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Phytochemical Screening, Thin Layer Chromatography and Antibacterial Activity of the Leaf Extracts of *Striga hermonthica*

R. Augustine*, B.S. Maikasuwa, A.C. Etonihu

Department of Chemistry, Nasarawa State University, P.M.B 1022, Keffi, Nasarawa State, Nigeria
*Author for correspondence; Email: augustinerosemary20@gmail.com

Abstract This study was performed to evaluate the phytochemicals, antibacterial properties of *n*-hexane and methanolic leaf extracts of *Striga hermonthica*, using standard methods and to determine the active ingredients present in the leaf extracts of *Striga hermonthica* using thin layer chromatography (TLC). The phytochemical screening carried out on the methanolic leaf extracts of *Striga hermonthica* revealed the presence of alkaloids, saponins, cardiac glycosides, tannins, flavonoids, carbohydrates and phenols; only cardiac glycosides were observed in the hexane leaf extracts. The methanolic and hexane leaf extracts had activity against Salmonella typhi, Escherichia coli and Staphylococcus aureus at 50mg/cm^3. Their zones of inhibition were Salmonella typhi (8.0mm and 10.00mm), Escherichia coli (6.1mm and 9.00mm) and Staphylococcus aureus (8.0mm and 7.00mm), respectively. The minimum concentration of methanolic leaf extracts of *Striga hermonthica* that inhibited visible growth was >50.0mg/cm^3 against *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* while that of hexane leaf extractwas >50.0mg/cm^3 against Salmonella typhiand Staphylococcus aureus. Thin Layer chromatography profiling of both the hexane and methanol extracts indicated the presence of bioactive constituents with different R_f values obtained from three different solvent systems (hexane/ethylacetate, 1:1; hexane / ethylacetate, 4:1 and hexane / ethylacetate / chloroform / acetic acid, 2:1:1:1). The antibacterial activity of the methanolic and hexane leaf extracts of *Striga hermonthica* observed in this study justifies its use for the treatment of urinary tract infections, skin disease, pneumonia and jaundice.

Keywords Antibacterial, phytochemical, TLC, *Striga hermonthica*.

Introduction

Microbial infections have caused a big burden of diseases. An alarming increase in microbial resistance against conventional antibiotic treatment has developed over the last forty years in both medical and livestock sectors [1]. This alarming increase in the development of resistance to the antibiotics in the clinical practice has led to the incidence of new and re-emergence of infectious diseases making them expensive and difficult to treat. Plants have great potential as antimicrobial agents due to the presence of a wide range of bioactive compounds such as alkaloids, flavonoids and saponins. However, since many medicinal plants are unevaluated, it is possible that the active compounds of many plants go undetected while some plants used therapeutically result in poisoning.

Striga hermonthica (Figure 1) is a flowering root parasitic plant considered as a hemi-parasitic plant. *Striga hermonthica* (Delile) Benth (Scientific name) belongs to the family Scrophulariaceae and is one of the most ubiquitous parasitic weeds of food crops, such as: rice (*Oryza sativa*), millet (*Pennisetum glaucum*), maize (*Zea*

mays), sorghum (*Sorghum bicolor*) and sugarcane (*Saccharum officinarum)* [2]. *Striga hermonthica* is an erect, annual shrub wide-spread in the tropics and subtropics of Gambia, Ghana, Mali, Nigeria, Niger Republic, Senegal and Sudan [3]. Despite its parasitic devastating impacts, *Striga hermonthica* extracts are rich in secondary metabolites and find broad use in traditional medicine, especially as a result of their antimicrobial activity [4]. It is well-known in some parts of Africa and India as a medicinal plant, where it is used for treatment of leprosy and leprous ulcers. In East Africa, a decoction or infusion of the roots is administered orally as an abortifacient and in the treatment of pneumonia [5] and anti-diabetic agent in western part of Sudan [6]. It has also been reported that *Striga hermonthica* extract has antimalarial activity [7].

