottles and sterilized with autoclave at 121°C for 15 min. Dimethyl sulfoxide (DMSO) was used in solubilizing the extract to get graded concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12 mg/ml, 0.625 mg/ml.

#### Antibacterial determination using agar diffusion method

An overnight broth culture was used to obtain 0.5 Macfarland standard of bacteria used to seed sterile molten nutrient agar medium maintained at 45°C. Five holes of 8mm respectively were bored on each of the segmented and labeled seeded agar plates with a sterile cork borer after solidification. With the aid of sterile syringe, the wells were filled with 0.1 ml of different dilutions of the extract and fractions. Thereafter, the preparations were allowed to stand for 30min to ensure pre-diffusion and then incubated at 37°C for 24 h for the bacteria and 25°C for 48h for fungi.

The inhibition zone diameter of each concentration of the test organisms were determined and recorded in triplicate using transparent ruler in millimeter (mm). The mean of these results were taken for the purpose of accuracy of values.

#### Results

##### Qualitative phytochemical analysis

The result of the qualitative phytochemical screening of the ethanol extract of *T. macro* indicated the present of alkaloids, flavonoids, cardiac glycosides, terpenoids, saponins, tannins, steroids and reducing sugar shown in Table 1.

**Table 1:** Results of Phytochemical analysis of ethanolic leaf extract of *Triclisia macrophylla*

Phytochemical constituents	Results
Alkaloids	+
Flavonoids	+
Saponins	+
Terpenoids	+
Cardiac glycosides	+
Reducing sugar	+
Tannins	+
Steroids	+

Key: + = Present

##### Quantitative phytochemical analysis

The result of the quantitative determination of some phytochemicals present in the ethanol extract of *T. macrophylla* has such as alkaloids, flavonoids, saponins and cardiac glycosides are presented in table 2

**Table 2:** Results of quantitative analysis on the ethanol extract of *triclisia macrophylla*

Phytochemicals	Alkaloids	Flavonoids	Saponins	Cardiac glycosides
% yield	14	20.8	17.4	23

### Antibacterial activity

The antibacterial activity of ethanol extracts of *T. macrophylla* was determined by the disc diffusion method against different bacteria. These bacterial strains are Gram-

positive and Gram-negative species frequently encountered in infectious diseases. The results of the diameters of inhibition zones are shown in the Table 3

**Table 3:** the antibacterial activity (inhibition zones, mm) of the ethanol leaf extract of *Triclisia macrophylla* against tested organisms

Extract conc. (mg/ml)	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>	<i>K. pneumonia</i>
50	2	4	-	-	10	15.7
25	-	2	-	-	7.5	14
12.5	-	-	-	-	5	9
6.25	-	-	-	-	2	7
3.125	-	-	-	-	-	3

### Discussion

This study investigated the phytochemical constituents of *T. macrophylla* leaves and the effect the ethanol extract on the pathogenic organism causing severe infectious diseases to humans. The analysis of ethanol extract of *T. macrophylla* revealed the present of bioactive photochemical constituents such as steroids, terpenoids, tannin, flavonoids, saponin, alkaloids, cardiac glycosides as shown in table 1. This validated the previous researcher that first documented the present of the same phytochemical constituents [11]. Some of the estimated bioactive constituents were present in reasonable amount as shown in table 2. These bioactive compounds have been established to show antibacterial activities through interrupting microbial membranes, weakening cellular mechanisms, controlling biofilm formation, inhibiting bacterial capsule production, and reducing microbial toxin production [18-19]. The antibacterial activities were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of microbial growth and it shows a positive and absence of zone as negative [20]. The antibacterial activity of *Triclisia macrophylla* showed that the ethanol extract inhibited some pathogenic microorganisms tested such as *Kl ebsiella pneumonia*, *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis*. *P. aeruginosa* and *s. aureus* were not inhibited at any concentrations of the extract. However, *k. pneumonia* and *E. faecalis* were strongly inhibited as shown in table 3. This indicated that the ethanol extract might contain bioactive constituent with potential in treating diseases caused by *k. pneumonia* and *E. faecalis*.

### Conclusion

It can be concluded from the results that ethanol extract of *Triclisia Macrophylla* in the present study possess significant bacterial activity against some tested organisms implicated in infectious diseases. This explains the reason for its folkloric uses as a remedy diarrhea and gastrointestinal infections. This medicinal plant has potential for alternative medicine or development of antibacterial agents for preventing and treating infectious diseases.

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