



Phytochemical and Antibacterial Studies on Aqueous Ethanol Extract of *Thesium viride* (Santalaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SS designed the study, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Authors MSA and GI analyses of the study and managed the experimental process. Author UI identified and collected the plant. All authors read and approved the final manuscript.

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ABSTRACT

Thesium viride Hill (Santalaceae) is a sub-shrub hemiparasite that grows up to 45 cm tall and widely distributed in Europe, Asia and Africa. It is used ethno medicinally in ulcer, jaundice and splenomegally. Phytochemical screening was carried out on the aqueous ethanol extract of the whole plant by using standard phytochemical methods. Antibacterial studies were also carried out on the aqueous ethanol extract by using diffusion method and broth dilution methods. The aqueous ethanol extract of the plant was found to contain alkaloids, flavonoids, anthraquinones, saponins and cardiac glycosides. The aqueous extract was found to be active against *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Helicobacter pylori* and *Shigella dysenteriae* and had minimum inhibitory concentrations (MIC) of 10, 5, 5, 5, and 5 mg/ml and also minimum bactericidal concentration (MBC) of 20, 20, 20, 10, and 10 mg/ml respectively. The extract was also found to be inactive against *Corynebacterium ulcerans* and *Salmonella typhi*. Zones of inhibition produced by aqueous ethanol extract of plant were less than those produced by

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ciprofloxacin (5 µg/ml). The aqueous ethanol extract of the plant could serve as an antibacterial agent on the sensitive bacteria based on the study.

Keywords: *Thesium viride*; Santalaceae; phytochemical; antibacterial; aqueous ethanol.

1. INTRODUCTION

Plants and their extracts have been used in treatment of several infectious diseases. These plants have been useful in the development of novel drugs which are more efficacious with lesser side effects than semi-synthetic and synthetic antimicrobial agents. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to an alarming increase in the incidence of new and re-emerging infectious diseases and development of resistance to the antibiotics in current clinical use [1].

Alternative antibacterial drugs from plants have been revived for disease management due to the increased prevalence of multidrug resistance strains of bacterial isolates. This increases prevalence have been attributed to the indiscriminate uses of commercial antibiotics and this in turn has forced scientist to search for new antibacterial substances from various medicinal plants [2].

Thesium viride belongs to the family Santalaceae commonly called "Huntu" among the Hausa in Northern Nigeria. It is distributed mainly in Europe, Asia and Africa [3]. It is a sub-shrub hemiparasite up to 45 cm tall, tufted stems starting from a woody rootstock, branched stems, about 2mm thick, greyish green [4]. It is prescribed to cure ulcers and jaundice [5], the aerial part of plant was used in the treatment of jaundice, liver enlargement and splenomegaly [6].

The genus *Thesium* has about 300 species with 60 species occurring in tropical African [7]. Phytochemical constituents associated with this genus include; cardiac glycoside in *T. lineatum*, alkaloids in *T. lineatum* and *T. humile* [8]. Flavonoids, phenylpropanes and terpenes in *T. chinense* [9].

The main objective of the present study was to carry out qualitative phytochemical screening and to assess the antibacterial potentials of *T. viride* extracts against some bacteria in order to use it as a source of new antibacterial agent.

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Preparation

The Fresh whole plant of *T. viride* was collected from Karau-karau village, Giwa Local Government Kaduna State, Nigeria in November 2014. It was authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria. The plant was dried and powdered using pestle and mortar.

2.2 Phytochemical Studies

2.2.1 Extraction of plant material

The ground powdered plant of *T. viride* (100 g) was weighed and macerated with 400 ml of aqueous ethanol (70% v/v) for 72 hours at room temperature and filtered. The filtrate obtained was evaporated to dryness on a water bath (75 °C) which yielded (16.7 g).

2.2.2 Phytochemical Screening

The aqueous ethanol extract of the plant was screened using standard phytochemical procedures to confirm the presence of phytochemical [10].

2.3 Antibacterial Studies

2.3.1 Collection of microbial cultures

Culture of *Staphylococcus aureus*, *Streptococcus faecalis*, *Corynebacterium ulcerans*, *Escherichia coli*, *Helicobacter Pylori*, *Salmonella typhi* and *Shigella dysenteriae* strains were collected from Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria.

