

## In Vitro Antibacterial Activity and Phytochemical Evaluation of *Acanthospermum hispidum* (Asteraceae) Root Extracts

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### ABSTRACT

*Acanthospermum hispidum* (Asteraceae) is used in traditional medicine for the treatment of microbial infections. This research was designed to investigate the phytochemicals and antibacterial potentials of the root extract of *A. hispidum*. The powdered root was sequentially extracted by soxhlet extractor using n-hexane, chloroform and methanol. Antibacterial profile of the crude extracts were examined against clinical isolates of the following bacterial; *Escherichia coli* 0:157:H7, *Streptococcus Pneumonia*, Methicilin resistant *Staphylococcus aureus* (MRSA) and *Mannheim* species using standard procedures. The chloroform crude extract show higher activity against all tested organisms with zone of inhibition ranging from 13-30mm at 12.5-100mg/mL concentration. The activity demonstrated can be compared with that of ciprofloxacin at 20 mg/mL with the zone of inhibition ranging between 30-35mm on the organism tested. The phytochemical evaluation reveals the present of the following metabolites; steroids, tannins, flavonoid, alkaloid, cardiac glycoside and saponins. *Acanthospermum hispidum* possessed antibacterial activity which resided in the chloroform extract of the root.

**Keywords:** *Acanthospermum*, Extraction, Phytochemical, Antibacterial, Activity.

### 1.0 INTRODUCTION

Throughout the ages humans have relied on nature for basic needs such as the production of food, shelter, clothing, fertilizers, flavors, fragrances, means of transportation and medicines (Newman *et al.*, 2000). The great ancient Chinese, Indian and North America civilizations provided written evidence of man's utilization of plants for treatment of wide range of diseases (Phillipson, 2001). *Acanthospermum hispidum* popularly known as Kashin Yawo in Hausa native of western Nigeria, is an annual plant in the family of Asteraceae, which is native to tropical America (Anup *et al.*, 2012). It was reported to be an important medicinal plant in India. It possessed antibacterial, abortifacient and teratogenic (Lemonica and Alvarenga, 1994), antiviral (Summerfield and Saalmuller, 1988), antimicrobial activity on the ethanolic extracts of the leaves and flowering parts (Fleischera *et al.*, 2003), antiplasmodial (Sanon *et al.*, 2003), anti-diarrheal

(Abdulkarim *et al.*, 2005), antitumour (Deepa and Rajendran, 2007), anthelmintic (Harekrishna *et al.*, 2010), antitrypsomal (Alicia *et al.*, 2011) and antifungal activity and constitute some plant secondary metabolites such as flavonoids, tannins, saponins, alkaloids etc, which are capable of modifying the activities of cells (Iwalewa *et al.*, 2007). The treatment of infectious diseases with antimicrobial agents continues to present problem in modern day medicine with many studies showing significant increase in the incidences of bacterial resistance to antibiotic (Cuilel, 1994; Sofowora, 1993). Nevertheless, so many scientific investigations has been done on various part of the plant such as leaves and stem bark but no work has been done on the root parts of *A. hispidum* (Anup *et al.*, 2012). This research was designed to evaluate the phytochemical and the antibacterial activity of *A. hispidum* root extracts against human pathogenic strains.

## 2.0 MATERIALS AND METHOD

### 2.1 Materials

The materials utilize for this research work includes *A. hispidum* roots, drugs, reagents, solvents, glassware's and equipment's which are of analytical grade.

#### 2.1.1 Sampling and Sample Treatment

The root of *Acanthospermum hispidum* were collected by first detaching the root from other part of the plant and was taken as the sample. The leaves and stems were also collected for the purpose of identification. Sample collection was done within Sokoto metropolis, Sokoto State, Nigeria in July, 2017. The plant sample was identified at the Herbarium Section of the Department of Pharmaceutics and Pharmacognosy of Usmanu Danfodiyo University Sokoto, with a voucher number (PCG/UDUS/ASTE/0001). The fresh samples were sun-dried for two weeks, ground into a fine powder and stored in a plastic container until required for further use.

#### 2.1.2 Drugs and Organism

Ciprofloxacin (Laborate pharmaceutical, India) was use as positive control against the activity of the crude extracts on the following clinical isolates; *Escherichia coli* 0:157:H7 from diaheric sample, *Streptococcus Pneumonia*, Methicilin resistant *Staphylococcus aureus* (MRSA) and *Mannheim* species while dimethyl sulfoxide was use as the negative control which were obtain from Department of Veterinary Microbiology Research Laboratory of Usmanu Danfodiyo University Sokoto, Nigeria.

