



Research Article

Phytochemical Quality and Antibacterial Activity of *Triclisia patens* Oliv. Root Extract

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ABSTRACT

The resistance to drugs by microorganisms has been on the increase despite the production of new antibiotics. Thus, it is necessary to further explore natural products to solve the problem of lingering treatment failures. This study aimed to assess the phytochemical quality and antibacterial efficacy of *Triclisia patens* Oliv. root extract. Cold maceration method was used to obtain the ethyl acetate and aqueous extracts. Standard methods were applied to assay for the phytochemical constituents who include; alkaloids, saponins, flavonoids, tannins, steroids, terpenoids and phenols using the ethyl acetate extract. The antibacterial activity and the minimum inhibition concentration were determined using agar well diffusion and agar dilution method respectively. The test organisms used were *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterobacter* sp. Following phytochemical screening, phenols, terpenoids, alkaloids and flavonoids were discovered to be present. The inhibition zone diameters of the ethyl acetate extracts were 13.00 mm, 13.70 mm, and 20.00 mm against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* respectively, while no inhibition zones were reported for *Pseudomonas aeruginosa* and *Enterobacter* sp. The aqueous extract revealed inhibition zone of 24.00 mm against *Staphylococcus aureus*, but no inhibition against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Enterobacter* sp. The minimum inhibitory concentration (MIC) of the ethyl acetate extract was 50.00 mg/mL, 3.12 mg/mL and 6.25 mg/mL against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* respectively. The MIC for the aqueous extract was greater than 100 mg/mL against *Staphylococcus aureus*. The ethyl acetate extract showed better antibacterial activity against the tested pathogens compared to the aqueous extract. The outcome of the study has validated the ethnomedicinal uses of *Triclisia patens* Oliv. and shown that it can be used as a source of pharmacological compounds.

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INTRODUCTION

Conventional antibiotics have been used to combat infectious diseases successfully for decades. However, currently, most antibiotics have been rendered almost ineffective in treating certain infectious diseases because of microbial resistance and toxicity problems (Adrien *et al.*, 2018). Therefore, effective and safe antimicrobial agents must be sourced for. The lingering treatment failures and the need to find a lasting solution to infectious diseases necessitates further exploration of natural products to uncover new grounds in drug production (Bisi-Johnson *et al.*, 2017). Natural products have served as important source of drugs since ancient times and about half of the useful drugs today are derived from natural sources. A number of drugs from plant species active against diseases have been discovered through ethnobotanical leads (Tringali, 2001; Bisi-Johnson *et al.*, 2017). The plant kingdom still contains numerous species of plants with yet-to-be-discovered medicinally valuable compounds. Large number of plants is being screened for their anti-inflammatory, hypotensive, hypoglycaemic, amoebicidal, antifertility, cytotoxic and antibiotic properties (Trease and Evans, 1989). It is estimated that a sizeable majority of the world population use herbal medicine for various chemotherapeutic purposes (Ripa *et al.*, 2009) and it is a major component in all indigenous peoples' traditional medicine (Okwori *et al.*, 2008; Abo *et al.*, 2011). The use of medicinal plants is crucial for maintaining the health of both people and entire societies. Plants have medical significance because of their bioactive components, which have a clear physiological impact on the body. These constituents include alkaloids, saponins, tannins, flavonoids and phenolic compounds (Hill, 1952). Bioactive compounds act through two main mechanism of action: their hydrophobic property facilitates their adhesion to the surface of the bacteria and they inactivate bacterial molecules such as enzyme or receptors

(Pandey and Kumar, 2013). Other compounds interact with the lipid bilayer in cell membrane, thereby separating the two membranes and consequently leading to swelling and cell death (Tshingani *et al.*, 2017) Many plants are yet to be explored scientifically.