2.3.2 Preparation of the aqueous ethanol extract

Dried aqueous extract (0.2 g) of the plant was weighed and dissolved in 10 ml of DMSO to make a stock solution of 20 mg/ml.

2.3.3 Determination of zone of inhibition

Diffusion method was used to screen the extract. Muller Hinton agar was used as the culture medium. It was sterilized at 121°C for 15 minutes and poured into sterilized petri dishes and was allowed to cooled and solidified. The sterilized medium was seeded with 0.1 ml of the standard inoculums of the test organisms, the inoculums was spread evenly over the surface of the medium with a sterile swab. Wells were bored into the agar plates using 6 mm sterile cork borer and 0.1 ml of the solution of the extract of the concentration of 40 mg/ml was then introduced into each well on the inoculated medium. The plates were incubated at 37°C for 24 hours. Thereafter the zone of inhibition of growth for each well was read to the nearest millimetre and recorded [11].

2.3.4 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the aqueous ethanol extract was determined using the broth dilution method. Mueller Hinton broth was prepared; 10 ml was dispensed into test tubes and was sterilized at 121°C for 15 minutes. The broth was allowed to cool. Dilution of the test micro organism was done in the normal saline until the turbidity marched with that of the Mcfarland standard by visual comparison. Two-fold serial dilution of the extract in the sterile broth was prepared and concentrations were obtained as 20, 10, 5, 2.5 and 1.25 mg/ml. The test microbe (0.1 ml) in the normal saline medium was inoculated at different concentrations; incubation was made at 37°C for 24 hours after which the test tubes was observed for turbidity (growth). The least concentration of the plant extract that did not permit any visible

growth of the inoculated test organism in broth culture was regarded as the MIC [12].

2.3.5 Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration was carried out and was determined whether the test microbes were killed or their growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121°C for 15minutes and poured into sterilized petri dishes and was allowed to cool and solidified. The contents of the MIC in the serial dilution was then sub-cultured onto the prepared medium and the medium was incubated at 37°C for 24 hours, after each plate of the medium was observed for colony growth, The MBC was determined as the plates with lowest concentration of the extract without colony growth [13].

3. RESULTS

3.1 Phytochemical Studies

Preliminary qualitative tests for presence of phytochemicals were carried out on the ethanol extract. The results from phytochemical tests on *T. viride* intimated the presence of alkaloids, flavonoids, anthraquinones, saponins, steroids and cardiac glycosides (Table 1).

3.2 Antibacterial Studies

3.2.1 Zone of inhibition

Zone of growth inhibition (mm) against *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Shigella dysenteriae* and *Helicobacter pylori* were found to be 18, 20, 21,

Table 1. Phytochemical constituents identified in aqueous ethanol extract of *Thesium viride*

Constituents	Test	Observation	Inference
Alkaloids	Drangendoff's	Orange-red precipitate	+
	Wagner's	Brown precipitate	+
	Mayer's	White precipitate	+
Flavonoids	Shinoda's	Orange colour	+
	Sodium hydroxide	Yellow colour	+
Anthraquinones	Borntrager's	Bright pink	+
	Modified Borntrager's	Bright pink colour	+
Saponins	Frothing	Froth formed	+
Phenolic compounds	Ferric Chloride	Dark greenish precipitate	+
Steroids	Liebermann-Burchard	Brownish ring	+
	Salkowski's	Reddish brown	+
Cardiac glycoside	Killer-killiani	Brown ring	+

Key: + = Present

22 and 24 respectively compared to control (ciprofloxacin 5 µg/ml) having a zone of inhibition of 32-37 mm. Also, *Corynebacterium ulcerans* (Gram positive) and *Salmonella typhi* (Gram negative) were found to be resistant to the extract (Table 2).

3.2.2 Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration (MIC) was 10 mg/ml against *Staphylococcus aureus* and 5mg/ml against other sensitive pathogens (Table 3).

3.2.3 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was found to be 20 mg/ml against *Staphylococcus aureus*, *Streptococcus faecalis* and *Escherichia coli* while 10 mg/ml against *Helicobacter pylori* and *Shigella dysenteriae* as shown in Table 4.