## 2.2 METHOD

### 2.2.1 Extraction and Determination of the Phytochemical Constituents

The powder root of *Acanthospermum hispidum* (240g) was sequentially extracted with n-hexane, then sterilized by auto clave at 121°C for 15minute. The mixture was allowed to cool and solidified as presented in appendix IV.

#### 2.2.2.4 Standardization of Overnight Culture

One loaf-hole colony was transferred into 5 cm<sup>3</sup> of nutrient broth then incubated at 37°C overnight.

#### 2.2.2.5 Serial Dilution of the Overnight Culture for Gram-Negative Bacterial

chloroform and methanol for 48hr each using the soxhlet extraction method. The extracts were concentrated in vacuum at 35 °C to evaporate and give n-hexane, chloroform and methanol crude extracts. These were then packed in separate clean dry bottles and stored at room temperature until required for use. The extracts were screened for the presence of steroids, tannins, flavonoid, alkaloid, cardiac glycoside and saponins as described by Evans (2006). The percentage yield of the crude n-hexane, chloroform and methanol extracts were calculated using the formula in Equation 1.

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100 \quad (1)$$

### 2.2.2 Preparation of Medium

#### 2.2.2.1 Nutrient Agar

The culture media was prepared according to the manufacturer's instruction as follows: Nutrient agar (5.6g) was weighed and dissolved in 200 cm<sup>3</sup> of distilled water. It was heated gently over Bunsen burner to completely dissolved, 20 cm<sup>3</sup> of the dissolve agar were dispensed into universal bottles each (10 bottles) which are then sterilized by auto clave at 121°C for 15minute. The temperature was allowed to drop before opening. The agar was allowed to cool and then poured into a clean petri dish, and allow to set.

#### 2.2.2.2 Preparation of Normal Saline

Normal saline (0.9g) was dissolved in 100 cm<sup>3</sup> of distilled water and stirred to dissolve completely. Nine (9) cm<sup>3</sup> of the mixture were dispensed into a clean test tube each and four (4) cm<sup>3</sup> of the mixture each into another clean test tube.

#### 2.2.2.3 Preparation of Nutrient Broth

Nutrient broth (1.3g) was dissolved in 100 cm<sup>3</sup> distilled water and stirred to dissolve completely. 5 cm<sup>3</sup> were dispensed into a clean test tube each and Four test tubes were set, three of which containing 9 cm<sup>3</sup> each and one containing 4 cm<sup>3</sup> normal saline. 1 cm<sup>3</sup> of the overnight culture was pipetted into the first 9 cm<sup>3</sup> normal saline to make 0.1 cm<sup>3</sup> concentration, from this 0.1 cm<sup>3</sup>, another 1 cm<sup>3</sup> was transferred into the second 9 cm<sup>3</sup> normal saline to make 0.01 cm<sup>3</sup>, from this 0.01 cm<sup>3</sup>, exactly 1 cm<sup>3</sup> was transferred into the third 9 cm<sup>3</sup> normal saline to make 0.001 cm<sup>3</sup>, from this 0.001 cm<sup>3</sup> another 1 cm<sup>3</sup> was

transferred into 4 cm<sup>3</sup> normal saline to make 0.005 cm<sup>3</sup> which was used for floatation.

### 2.2.2.6 Serial Dilution of the Overnight Culture for Gram-Positive Bacterial

Three test tube each containing 9 cm<sup>3</sup> normal saline were prepared, using a graduate pipette, 1 cm<sup>3</sup> of the overnight culture was transferred into the first 9 cm<sup>3</sup> normal saline to make 0.1 cm<sup>3</sup>, again 1 cm<sup>3</sup> taken from this 0.1 cm<sup>3</sup> into the second 9 cm<sup>3</sup> normal saline to make 0.01 cm<sup>3</sup> and then from this 0.01 cm<sup>3</sup>, another 1 cm<sup>3</sup> was transferred into the third 9 cm<sup>3</sup> normal saline to make 0.001 cm<sup>3</sup> which was used for floatation.