Triclisia patens Oliv., a dioecious liana with a stem up to 6 cm in diameter and a length of up to 12 m, belongs to the Menispermaceae family. The leaves alternate, are whole, and have pinnate veins. The fruit is made up of spheroid, flattened drupes and the flowers are unisex (Mosango, 2008). About 12 of the 20 species of *Triclisia* are found in tropical Africa. *T. macrophylla* Oliv. has an unclear spreading from Sierra Leone to Cameroon and Equatorial Guinea. *T. subcordata* Oliv. occurs throughout West and Central Africa and has similar uses to *T. patens*. *T. patens* can be found in rainforest, secondary forest and on fallow land. It has a wide distribution and grows in both primary and secondary vegetation; there are no indications that it is endangered (Mosango, 2008).

Plants belonging to this genus possess potential therapeutic values known since ancient times to cure various ailments and infectious diseases such as malaria, venereal diseases, epileptic attacks, diarrhea, stomach problems, leprosy, mental health problems, dysentery, respiratory diseases, convulsive coughing, and renal related ailments including edema, anemia, gout, urinogenital infections, and swelling of extremities (Asuzu *et al.*, 1995; Abo *et al.*, 2011; Samita *et al.*, 2017; Tiam *et al.*, 2019). Camacho *et al.* (2003) screened extracts derived from 43 plant species for their antiprotozoal activity against *Leishmania donovani* and *Trypanosoma brucei brucei* and discovered that the most active was *Triclisia patens* with an IC₅₀ value of 1.5µg/mL against *Leishmania donovani*. Crude ethanolic extracts of the wood and bark of *Triclisia patens* showed significant anti-amoebic effects. An aqueous extract of the roots has also showed antispasmodic activity on the respiratory tract and intestinal smooth muscle tissue of

several test animals (Mosango, 2008). In Ukwuani, Delta state, it is commonly called “*ikei-ani*” where it is used to treat diarrheal problems. There is little information about *T. patens* in the literature and no report on its antibacterial activity. Due to the acclaimed pharmacological relevance of several species of *Triclisia* by Africans, this paper is aimed at investigating the phytochemical quality and antibacterial activity of *Triclisia patens* Oliv. roots.

MATERIALS AND METHODS

Sample Collection and Preparation

Triclisia patens Oliv. were obtained from Boundary Road, Ovbiogie, Benin City and authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City by Prof E.I. Aigbokhan. The plants were taken to Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Benin, Nigeria, for analysis. The plant roots were thoroughly washed with clean water and sundried. The dried plant roots were pulverized into powdered form using a sterile electric blender and stored in an airtight container.

Aqueous extraction of *Triclisia patens* Oliv. root

The method of Olayemi and Opaleye (1999) was used for both the aqueous and ethyl acetate extraction of *Triclisia patens* Oliv. root. Weighed 500 g of the pulverized plant root was suspended in 2.5 L of sterile water. The suspension was stirred with a sterile glass stirrer. The jar was left for 72 hr for complete extraction. The suspension was continuously stirred at an 8 hr interval for a period of 72 hr. The solution was filtered with Whatman filter paper (No. 1). The filtrate was thereafter concentrated using a rotary vacuum evaporator, and the crude extract obtained was stored at 4 °C until required.

Ethyl acetate extraction of *Triclisia patens* Oliv. root

Weighed 500 g of the pulverized plant root was suspended in 2.5 L of ethyl acetate. The suspension was also stirred thoroughly with a glass stirrer and stored in a cupboard away from sunlight to prevent evaporation. The suspension was continuously stirred at an 8 hr interval during the period of the maceration for 72hr. The filtrate was obtained by using Whatman filter paper (No.1). The filtrate was thereafter concentrated with a rotary vacuum evaporator (Olayemi and Opaleye, 1999).

Phytochemical Quality of *Triclisia patens* Oliv. root extract

The phytochemical screening of the *Triclisia patens* root extract was determined using the standard methods as described by Soforowa (2006) and Trease and Evans (1989).

