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Phytochemical and biological activities of leaf extracts of *Cnidoscolus aconitifolius*

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Abstract
Preliminary phytochemical investigation of sap, aqueous and ethanolic leave extract of *Cnidoscolus aconitifolius* revealed the presence of saponin, glycosides, flavonoids, tannins, phenolics, protein, carbohydrate, reducing sugar and protein. The ethanolic extracts of the leave gave a clear zone of inhibition against the growth of the test bacteria (*Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa*). The sap extract showed considerably low zones of inhibition while the aqueous extract of the leave had no inhibition at all. Both ethanolic and aqueous extract had great antifungal property against tested fungi (*Aspergillus Niger, Aspergillus flavus*).

Keywords: Phytochemicals, *Cnidoscolus aconitifolius*, antifungal, antibacterial

Introduction
Medicinal plants contain organic compounds which could produce definite physiological action on the human body. These bioactive substances could include tannins, alkaloids, terpenoids, steroids and flavonoids etc. (Edeoga *et al.*, 2005) [5]. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas (Vasu *et al*., 2009) [16]. In Nigeria many spices are used as food and medicine. Medicinal plants play a significant role in the health of humanity (Sofowora, 1993) [11]. The medicinal value of these plants lies in their phytochemical content. Phytochemicals are chemical constituents of plants and exert physiological action on man and animals (Subhashini *et al.*, 2010) [12]. The active principles isolated, have provided leads in the development of several lifesaving drugs, which are in use today. Different civilizations have developed their own indigenous system of medicines, across the various ages (Gupta *et al.*, 2010). *Cnidoscolus aconitifolius*, commonly known as chaya plant, is a large, fast growing leaf perennial shrub that is believed to have originated in Yucatan Peninsula of Mexico, (Grubben *et al.*, 2004). To the best of our knowledge little or no work has been done on the plant *C. aconitifolius* in Taraba, Nigeria. This work is designed to enrich the available scientific data on the phytochemistry and antimicrobial activities of *C. aconitifolius* leaves. This paper reports the phytochemistry and antimicrobial activities of *C. aconitifolius* leaves on some bacterial and fungal isolates.

Materials and Methods
Collection and Preparation
Fresh leaves of the sample were collected from their natural habitat in Wukari Local Government Area of Taraba State. The leaves stem were washed under running tap water to eliminate dust and other foreign particles and then dried. After the leaves were shade dried and blended using mortar and pistol, extraction was carried out with a modified method of (Swain 1988) in which 20g of powder dried leaves was soaked in 200ml of ethanol and kept for 72hrs. After 72hrs it was filtered. The same procedure was carried out for aqueous extraction process.

Phytochemical screening
Phytochemical screening was carried out using the method described by Tiwari, *et al.*, 2011, Ushie *et al*., 2013 and Ushie *et al*., 2019 [14].

Test for Tannins
To each extract, 2 drops of 5% $FeCl_3$ were added. A greenish precipitate indicated the presence of tannins in the extract.

Test for Saponins
2cm^2 of aqueous extracts in a test tube was vigorously shaken for two minutes. Frothing observed in two extract tested indicate the presence of saponin.

Test for Flavonoid
To 3ml of extracts 1ml of 10% of NaOH was added to the extract, the extract turns yellow.

Test for Alkaloids
To 1ml of each extract 2 drops of Hager's reagent was added. A reddish brown precipitate observed indicate the presence of alkaloid in the extract.

Test for Anthraquinones
About 1ml of extract was shaken with 2cm^3 of benzene and 4cm^3 of 10% NH_3 solution was shaken. The presence of a pink color in the ammonia solution (lower layer) phase indicates the presence of anthraquinones.

Test for Glycosides
To 10ml of the extracts in a test tube, 10ml of 50% H_2SO_4 was added. The mixture then heated in boiling water for 15 minutes. 10mlof Fehling's solution was added and mixture boiled. A brick red precipitated was observed in the extracts showing the presence of glycosides.

Test Micro-organisms
The organisms*: Staphylococcus aeruginosa, Salmonella typhi, Escherichia coli, Aspergillus Niger, Aspergillus Flavus* and *Penicillium* spp were gotten from the Microbiology department, Federal University Wukari, Taraba state.

Sterilization of materials
All glass wares used in this study were washed with detergent, rinsed and sterilized in a dry ventilated oven at 160 ºC for 2 hours. All media were sterilized by autoclaving at a temperature of 121 ºC and 15 psi for 15 minutes. The scalpel, cork borer, inoculating needle were sterilized by dipping them into 70% ethanol and passing them over a Bunsen burner flame until red hot.

