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Phytochemical Screening and *In Vitro* Antibacterial Activity of Methanol and Aqueous Extracts of *Acalypha Racemosa* Leaves

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Abstract - Drug resistance microorganisms have posed enormous public concern there is therefore a need to screen plants that could have the potency in the control of the pathogenic organisms. The extracts obtained from the *Acalypha racemosa* were screened for phytochemical properties and *in vitro* investigation of methanol and aqueous extracts. The phytochemical screening revealed the presence of saponins, phlobatanins, flavonoids, and alkaloids. Glycoside and cardiac glycoside, tannins and sterols were in traced amount. The *in vitro* antibacterial investigation of the crude extract was conducted. The studies showed that the plant contains compounds that have antibacterial activity. The extracts revealed greater potential against the test bacteria with zones of inhibition ranging from 20-30 mm for methanol extract in the order *Escherichia coli* > *Klebsiella pneumoniae* > *Proteus mirabilis* > *Bacillus subtilis* > *Pseudomonas aeruginosa* and 18-27mm for aqueous extract in the order *Escherichia coli* > *Bacillus subtilis* > *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* > *Proteus mirabilis*. All the bacteria were more susceptible to methanol extract than the aqueous extract as indicated in the zones of inhibition with exception of *Pseudomonas aeruginosa* in which there was no inhibition. The aqueous extract had effect on all the test bacteria although having zones of inhibition lower than the methanol extract. The minimum inhibitory concentration (MIC) of both extracts range from 12.5-50 mg/ml and the minimum bactericidal concentration (MBC) range from 25-100 mg/ml. *Escherichia coli* was more susceptibility to both extracts and less was seen in the case of aqueous extract against *Proteus mirabilis*. The screening of the plant has revealed its potency in the treatment of pathogenic infections that may be caused by these pathogens.

Keywords: Drug resistance; Pathogenic organisms; Antibacterial; Zones of Inhibition; Microorganisms, MIC and MBC

Introduction

The used of plants and herbs in the treatment of pathogenic diseases in most of the developing world. Researchers around the world have been engaged in the findings and formulation of new drugs; this is as a result of every day challenge of drug resistivity by microorganisms (Maureer-Gimes *et al.*, 1996). Incidents due to drug resistant microorganisms and the emergence of unknown diseases causing microbes, posed enormous global public health concern (Iwu, 1993). This resistance can be due to inappropriate administration of drugs commonly used on the treatment of these infectious diseases (Afolayan and Aliero, 2006). Furthermore some antibiotics have serious undesirable side effects which limit their applications,

so there is serious need to develop new antimicrobial agents that are very effective with minimal unwanted side effects and higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs (Ezekiel *et al.*, 2009). Also, there has been little or no report of any form of microbial resistance during the use and administration of herbal medicines (Stephen *et al.*, 2009). More importantly in Africa, particularly West Africa, new drugs are often beyond the reach of the poor such that up to 80% of the population use medicinal plants as remedy against infections and diseases (Kirby, 1996; Hostettmann and Maston, 2002). The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs. There is a need for everyday research on the development and formulation of new drugs to counterfeit/curtail resistant of pathogenic microorganisms to antibiotics (Hammuel *et al.*, 2011). Apart from employing the medicinal plants (herbs) for treatment of pathogenic disease it can be used to treat various ailments that are not directly caused by microorganisms such as jaundice, fever, rheumatism, epilepsy (Igbinosa *et al.*, 2009). The world health organization has recorded over 20,000 species of the plant *Acalypha* with medicinal properties providing treatment for such complaints as pneumonia, ulcers, diarrhoea, bronchitis, cold and diseases of the respiratory tract (Yebpella *et al.*, 2011).

Acalypha plant is a genus of the family Euphorbiaceae and grows as an annual bedding plant (Oladunmoye, 2006). There are about 570 species, a large proportion of which are weeds that is open to forest zones of Nigeria and it is widely spread across tropics of Africa for example the macrophylla, hoffamanii, godseffiana, macafeeana, hispida, marginata and racemosa are peculiar cultivars within Nigeria (Oladunmoye, 2006; Yusha'u *et al.*, 2008; Iniaghe *et al.*, 2009). Euphorbiaceae species are large, fast-growing, evergreen shrub provides a continuous splash of colour in the landscape with the bronze red to muted red, 4 to 8 inch long, heart-shaped leaves available in varying mottled combinations of green, purple, yellow, orange, pink, or white, depending upon cultivar (Gilman, 1999). Others are ornamental plants or called as horticultural shrubs (Iniaghe *et al.*, 2009).