Test for Alkaloids

0.5 g of the extract was stirred with 5 mL of 1% aqueous HCl on water bath and then filtered. One (1) mL each of the filtrate was taken into two test tubes. In the first tube, few drops of Dragendorff’s reagent were added and the occurrence of an orange-red precipitate was taken as positive. To the second tube, Mayer’s reagent was added and a buff-coloured precipitate was an indication of the presence of alkaloids (Sofowora, 1993)

Test for Saponins

1 mL of the filtrate was diluted with 10 mL distilled water and boiled. The filtrate was mixed with 5 mL of distilled water and shaken vigorously for about 5 min. A stable persistent froth on warming was indicative of the presence of saponins.

Test for Reducing Sugars.

To 2 mL of the extract, two drops of Benedict’s reagent was added. The resulting solution was heated over a boiling water bath for 5 min. The formation of a brick red precipitate was a positive result for the test of reducing sugars.

Test for Phenolic compounds

One (1) mL of the filtrate was added to 5 mL of 90 % ethanol. Thereafter, one drop of 10 % ferric chloride solution was added. A pale yellow color is indicative of a positive result.

Tests for Flavonoids

One (1) mL of filtrate was boiled in 5 mL of distilled water and filtered. 3 mL of 10 % of lead acetate solution was added. The formation of a yellow colored precipitate indicates the presence of flavonoids.

Test for Tannins

Two (2) mL of the extract was added to distilled water (10 mL) and boiled for 5 min. The solution was filtered and two drops of ferric chloride solution were added. The formation of a bluish precipitate is indicative of the presence of tannins.

Test for Steroids

Two (2) mL of acetic anhydride was added to 0.5 g plant extract in 2 mL of dilute hydrogen tetraoxosulphate (VI) (H_2SO_4). A color change from violet to blue or green is indicative of the presence of steroids.

Test for Terpenoids

Five (5) mL of extract was mixed in 2 mL of chloroform and 3 mL of concentrated hydrogen tetraoxosulphate (VI) (H_2SO_4) was cautiously added to the test tube to form a layer. A reddish brown color of the inter-phase is an indication of the presence of terpenoids.

Determination of the Antimicrobial Activity**Preparation of inoculum**

The antibacterial activity of the root's extract was determined using five bacterial species. The pathogens were collected from the stock culture of the Pharmaceutical Microbiology Laboratory, Department of Pharmaceutical Microbiology, University of Benin, Benin City, Nigeria, and they included *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Enterobacter sp.*, and *Pseudomonas aeruginosa*. The viability and purity of each isolate was carried out by streaking and sub-culturing on Mueller-Hinton agar. After incubation for 24 hr at 37 °C, the

inocula were standardized to 1.5×10^8 cells/mL (0.5 McFarland turbidity).

Antimicrobial Activity Testing

The agar well diffusion method was employed for the screening of the extracts for antibacterial activity as described by Valgas *et al.* (2007). With the aid of a sterile cotton swab, the bacterial inoculum (1.5×10^8 cells/mL) was spread on Petri dishes containing Mueller-Hinton agar. Wells of 10 mm in diameter were bored in the agar, and 0.2 mL (concentrations of 50 mg/mL and 100 mg/mL) of the root's extract were added into the wells. The Petri plates were incubated at 37 °C for 24 hr and the inhibition zone diameter (IZD) was measured in mm. Chloramphenicol was used as a control.

Minimum Inhibitory Concentration (MIC) Test

The Afolayan and Meyer (1997) agar dilution method was used to find the lowest concentration of plant root extract that would completely inhibit the growth of the bacteria. The Mueller-Hinton agar was also employed and the inocula were prepared as previously described. Serial dilutions of the extract in concentrations of 1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL were prepared. Further 1:20 dilutions were carried out by introduction into sterilized molten Mueller-Hinton agar, thereby diluting the concentration to 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.12 mg/mL respectively. The plates were allowed to solidify and were dried. The inoculum was streaked on each of the plates containing each of the concentrations and incubated at 37 °C for 24 hr. Tubes without the root's extract were used as control. The highest dilution that inhibited bacterial growth was referred to as the MIC. Results were expressed in mg/mL