The use of plant extracts and phytochemicals can be of great significance in therapeutic treatments due to their antimicrobial activities. The World Health Organisation [1] advocates for traditional medicine as a safer remedy for ailments of microbial and non-microbial origin. Most modern medicines were discovered through study of plants which were used traditionally to treat specific illnesses. In addition, very few medicinal plants have been analyzed chemically and their bioactive constituents are yet to be validated. As a result, knowledge from traditional medicine can be very essential in the development of cheap and effective antibiotics. This present study was carried out to determine the phytochemicals, antibacterial activity and the active ingredients present in the leaf extracts of *Striga hermonthica* using thin layer chromatography (TLC).

Figure 1: Striga hermonthica Plant

Materials and Methods

Collection and Treatment of Plant Material

The leaves of *Striga hermonthica* were collected from Nasarawa State University in Keffi Local Government Area (L.G.A) of Nasarawa State in Nigeria. The plant sample collected was taken to the Department of Plant Science and Biotechnology for identification. Thereafter, the sample was taken to Chemistry and Microbiology laboratories of Nasarawa State University, respectively for chemical and antibacterial analyses.

The leaves of *Striga hermonthica* collected from the wild were air-dried in an oven at 40^{o}C for 4 days to remove water content. The dried leaves were pulverized into powder using a wooden mortar and pestle. The powered materials were kept in a cellophane bag and stored in a cool dry place until needed for extraction. The dried powdered leaves were submitted to a continuous extraction in a soxhlet extractor for 5 days using 100% methanol and n-hexane as solvents.

Extraction of Plant Material

The plant sample (100g) was weighed and transferred into a clean thimble fixed to the soxhlet extractor. 250 cm^3 of n-hexane was poured into the round bottom flask with small quantity of boiling chips (to prevent bumping), connected to the soxhlet apparatus and heated on a heating mantle at about 60-80^oC. The system was allowed to run for about 8h until the plant material/sample was colourless. The same procedure was repeated using methanol as solvent.

Qualitative Phytochemical Screening of the Extracts

The leaf extracts of *Striga hermonthica* were subjected to qualitative phytochemical screening to identify the presence of secondary metabolites such as flavonoids, saponins, tannins, alkaloids, cardiac glycosides, steroids, reducing sugars, carbohydrates, anthraquinones, volatile oils, resins and phenols as described by [8-10].

Thin Layer Chromatography (TLC)

This is the simplest technique used to determine the overall nature of the number and type of metabolites in plant extract [11]. The stationary phase consisted of a thin layer of silica gel adsorbent on a flat, thick glass TLC plate $60F_{254}$ (Merck). This means that the TLC plates were pre-coated with a fluorescent material to absorb UV light as low as 254 nm and to aid visualization of spots. The crude plant extracts were screened for flavonoids, alkaloids, phenols, saponin, tannins and cardiac glycosides. Using capillary tubes, spots of the dissolved extracts were made on the base line of the TLC plates. The plates were placed into the TLC development tank (chamber) containing hexane, ethylacetate, chloroform and acetic acid in definite proportions. Visualization of compounds was done using iodine vapour.

TLC Plates

Pre-coated TLC plates with dimensions 9cm by 4cm were used for the analyses. Each of the extract was spotted by the use of a capillary tube on the origin line drawn about 1cmfrom the base of the plate.

Solvent Development

Different solvent systems which were carefully chosen based on the results of the phytochemical screening were employed. The solvent system includes:

Hexane: Ethylacetate (1:1)

Hexane: Ethylacetate (4:1)

Hexane: Ethylacetate: Chloroform: Acetic acid (2:1:1:1)

One drop of glacial acetic acid was added to each solvent system to aid the distinct separation of spots into their different components. These solvent systems gave good resolution (separation). The inside of the developing tank was lined with a piece of filter paper which was soaked with the solvent systems. It keeps the chamber saturated with solvent vapors, there by speeding up the development. The spotted plate was then placed in a slanting position inside the tank which was then covered. The developed plate was removed from the tank after the solvent has travelled four-fifth of its height. The solvent front was quickly marked and the plate was air-dried.