Some of the species *Acalypha* are known to be used in traditional medicine. Musa *et al.* (2000) reported the medicinal importance of the *A. racemosa* to have exhibited the antibacterial activity. Iniaghe *et al.* (2009) also reported the use of the decocted leaf of the plant by traditional practitioners in Ilorin metropolis, Nigeria to treat liver disorders and other disease condition which resulted in jaundice. The aqueous extract of the leaves of *A. racemosa* has also been reported to have hepatoprotective effect in carbon tetrachloride induced liver of rats (Iniaghe *et al.*, 2009). The aim of this research was to screen *A. racemosa* for bioactive compounds and antimicrobial property.

Materials and Methods

Preparation of the extract

Fresh leaves of *A. racemosa*, were collected from a parish house Zaria and were identified by the Department of Botany, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria. The collected leaves were dried at 40°C using Gallenhamp drying cabinet. The dried leaves were ground into powdered form using mortar and pestle. Three hundred grammes (300 gm) of the powdered sample was dissolved in 400 ml each of distilled water and methanol, the process was allowed to stay for 2 days to ensure proper extraction. The mixture was then filtered using Whatman No. 1 filter paper. The solvents were evaporated using water bath at temperature of 50 °C in order to concentrate the extract.

The phytochemical screening of the plant extracts

Conventional standard protocols (Sofowara, 1993; Trease and Evans, 1989) for detecting the presence of different chemical constituents in plant extract were employed. The tests for the secondary metabolites viz. tannins, alkaloids, saponins, glycosides, flavonoids, cardiac glycoside, phlobatannins, and steroids were carried out.

Test for chemical contents and organism

Tanning test (FeCl₃ test): About 0.5g of the dried powdered sample was dissolved in 20 ml of distilled water in a test tube, boiled and filtered. To the filtrate few drops of 1.0% Iron II chloride solution was added and observed for a blue-green precipitate. *Flavonoid test:* For flavonoid test was conducted in two different ways or methods: To 1.0 ml of the extract 1.0 ml of 10% lead acetate was added, and observed for yellow precipitation. And to 1.0 ml of the extract of the plant 1.0ml of dilute NaOH was added and observed for precipitation. *Alkaloids test:* Two different reagents (Wagner's and Mayer's reagents) were used to ascertain the presence of alkaloids in the sample: Two (2.0) ml of the mixed and shook with 10.0 ml of 2% HCl on a steam bath and filtered. The filtrate was divided into two equal portions. To the first portion Wagner's reagent was added in drops and observed for a brown precipitate. To the second portion of the filtrate, Mayer's reagent was added also in drops and observed for white to yellow or creamy with precipitate. *Saponins test (frothing test):* One (1.0) ml of extract was boiled with 5.0 ml of distilled water for five (5) minutes and decanted while still hot. Then was filtered, and 1.0 ml of the filtrate was diluted with 4.0 ml of distilled water and was shook vigorously. It was observed on standing for stable froth. *Glycoside test (FeCl₃ test):* A total of 5.0 ml of the extract in a test tube 2.5 ml of dilute H₂SO₄ acid was added and boiled in water bath for 15 minutes. It was cooled and neutralized with 20% KOH solution. Into the mixture of the extract, acid and base, 5 ml of a mixture of Fehling's solution A and B were added, boiled and observed for reddish brown colour at the interphase. *Cardiac glycoside test:* To 1.0 ml of the extract 8% of methanol was added and mixed with 1.0 ml of 2% solution 3, 5-dinitrobenzoic acid in 95% alcohol. The solution was made alkaline with 5% NaOH. It was observed for violet colour which faded through reddish brown to brownish yellow. *The test organisms:* the test organisms for the study are bacteria which include the *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli* and *Klebsiella pneumoniae*. These organisms were obtained from patients in the Department of Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. They were transported in slants to National Research Institute for Chemical Technology, Zaria, Nigeria.