Media preparation
The medium used was Mueller Hinton Agar (MHA) prepared according to the manufacturer's instruction. About 38 g of powdered PDA medium was dissolved in 1 L of sterile distilled water and sterilized by autoclaving at 121 °C at 15 psi for 15 min and allowed to cool before pouring carefully into 100 sterile Petri dishes. The Petri dishes that contained the medium were incubated for 24 h at room temperature (37 °C) to check for sterility before use as described by Cheese brough (2004) [3].

Antimicrobial Activity
Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of anti-bacterial activities of the plant samples (Perez *et al.,* 1990). A sterile swab was used to evenly distribute bacterial culture over the appropriate medium (Mueller Hinton Agar). Muller Hinton agar was prepared as per the instructions by the manufacturer. The plates were allowed to dry for 15 minutes before use in the test. Once the media solidified then it was then inoculated with the bacteria species. The media was then punched with 6 mm diameter hole and was filled with extract; a pipette was used to place 30ul of the extract into the well. A total of two extracts was used on a particular bacterial species; with a total of three plates used for each extract including. The positive control was the same on all isolate. The plates were incubated at 37C for 24 hours after which they were examined for inhibition zones. A ruler was used to measure the inhibition zones.
Effect of plant extract on mycelia growth of the test fungi was studied using the food poisoning techniques (Sangoyomi, 2004) [10]. One milliliter of each plant extract was dispensed per petri dishes and 9ml of the media (molten PDA) was added to each of the petri dishes containing extract and carefully spread evenly over the plate, this gave rise to PDA–extract. This was used for the inhibition of mycelia growth. The plates were gently rotated to ensure even dispersion of the extracts. The agar extract mixture was allowed to solidify and then inoculated at the center with a 4mm diameter mycelia dish obtained from the colony edge of 7-day old pure cultures of each of the four test fungi. Each treatment consists of three replicates. The negative control set up consist of blank agar plate (no extract) inoculated with the test fungi as described above. Petri-dishes dispensed with molten PDA and one ml of ketoconazole dissolved in distilled water inoculated with each test fungus served as the commercial fungicides. All the plates were incubated at 28 ºC for 5days and examined daily for growth and presence of inhibition. Colony diameter was taken as the mean growth along two directions on two pre-drawn perpendicular lines on the reverse side of the plates. The effectiveness of the extract was recorded in terms of percentage inhibition, which was calculated according to the method described by Whips (1987).

$$\text{Percentage inhibition} = R1 - R2 \times \frac{100}{R2}$$

Where R1 is the farthest radial distance of Pathogen in control plate while R2 is the farthest radial distance of Pathogen in extract incorporated agar plates.

Table 1: Results of phytochemical analysis

Constituent	Aqueous extract	Ethanolic extract
Tanniins	+	+
Saponins	+	+
Flavonoids	+	-
Alkaloids	-	+
Steroids	-	-
Phenolics	+	+
Proteins	-	-
Carbohydrates	+	-
Reducing sugar	+	+
Glycosides	+	-

Keys:+ = Present, -= Absent

Table 2: Sensitivity test for fungi

Organism	*A. niger*	P. Spp	*A. flavus*
Aqueous extract	70.93±3.93	85.66±1.77	68.22±1.69
Ethanol extract	82.56±5.82	95.74±1.77	76.91±2.99
Positive control	89.92±1.15	78.00±2.00	86.00±0.00

Table 3: Sensitivity test for Bacteria

Organisms	*E. coli*	*S. typhi*	*P. aeruginosa*
Aqueous extract	0.00±0.00	0.00±0.00	0.00±0.00
Ethanolic extract	21.67±2.08	16.67±1.53	18.33±1.53
Sap extract	13.00±3.00	8.00±2.00	8.67±2.08
Ciprofloxacin	26.00±1.00	28.67±0.58	29.00±1.00