4. DISCUSSION

The results of phytochemical analysis indicated the presence of alkaloids, flavonoids, anthraquinones, saponins, cardiac glycosides and steroids. These phytochemical constituents identified are considered significant important biological compounds of plant origin. Phenolic compounds (tannins, flavonoids and

anthraquinones), alkaloids and terpenoids are secondary metabolites that are major group of antimicrobial agents in plant [14].

The zone of inhibition indicated that the aqueous ethanol extract of *Thesium viride* was active against *Staphylococcus aureus* (Gram positive), *Streptococcus faecalis* (Gram positive), *Escherichia coli* (Gram negative), *Helicobacter Pylori* (Gram negative), and *Shigella dysenteriae* (Gram negative) while it was inactive against *Corynebacterium ulcerans* (Gram positive) and *Salmonella typhi* (Gram negative) as shown in table 2. The extract shows a higher degree of activity of 24 mm of zone of inhibition against *Helicobacter pylori* as compared to 30 mm of the standard ciprofloxacin. The antibacterial activity of the extract against the sensitive test organisms is greater than 18 mm which makes it a very active agent [15].

The minimum inhibitory concentration that is the lowest concentration that inhibits the growth of microorganisms of the aqueous ethanol extract was observed (10 mg/ml) against *Staphylococcus aureus* while it is more effective against other organisms (5 mg/ml) as shown in table 3. The minimum bactericidal concentration (concentration that kills the bacteria) was found to be 20 mg/ml against *Staphylococcus aureus*, *Streptococcus faecalis* and *Escherichia coli* while it is 10 mg/ml against *Helicobacter pylori* and *Shigella dysenteriae*.

Table 2. Zones of inhibition (mm) produced by ethanol extract of *Thesium viride* against test organisms

Test organisms	Ethanol extract (20 mg/ml)	Ciprofloxacin (5 µg/ml)
<i>Staphylococcus aureus</i>	18	35
<i>Streptococcus faecalis</i>	20	32
<i>Corynebacterium ulcerans</i>	0	32
<i>Escherichia coli</i>	21	37
<i>Helicobacter pylori</i>	24	30
<i>Salmonella typhi</i>	0	41
<i>Shigella dysenteriae</i>	22	39

Table 3. Minimum Inhibitory Concentration (MIC) of aqueous ethanol extract of *Thesium viride* against the test organisms

Test organism	Concentrations of aqueous ethanol extract of <i>T. viride</i> (mg/ml)					
	20	10	5	2.5	1.25	MIC
<i>Staphylococcus aureus</i>	-	-	+	+	+	10
<i>Streptococcus faecalis</i>	-	-	-	+	+	5
<i>Escherichia coli</i>	-	-	-	+	+	5
<i>Helicobacter pylori</i>	-	-	-	+	+	5
<i>Shigella dysenteriae</i>	-	-	-	+	+	5

Keys: + = No inhibition of growth; - = Inhibition of growth

Table 4. Minimum Bactericidal Concentration (MBC) of aqueous ethanol extract of *Thesium viride* against the test organisms

Test organisms	MBC (mg/ml)
<i>Staphylococcus aureus</i>	20
<i>Streptococcus faecalis</i>	20
<i>Escherichia coli</i>	20
<i>Helicobacter pylori</i>	10
<i>Shigella dysenteriae</i>	10

Successful antimicrobial therapy of an infection ultimately depends on the concentration of antibiotic at the site of infection. This concentration must be sufficient to inhibit growth of the offending microorganism. If host defences are intact and active, a minimum inhibitory effect, such as that provided by bacteriostatic agents may be sufficient. On the other hand, if host defences are impaired, antibiotic-mediated killing (*i.e.*, a bactericidal effect) may be required to eradicate the infection. The concentration of drug at the site of infection not only must inhibit the organism but also must remain below the level that is toxic to human cells. If this can be achieved, the microorganism is considered susceptible to the antibiotic [16].

5. CONCLUSION

Aqueous ethanol extract of *T. viride* was found to contain some phytochemicals responsible for the antibacterial activity as such it could therefore serve as a potent source antibacterial agent of medicinal plant origin.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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