RESULTS AND DISCUSSION

The qualitative phytochemical screening of the ethyl acetate extract of *Triclisia patens* root, as presented in Table 1, revealed the presence of phenolic compounds, eugenols, alkaloids, and

flavonoids. Previous studies on *T. patens* have reported the presence of a number of bisbenzylisoquinoline alkaloids including phaenthine, *N,N*-dimethyl-phaenthine, cocsuline, pycnamine, triglletimine and isotetrandine as well as the oxo-aphorphine alkaloid, o-methyl-moschatoline (Dwuma-Badu *et al.*, 1975a; Tackie *et al.*, 1974; Dwuma-Badu *et al.*, 1975b). Camacho (2003) reported similar results of compounds which include; alkaloids, terpenoids and flavonoids amongst others. Menispermaceae are renowned for the presence of alkaloids

(Partridge *et al.*, 1988). Abo *et al.* (2011) investigated the phytochemical quality of the roots of *Triclisia subcordata* and revealed the presence of alkaloids, tannins and trace amounts of saponin. Alkaloids play an important role in the defense and survival of plants because they provide protection from microbes (antibacterial and antifungal activities), insects, herbivores, and other plants through the usage of compounds that allelopathically active (Molyneux *et al.*, 1996).



Plate 1: *Triclisia patens* Oliv.



Plate 2: Appearance of *Triclisia patens*

Table 1: Qualitative Phytochemical Screening of *Triclisia patens* Oliv. root

Phytochemical Constituents	Inference
Saponins	-
Phenols	+
Terpenoids	+
Steroids	-
Alkaloids	+
Flavonoids	+
Tannins	-
Eugenols	-
Glycosides	-
Reducing sugar	-

Key: + = present, - = absent

The wide-ranging biological activity of flavonoids have been demonstrated, including effects that are anti-allergic, antibacterial, anti-inflammatory, anti-mutagenic, antioxidant, antiproliferative, antithrombotic, antiviral, and hepatoprotective. Studies on flavonoids as a potential cancer chemo-preventive drug have multiplied tremendously (Tringali, 2001).

In recent years, phytochemical studies have reported that plants contain secondary metabolites that possess pharmacological potentials. Some of these secondary metabolites include alkaloids, flavonoids, terpenoids, phenols and eugenols. Some of the biological activities of phenolic acids were reported to include an increase in bile production, a decrease in blood cholesterol, and antibacterial activity against some strains of bacteria, including *Staphylococcus aureus* (Glyglewski *et al.*, 1987). According to Ghasemzadeh *et al.* (2010), phenols are also linked to anti-ulcer, anti-inflammatory, antioxidant, cytotoxic and anticancer, antispasmodic, and antidepressant properties. Terpenoids have medicinal properties such as anticarcinogenic (diterpenoid anticancer drug, taxol), antimalarial (the sesquiterpenoid antimalarial drug, artemisinin), anti-ulcer, hepaticidal, antimicrobial and diuretic (glycyrrhizin) activities (Langerhein, 1994; Dudareva, 2004).

In rural areas, decoctions of plant parts (such as roots, rhizomes, tubers, stems, leaves, and barks)

are used to treat ailments including diarrhea. These bioactive components have been revealed to be the cause of many plants' anti-diarrheal properties (Njume and Goduka, 2012). The presence of these bioactive compounds justifies its ethnomedicinal uses in treatment of various ailments.

Antimicrobial activity testing is usually conducted to demonstrate the antimicrobial potential of plants' extracts. The antimicrobial property of any plant extract is evidenced through the inhibition of the test organisms, which is represented as the diameter of the inhibited zone, also called inhibition zone diameter (IZD). *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter* sp. and *Staphylococcus aureus* were used to assay for the antibacterial activity of the aqueous and ethyl acetate extract of *T. patens* roots. The ethyl acetate root extract of *Triclisia patens* Oliv. had activity on only three of the tested pathogens ranging from 13.00 mm (*Staphylococcus aureus*) to 20.00 mm (*Bacillus* sp.). The inhibition zone diameters for the ethyl acetate root extract of *Triclisia patens* against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* were 13.00 mm, 13.50 mm and 20.00 mm respectively, while that of aqueous extract was 24.00 mm against *Staphylococcus aureus* only as shown in Table 2