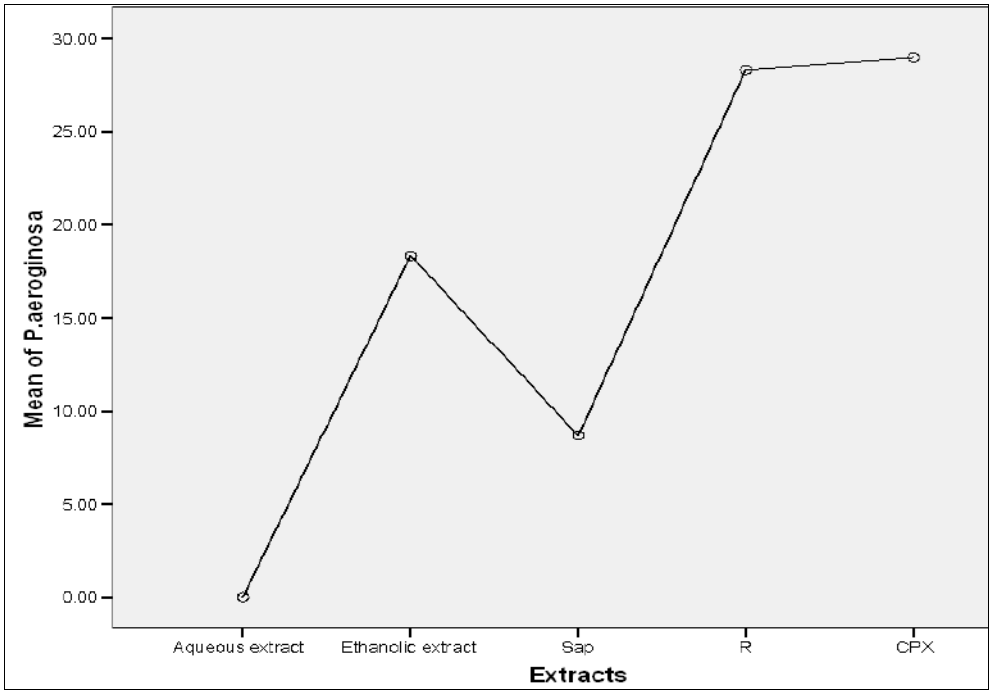

Fig 1: Mean value of *P. aeruginosa*

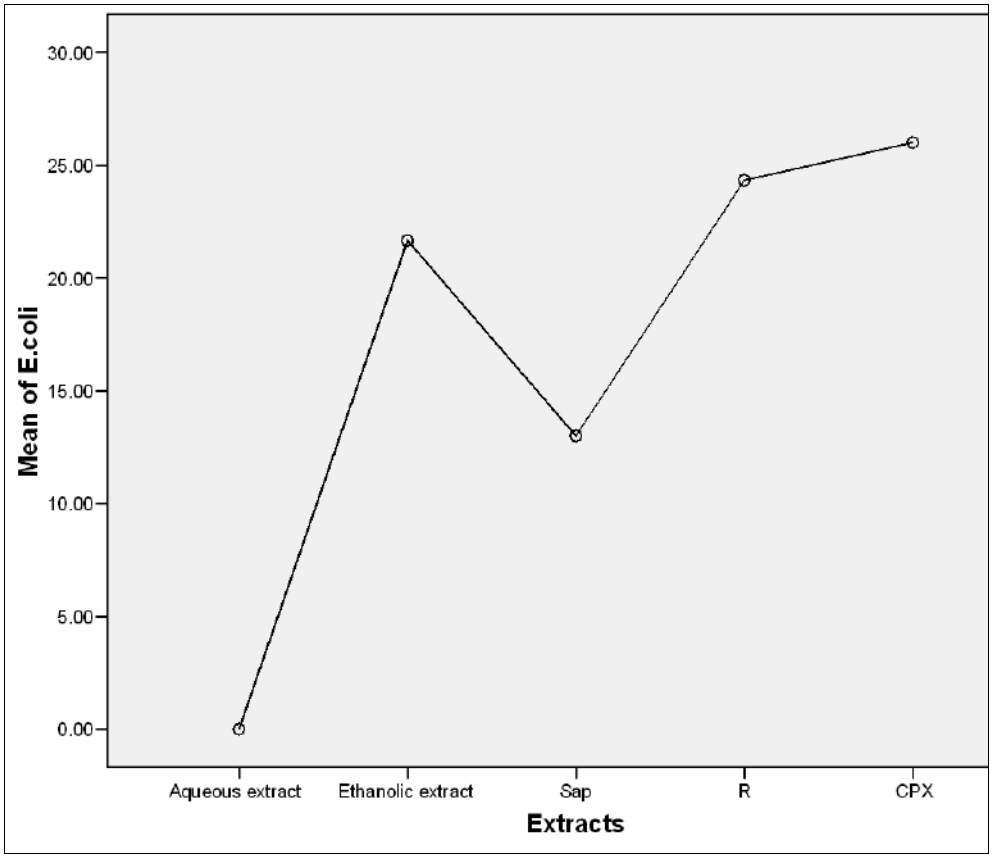

Fig 2: Mean value of *E. coli*

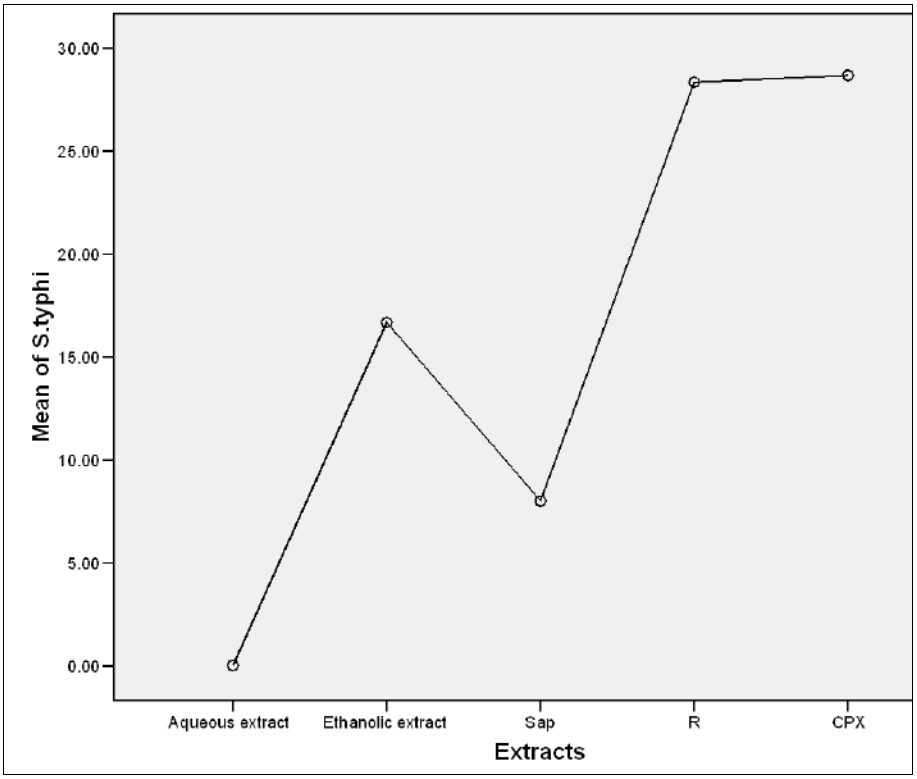

Fig 3: Mean value of *S. typhi*

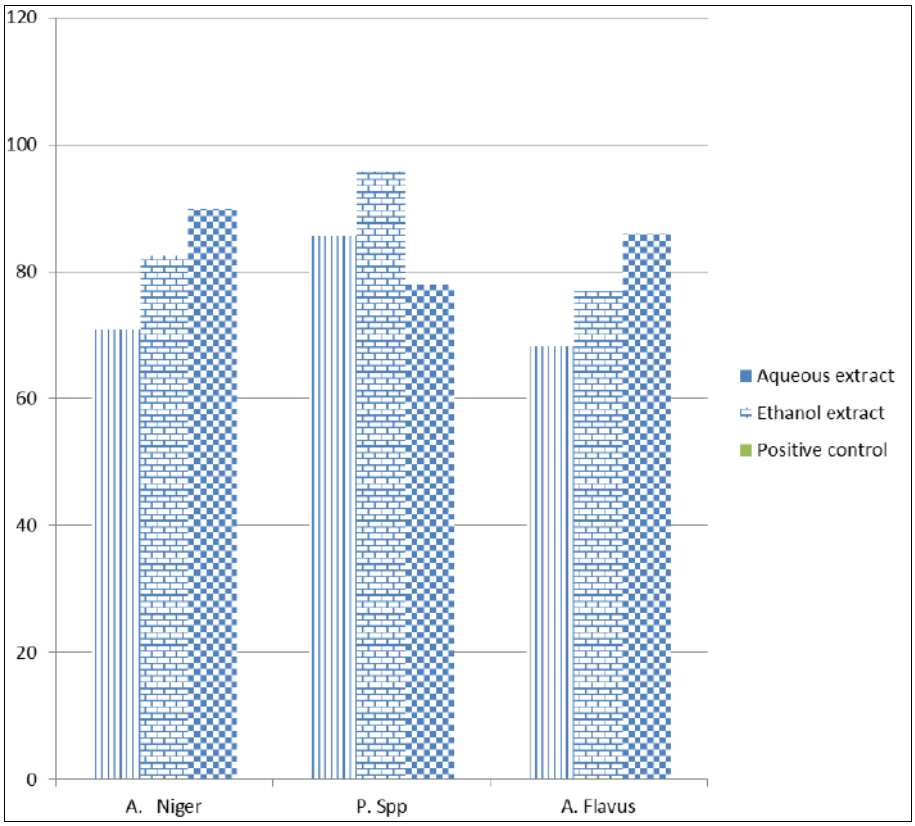

Fig 4: Sensitivity test for fungi

Discussion

The phytochemical analysis carried out on the aqueous extract and ethanolic extract showed the presence of some bioactive compounds in the plant. In the two forms of extract, ten bioactive constituents were tested for, out of which six were present in the aqueous extract and five in the ethanolic extract (Table 1). Analysis of tannins in the two extracts was positive. Presence of tannins suggests the ability of this plant to play a major role as antidiarrhoec and antihaemorrhagic agent (Asguith and Butler, 1986) [1]. Saponins was positive for both extracts. This compound has been shown to have immense significance as antihyper cholesterol, hypotensive and cardiac depressant properties (Trease and Evans, 1985., Price, 1987) [15]. Hence this plant

could be suitable for these purposes. Cardiac glycosides showed positive results only in the ethanolic extracts with no clear intensity indication in the extracts. The cardiac glycosides have been used for over two centuries as stimulants in cases of cardiac failure (Trease and Evans, 1985; Olayinka *et al*., 1992) [15, 8]. This perhaps justifies the already locally established function of the plant in the treatment and management of hypertension. It was also found that alkaloids were only present in ethanolic extracts while absent