The air-dried plates were viewed at 254 and 366nm in a UV Lamp and placed in spraying chamber (Iodine Vapor Tank) for visualization of spots and photographed. The separated spots were located, marked with a lead pencil (it is non-colour pigmented material and doesn't react with the solvent). The retention factor (R_f) for each spot was calculated thus:

$$Retention\ factor\ (R_f) = \frac{Distance\ travelled\ by\ the\ component\ (spot)}{Distance\ travelled\ by\ the\ solvent\ front}$$

Antibacterial and Sensitivity Test

Sample Collection

The enterobacterial isolates used were 12 clinical isolates obtained from Microbiology Department of Nasarawa State University Keffi in Nigeria. The isolates consisted of the bacteria *Salmonella typhi, Staphylococcus aureus, and Escherichia coli.*

Preparation of Medium Used

The medium used was nutrient agar. The medium was prepared according to the manufacturer's specification [12]. 28g of the agar was dissolved in 1 litre of distilled water, boiled to dissolve completely and was sterilized in an autoclave at 121^{o}C for 15 minutes. It was allowed to cool to about 45^{o}C and poured into the sterilized plates.

***In-vitro* Antibacterial Susceptibility Testing**

The isolates were screened for antibacterial susceptibility test using the Cup-plate agar diffusion method. Different concentrations (50.0 to 3.125mg/cm^3) of methanol and n-hexane extracts of *Striga hermonthica* leaves were prepared in 10% dimethyl sulphoxide. The standardized clinical bacterial isolates (10^5 CFU) were uniformly streaked on the entire surface of Mueller-Hinton Agar (MHA) plates and wells were bore in the MHA plates streaked with bacteria suspension using a pair of sterile core borer. The base of the well was sealed with sterile melted nutrient agar and 0.1 cm^3 of the extracts was dispensed into the wells. Inoculated plates were then incubated at 37^{o}C for 18 h. After incubation, the diameters of the zones of microbial growth inhibition were measured in millimeters (mm) using a transparent ruler. The inhibition zones were then interpreted as not sensitive or sensitive.

Minimum Inhibitory Concentrations (MICs)

The MIC of methanol and n-hexane leaf extracts of *Striga hermonthica* was carried out using Micro broth dilution method. 100µL of sterile Mueller-Hinton Broth (MHB) was prepared in 96 sterile wells microtitre plates and 100µL of the extract (50mg/cm^3) was diluted (usually two-fold dilution) in 100µL of double strength MHB in the microtitre plates from well 1-5 and wells 6 and 7 serve as organisms viability control and medium sterility control. 5µL of standardized clinical bacteria suspension (10^5CFU) were dispensed into each well (1-6) in the microtitre plate and the plate was incubated at 37^{o}C for 18 h. The minimum concentrations of the extract that inhibited visible growth were read as the MICs.

Results and Discussion

The phytochemical screening carried out on the methanolic leaf extracts of *Striga hermonthica* revealed the presence of alkaloids, saponins, cardiac glycosides, tannins, flavonoids, carbohydrates and phenols whereas, only the presence of cardiac glycosides was observed on the hexane leaf extracts (Table 1). This implies that the leaf of *Striga hermonthica* contain more of the polar plant metabolites hence, more constituents from the methanolic extracts than from the hexane extracts, since hexane is non-polar. From Table 1, the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides in the methanolic leaf extracts of *Striga hermonthica* agrees with the findings of [4, 13] who reported the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides in the ethanolic leaf extracts of *Striga hermonthica*. The presence of cardiac glycosides in the hexane leaf extracts of *Striga hermonthica* agrees with the findings of [13] who reported the presence of cardiac glycosides in the hexane leaf extracts of *Striga hermonthica* obtained from Bara, North Kordofan in Sudan.