Table 2: Antibacterial activity of ethyl acetate and aqueous extract of *Triclisia patens* Oliv. root

Bacteria	Extract	Concentration		Control (Chloramphenicol 30 ug)
		100 mg/mL	50 mg/mL	
<i>Enterobacter</i> sp.	Ethyl-acetate	-	NZ	27.00
	Aqueous	NZ	NZ	
<i>Klebsiella pneumoniae</i>	Ethyl-acetate	-	NZ	19.00
	Aqueous	NZ	NZ	
<i>Pseudomonas aeruginosa</i>	Ethyl-acetate	-	NZ	24.00
	Aqueous	NZ	NZ	
<i>Bacillus subtilis</i>	Ethyl-acetate	-	13.50	26.00
	Aqueous	NZ	NZ	
<i>Escherichia coli</i>	Ethyl-acetate	-	13.00	21.00
	Aqueous	NZ	NZ	
<i>Staphylococcus aureus</i>	Ethyl-acetate	-	20.00	29.00
	Aqueous	24.00	NZ	

Key: NZ = No zone

Table 3: Minimum inhibitory concentration (mg/mL) of the aqueous and ethyl acetate extracts of *Triclisia patens* Oliv. root

Bacteria	Extracts	Concentration MIC (mg/mL)
<i>Escherichia coli</i>	Ethyl-acetate	50.00
	Aqueous	ND
<i>Bacillus subtilis</i>	Ethyl-acetate	6.25
	Aqueous	ND
<i>Staphylococcus aureus</i>	Ethyl-acetate	3.12
	Aqueous	>100

Key: ND = Not Determined

According to Biyiti *et al.* (2004), when using a diffusion method, an extract is deemed to be active when it induces a zone of inhibition greater than or equivalent to 10 mm. The extract contains secondary metabolites such phenols and alkaloids that are responsible for the antibacterial effects seen. This is consistent with the findings of Gryglewski *et al.* (1987) who reported that some bacterial strains, including *Staphylococcus aureus*, were resistant to the antibacterial effects of phenolic chemicals. Alkaloids also have antibacterial properties, and research has shown that this makes them important for preserving plants because it ensures their survival against pathogens

(Molynuex *et al.*, 1996). In comparison to the aqueous extract, the *Triclisia patens* Oliv. ethyl acetate extract was active against the majority of the tested microorganisms. Ethyl acetate may have extracted more of the bioactive components as a result.

The lowest extract concentration or greatest extract dilution that can prevent the growth of the organism being examined is known as the minimum inhibitory concentration (MIC). According to Adrien *et al.* (2018), it appears that there is no standardization of the MIC values for plant extracts and essential oils, and there is also no agreement on the inhibition concentration for natural products. As a result, the consistency of

the antimicrobial activity results is based on the growth inhibition zones observed and the estimated MBC/MIC ratio. From Table 3, the MIC of the root's ethyl acetate extract was found to be >100 mg/mL against *Escherichia coli* and 3.12 mg/mL and 6.25 mg/mL, respectively, against *Bacillus subtilis* and *Staphylococcus aureus*. Abo *et al.* (2011) also reported that the roots of *T. subcordata* had good antibacterial activity at concentration range 50-200 mg/mL, although it was only recorded against strains of *S. aureus* and *E. coli*.

CONFLICT OF INTEREST

No conflicts of interest exist among the authors

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CONCLUSION

This study revealed that the roots of *Triclisia patens* Oliv. had good antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*. The activity could be attributed to the presence of flavonoids, terpenoids, alkaloids, and phenols as established by the phytochemical screening. The highest inhibitory activity was detected in *Staphylococcus aureus*. Therefore, the roots of *T. patens* can be explored as a promising source of pharmacological compounds.

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