in the aqueous extract. The main reason that can be adduced for this is the mode of extraction. On this premise it will be advisable to extract the leaf of *C. aconitifolius* with ethanol in an attempt to exploit its detoxifying and antihypertensive properties since alkaloids is known to be effective for these purposes (Trease and Evans, 1978; Zee- cheng, 1997) [15, 19]. However flavonoid was found to be positive only in the aqueous extracts while in the ethanolic extract, this test was negative. This is also an indication that this compound can only be derived with the aqueous extract of the plant. The presence of phlobatannins suggests the diuretic property of the plant (Okuda, 1991) [7]. The foregoing would suggest the possible utilization of *C. aconitifolius* as diuretic agent.

The antimicrobial investigation carried out on the sap, ethanolic extracts of the leaves showed a broad spectrum of activity as presented in Table 1. They showed appreciable activity against *S. typhi, P. aeruginosa* and *E. coli.* While the aqueous extract of the leave exhibited no activity against the different strains of bacteria used. Ethanolic extracts showed considerable effectiveness compared to some of the commercial antibiotics (Ciprofloxacin and Ceftriaxone). Ethanolic extract of the leave exhibited 21.67±2.08 inhibition on *E. coli*, 16.67±1.53 on *S. typhi* and 18.33±1.53 on *P. aeruginosa*. The sap extract exhibited 13.00±3.00 inhibition on *E. coli*, 8.00±2.00 on *S. typhi* and 8.67±2.08 on *P. aeruginosa*. The mean zone of inhibition of ethanolic extract on *E. coli* in this study is