### 2.2.2.7 Antibacterial Activity (Agar well Diffusion Method)

The standard inoculum (0.1 mL, 0.5 Mcfarland turbidity standard = 1.0x10<sup>8</sup> cfu/mL) of each test bacteria was spread into sterile Muller thin iron broth Agar plates so as to achieve even growth. The plates

were allowed to dry and a sterile corks borer (8.0 mm diameter) was used to bore wells aseptically in the Agar plates. The extracts were prepared and severally diluted using 10% dimethylsulphoxide (DMSO) to achieve different concentrations of 12.5, 25, 50 and 100 mg/L respectively for each extracts. Subsequently, 200 µL of each concentration of the extract prepared was introduced into the wells earlier bored in the Agar plates. The plates were kept on the bench for 1h and then incubated at temperature of 37 °C 24 h growth. Ciprofloxacin was used as a positive control (20mg/mL was prepared by dissolving 200 mg of the drug powder in 10 mL of DMSO) and introduced at the center hole of each plate. Antibacterial activity of the extracts were determined by measurement of zones of inhibition produced around the wells (meter rule was placed in 3 different directions and an average was calculated). The presence of zones of inhibition produced around the wells indicates activity. (Lino and Deogracous, 2006).

## 3.0 RESULT AND DISCUSSION

### 3.1 Result

**Table 1. Percentage Yield of *A. hispidum* Root extracts**

Solvent	Weight of extracts (g)	Yield (%)	Color	Weight of residue(g)
n-hexane	13.688	5.557	Orange yellow	246.312
Chloroform	3.162	1.231	Light green	256.838
Methanol	19.926	8.300	Dark brown	240.074
Total	36.776	15.088		

**Table 2. Antibacterial Activity Test of *A. hispidum* Root extracts**

Extracts	Organism					+ve control	-ve control
		100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	Ciprofloxacin (20 mg/mL)	10 % DMSO
Methanol	<i>E. coli</i>	19±0.71	17±0.71	15±0.71	10±0.71	30±0.71	8±1.41
	<i>S. Pneumonia</i>	18±1.41	15±0.71	13±1.41	12±1.41	30±0.71	8±1.41
	MRSA	21±1.41	12±0.71	10±0.71	10±1.41	30±0.71	8±1.41
	<i>Mannheim</i> spp.	20±1.41	15±0.71	11±1.41	10±1.41	30±0.71	8±1.41
Chloroform	<i>E. coli</i>	20±1.41	15±2.12	08±1.41	8±1.41	30±0.71	8±1.41
	<i>S. Pneumonia</i>	20±1.41	19±1.41	15±2.12	12±0.71	30±0.71	8±1.41
	MRSA	25±0.71	19±2.12	15±2.12	13±0.71	30±0.71	8±1.41
	<i>Mannheim</i> spp.	30±0.71	25±0.71	15±2.12	11±0.71	35±0.71	8±1.41
n – hexane	<i>E. coli</i>	21±1.41	21±1.41	19±1.41	15±2.12	30±0.71	8±1.41
	<i>S. Pneumonia</i>	21±0.71	17±1.41	15±2.12	11±1.41	30±0.71	8±1.41
	MRSA	17±1.41	16±1.41	15±2.12	13±0.71	30±0.71	8±1.41
	<i>Mannheim</i> spp.	15±2.12	14±1.41	13±1.41	12±0.71	31±0.71	8±1.41

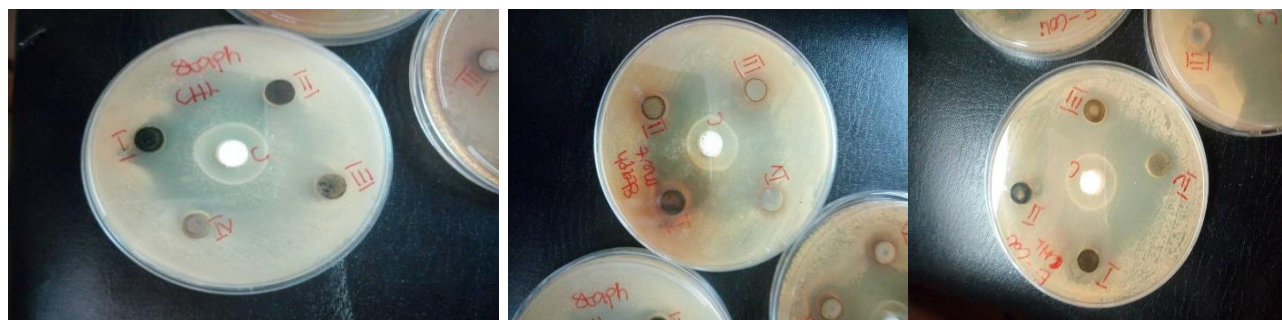
Zone of Inhibition in (mm)