The curative properties of the methanolic leaf extracts of *Striga hermonthica* may be due to the presence of secondary metabolites such as saponins, alkaloids, tannins, flavonoids, carbohydrates, phenols and cardiac glycosides [14]. Flavonoids are water-soluble antioxidants that prevent oxidative cell damage and have strong anti-ulcer activity [15]. Tannins coagulate proteins and hasten the healing of wounds and inflamed mucous membrane [16]. The presence of tannins in methanolic leaf extracts of *Striga hermonthica* explains its use in traditional medicine for the treatment of wounds and burns. The presence of saponins aids in the treatment of wounds and stopping of bleeding. Saponins have the property of precipitating and coagulating red blood cells [17]. Alkaloids,

comprising a large group of nitrogenous compounds, are widely used as cancer chemotherapeutic agents [18]. The presence of alkaloids in the methanolic leaf extracts of *Striga hermonthica* may be responsible for antimicrobial activity of the plant.

The results of the phytochemical screening of crude extracts of *Striga hermonthica* are presented in Table 1.

Table 1: Phytochemical screening of the Extracts of *Striga hermonthica*

S/N	Constituent	Hexane Extract	Methanol Extract
1	Flavonoids	-	+
2	Saponins	-	+
3	Tannins	-	+
4	Alkaloids	-	+
5	Cardiac Glycosides	+	+
6	Steroids	-	-
7	Reducing Sugars	-	-
8	Carbohydrates	-	+
9	Anthraquinones	-	-
10	Volatile oils	-	-
11	Resins	-	-
12	Phenols	-	+

+ = present; - = absent

Thin Layer Chromatographic Studies

A large number of solvent systems were tried to achieve a good resolution. Finally, three solvent systems Hexane: Ethylacetate (1:1), Hexane: Ethylacetate (4:1) and Hexane: Ethylacetate: Chloroform: Acetic acid (2:1:1:1) were used.

TLC studies of the hexane extract of *Striga hermonthica* leaves (Table 2) showed that in Solvent System I, 2 spots were detected with R_f values of 0.46 and 0.80. In Solvent System II, 5 spots were detected with R_f values of 0.84, 0.35, 0.47, 0.59 and 0.75. In Solvent System III, 4 spots were detected with R_f values of 0.58, 0.73, 0.86 and 0.91.

TLC studies of the methanol extract of *Striga hermonthica* leaves (Table 2) showed that in Solvent System I, 2 spots were detected with R_f values of 0.50 and 0.70. In Solvent System II, 1 spot was detected with R_f value 0.18. In Solvent System III, 2 spots were detected with R_f values of 0.73 and 0.86.

Under the same experimental conditions, compounds with the same R_f value show that they are the same compound [19]. Less polar compounds have larger R_f values in the solvent system while more polar compounds have smaller R_f values.

Plate 1: TLC Chromatogram of Solvent System I of Hexane and Methanol Extracts

Plate 2: TLC Chromatogram of Solvent System II of Hexane and Methanol Extracts

Plate 3: TLC Chromatogram of Solvent System III of Hexane and Methanol Extracts

Table 2: R_f values of some active ingredients obtained from the leaf extract of *Striga hermonthic*

Solvent System	**Hexane Extract**		**Methanol Extract**	
	No. of spots	**R_f Value**	**No. of spots**	**R_f Value**
Solvent System I	2	0.46, 0.80	2	0.50, 0.70
Solvent System II	5	0.35, 0.47, 0.59, 0.75, 0.84	1	0.18
Solvent System III	4	0.58, 0.73, 0.86, 0.91	2	0.73, 0.86

The antibacterial activity of methanolic leaf extracts of *Striga hermonthica* had activity against *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus* at 50mg/cm^3. Their zones of inhibition were 8.0mm (*Salmonella typhi*), 6.1mm (*Escherichia coli*) and 8.0mm (*Staphylococcus aureus*), respectively (Table 3). The antibacterial activity of methanolic leaf extracts of *Striga hermonthica* against the clinical bacteria isolates observed in this study is in agreement with the study earlier described by [13], who reported that the ethanolic extracts of *Striga hermonthica* were active against *Escherichia coli* and *Staphylococcus aureus* at 20mg/cm^3.