higher than that reported by Adeniran *et al.,* (2013). They treated *E. coli* with ethanolic extract of *C. aconitifolius* leaves. The differences may be attributed to the method of extraction. The mean zone of inhibition of ethanolic extract on *S. typhi* is higher than that reported by Awoyinka *et al*. (2007) [2].

The aqueous and ethanolic extract showed great antifungal activity on the test organisms. Aqueous extract inhibited 70.93% growth of A. Niger, 85.66% on Penicillin spp and 68.22% on A. Flavus. To some degree it showed effectiveness compared to commercial fungicide (ketoconazole) used in this experiment. Ethanolic extract was very effective against the test organisms; it has inhibition range of between 76.91 to 95.74%. It tends to be more effective than the control.

Conclusion

This study revealed the ability of *C. aconitifolius* to inhibit bacteria; thereby suggesting that it could serve as a broad spectrum antimicrobial agent and paving way for further investigation to identify the active compounds responsible for the plant biological activity with the required Minimum Inhibitory Concentration (MIC) for Use in drug development for safe health care delivery. It can be concluded that extracts from the aerial parts of *Cnidoscolus aconitifolius* have antimicrobial and antifungal activities, which support the ethno medicinal uses of the plant for the treatment of back pain, kidney stones, biliousness, jaundice, boils, warts, pimples or other skin conditions, kidney pain, gum disease and toothache (Obi and Onuoha, 2000).

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