**Key:** Zone of inhibition ≤ 8mm is considered inactive

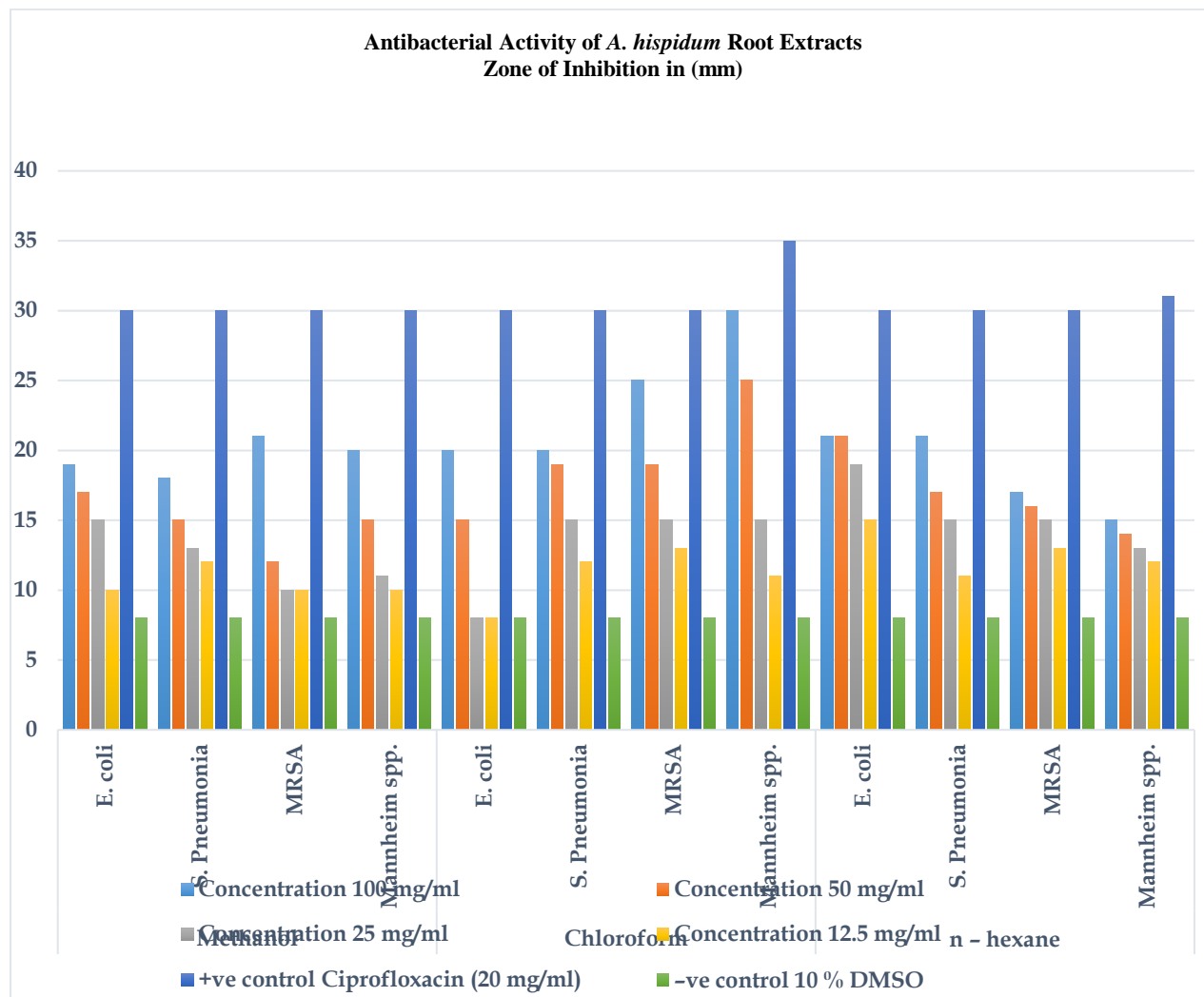
**Table 3: Phytochemical Evaluation of *A. hispidum* Root extracts**

Metabolite	Extracts			Test
	n-hexane	Chloroform	Methanol	
Tannins	–	–	+++	Ferric chloride Test
Saponins	–	–	+++	Frothing Test
Steroids	+++	+	+	Salkowski's Test
Flavonoids	–	–	+++	Alkaline Test
Alkaloids	–	–	+++	Hager's Test
Cadiac Glycoside	+++	+	++	Keller-killiani's Test