Table 3: Antibacterial Activity of *Striga hermonthica* Methanolic Extract

Diameter zone of Inhibition (mm)					
Bacterial Isolates	**Concentration of extracts (mg/cm^3)**				
	50	**25**	**12.5**	**6.25**	**3.125**
Salmonella typhi	8.0±0.4	-	-	-	-
Escherichia coli	6.1 ±0.1	-	-	-	-
Staphylococcus aureus	8.0 ±1.0	-	-	-	-

- = Not sensitive

The antibacterial activity of hexane leaf extracts of *Striga hermonthica* had activity against *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus* at 50mg/cm^3. Their zones of inhibition were 10.00mm (*Salmonella typhi*), 9.00mm (*Escherichia coli*) and 7.00mm (*Staphylococcus aureus*), respectively (Table 4). The antibacterial activity of hexane leaf extracts of *Striga hermonthica* against the bacteria although lacking most of the bioactive constituents could be because many plants store bioactive principles in the form of inactive glycosides. These glycosides could have been activated by enzyme hydrolysis which caused the sugar part to be broken off and made the chemical available for use. Many plant glycosides are used as medications in animals and humans [20].

The antibacterial activity of hexane leaf extracts of *Striga hermonthica* against the clinical bacteria isolates observed in this study is in agreement with the study earlier described by [13], who reported that the hexane extracts of *Striga hermonthica* were active against *Escherichia coli* and *Staphylococcus aureus*. Urinary tract infection is caused by *Escherichia coli*; skin infection (pimples and boils) is caused by *Staphylococcus aureus* while diarrhea, fever and typhoid are caused by *Salmonella typhi.* The antibacterial activity of methanolic and hexane leaf extracts of *Striga hermonthica* observed in this study justifies its use for the treatment of urinary tract infections, skin disease, pneumonia and jaundice [5, 7, 21].

Table 4: Antibacterial Activity of *Striga hermonthica* Hexane Extract

Diameter zone of Inhibition (mm)					
Bacterial Isolates	**Concentration of extracts (mg/cm^3)**				
	50	**25**	**12.5**	**6.25**	**3.125**
Salmonella typhi	10.00±2.10	-	-	-	-
Escherichia coli	9.00 ±1.10	-	-	-	-
Staphylococcus aureus	7.00 ± 0.02	-	-	-	-

- = Not sensitive

From Table 5, the minimum concentration of methanolic leaf extracts of *Striga hermonthica* that inhibited visible growth was >50.0mg/cm^3 against *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* while that of hexane leaf extract was >50.0mg/cm^3 against *Salmonella typhi* and *Staphylococcus aureus*. The inability of the methanolic and hexane leaf extracts of *Striga hermonthica* to inhibit the growth of bacteria at concentrations less than 50.0mg/cm^3 does not make the leaf extracts of *Striga hermonthica* a good antibacterial agent.

Table 5: Minimum Inhibitory Concentrations (MICs) of Methanolic and Hexane Extracts of *Striga hermonthica* (mg/cm^3)

Bacterial Isolates	**Methanol Extract**	**Hexane Extract**
Salmonella typhi	>50.0	>50.0
Escherichia coli	>50.0	50.0

Staphylococcus aureus	>50.0	>50.0

Conclusion

The phytochemical screening of the methanolic and hexane leaf extracts of *Striga hermonthica* revealed the presence of alkaloids, saponins, cardiac glycosides, tannins, flavonoids, carbohydrates and phenols which justifies the plantfor medicinal and pharmacological importance. The antibacterial susceptibility test showed that the methanolic and hexane leaf extracts of *Striga hermonthica* inhibited the growth of *Salmonella typhi*, *Staphylococcus aureus, and Escherichia coli.* This means that the plants could be used for therapeutic purposes.

TLC profiling of the hexane and methanol extracts of the bioactive phytochemicals gave different R_f values in three different solvent systems. This variation in R_f values provides us with an understanding of the various bioactive compounds due to their polarities. The results of the present study also supplement the folkloric usage of the plant leaves in herbal medicine.

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