Key: + = slightly present; ++ = averagely present; +++ = highly present; – = completely absent



**Figure 1: Chloroform extracts and control at the center**



## 4.0 DISCUSSION

### 4.1 Percentage yield of *A. hispidum* Root Extracts

Many naturally occurring compounds found in plants have been shown to possess antimicrobial activities and could thus serve as a source of traditional drugs (Kim *et al.*, 1995). As shown in **Table 1**, n-hexane had percentage yield of 5.56%, chloroform had 1.23% while methanol had percentage recovery yield of 8.3%. This is perhaps as a result of their difference in polarity. N-hexane being less polar is expected to extract less followed by chloroform being moderately polar while methanol being a polar solvent is expected to extract more. This agrees with the report of (Abdulkarim *et al.*, 2005). The aqueous methanol extracts of *Acanthospermum hispidum* (leaves and stem-bark) has percentage recovery of 7.3%. This may be as a result of the chemical composition of the

plant and the way they are held in the plant cell wall matrix (Dutta and Evans, 2005). The highest yield is seen in the methanol extract (8.3%) and this is due to its high polarity as seen in the case of methanol fraction. The plant is richer in polar compounds because methanol extracts more bioactive polar compounds which is useful in ethnomedicine.

### 4.2 Antibacterial Activity of *A. hispidum* Root Extracts

The antibacterial activity of *A. hispidum* Root Extracts is presented in Table 2; the extracts show activity against the following bacterial isolates (*Escherichia coli* 0:157:H7, *Streptococcus Pneumonia*, Methicilin resistant *Staphylococcus aureus* (MRSH) and *Mannheim* Species). The plant has recently been a research focus as potential source

for drug development due to its antibacterial potential (Alicia et al., 2011). *Acanthospermum hispidum* have demonstrated antibacterial activities against the tested clinical isolates, thus justifying its use in traditional medicine for treating different diseases associated with the tested isolates, as it could also serve as a new and cheaper alternative for antibacterial drugs. All the extracts show activity on all test organism at different concentration (12.5, 25, 50 & 100mg/mL) which was in line with procedure used by (Harekrishna et al., 2010) where 5-25mg/mL of each extracts were used; chloroform extract is inactive on *Escherichia coli* 0:157:H7 at 12.5 & 25mg/mL concentration which is a gram-negative bacterium as reported in (Alicia et al., 2011) findings. Chloroform extract showed more activity with zone of inhibition ranging between 13-30mm than the other extracts. This may be as a result of the synergistic behavior of the plant extracts. Therefore the trend of the zone of clarity is concentration dependent as seen in **figure 1** and **2** and comparing the activity of the extracts with an antibiotic drug ciprofloxacin with zone of inhibition ranges between 30-35mm, it implies that *A. hispidum* root extracts has antibacterial activity resided in the chloroform extracts and if subjected to further screening and

## 5.0 CONCLUSION

The extracts of *A. hispidum* root yield more when extracted with methanol and the methanol extract reveal the present of all the metabolites (steroids, tannins, flavonoids, alkaloids, cardiac glycoside and saponins). The plant root extracts show antibacterial

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analysis, it can be recommended to be used for development of new antibiotic drugs as was reported by (Fleischera et al., 2003).

## 4.3 Phytochemical Evaluation of *A. hispidum* Root Extracts

The result of the phytochemical evaluation of the root of *A. hispidum* presented in Table 3 reveal the presence of steroids, tannins, flavonoids, saponins, alkaloids, and cardiac glycoside. These bioactive compounds have been demonstrated to be responsible for the antimicrobial activity of medicinal plants (Mathias et al., 2007). These constituents were found to be similar to those reported for most medicinal plants. However, chloroform fraction of the root of *A. hispidum* shows the presence of only steroids and cardiac glycoside. Presence of steroids in the root of *A. hispidum* was also reported by (Isha et al., 2007) and presence of cardiac glycoside was reported by (Harekrishna et al., 2010). Methanol extract showed the presence of all the phytochemicals tested for. It is therefore recommended to isolate, purify and characterize these chemical constituents.

activity in concentration dependent on different pathogenic strains which reside in the chloroform extracts. Therefore, it can be used for the development of new antibiotic drugs. Further studies are needed for confirmation of antibacterial action by isolating pure chemical constituents and also identify which compound is responsible for antibacterial action of *A. hispidum* root.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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