The antimicrobial screening of the extracts

The antimicrobial activities of the plant extracts were determined using agar well diffusion method as described by Reuben *et al.* (2008). The bacterial and the fungal isolates collected in prepared slants of nutrients agar were sub-cultured into prepared nutrients broth and incubated at 37°C for 24 hours and standardized to 0.5 Mc-Farland scale (1.5×10^8 CFU/ml) in a prepared normal saline. The cell suspensions were swabbed onto nutrient agar plates MacConkey agar for *Klebsiella pneumoniae*. Wells were then bored into the plates of the inoculated organism using sterile cork-borer of 6mm in diameter. 1.0 g of each of the methanol and aqueous concentrated extracts were constituted into 10 ml of their respective solvents of extraction (methanol and water) to obtain the initial concentration of 100 mg/ml. Then 1.0 ml of the concentration of the both extracts was introduced to fill the wells created, allowed to stand for 30 minutes at room temperature for proper diffusion and then incubated at 37 °C for 24 hours in an incubator. Controls were also set up in parallel, but using the solvents of extractions only. After the incubation 24 hours the plates were observed for zones of inhibition and recorded in millimetre (mm).

Minimum inhibitory concentration (MIC)

The MIC of the crude extracts was determined using the method as given by Yebpella *et al.* (2011); 100 mg/ml of each of the extracts were reconstituted into nutrient broth in test tubes and the 100 mg/ml was taken as the initial concentration. Four more tubes of 5ml nutrient broth were set up and 5 ml of 100 mg/ml of the extract was taken and used for two-fold dilution of the four tubes of nutrient broth forming concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml.

Normal saline was used again to prepare turbid suspensions of the microbes; the dilution was done continuously and incubated at 37 °C for 30 minutes. Until the turbidity matched that 0.5 Mcfarland's standard by visual comparison. At that point the number of cells is assumed to be 1.5×10^8 CFU/ml. Of the cell suspension 0.1ml was inoculated into each of the tubes with the varied concentrations of extracts. All the tubes were incubated at 37 °C for 24 hours. The tube with the lowest concentration which has no growth (turbidity) of the microbes was taken to be the minimum inhibitory concentration (MIC).

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of the plant extract against the microbes was determined using the method of Ravikumar *et al.* (2007). The tubes of the MIC that showed no growth of the microbes were sub-cultured by streaking using sterile wire loop on nutrient agar plates, MacConkey agar plate for *Klebsiella pneumoniae*. The plates were incubated at 37°C for 24 hours. MBC was the lowest concentration of the extract that showed not any colony growth on the agar plates. It was obtained and recorded.

Results and Discussion

The phytochemical screening of both the extracts revealed the presence of saponins, alkaloids, flavonoids. Steroids and tannins were found in methanol extract in trace amounts as presented in Table 1. In the screening for the biological active compounds there was no information about glycoside and cardiac glycoside. These results agreed with the report of Iniaghe *et al.* (2009). These secondary metabolites exert antimicrobial activity in different mechanism; flavonoids have also exhibited a wide range of biological activities; such as antimicrobial, antioxidant, anti-inflammatory, anti-agionic, analgesic, anti-allergics and cytostatic properties (Hodek *et al.*, 2002). Tannins has been found to react with praline-rich protein to form irreversible complexes (Shinwari, 2010), resulting in the inhibition of cell protein synthesis. Herbs that have tannins as their major components are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003). The presence of saponins lends credence to the use of this plant in managing inflammation (Quinlan *et al.*, 2000).

Table 1. The phytochemical screening of the extract

No.	Components	Methanolic extract	Aqueous extract
1.	Saponins	+	+
2.	Flavonoids	+	+
3.	Alkaloids	+	+
4.	Sterols (Steroid nucleus)	T	+
5.	Cardiac glycoside	-	-
6.	Tannins	T	+
7.	Glycoside	-	-

Key: + = Present, - = Absent, T = Trace

The zones of clearance of the pathogens by the extracts as seen in Figure 1a indicated that both of the extracts had good activity against the pathogens. This agreed with the report of Verastegui *et al.* (2008) that ethanol extract is active against range of bacteria including fungi.

Pseudomonas aeruginosa in this study was resistant methanol extract this could be as a result of inadequate extraction of the specific bioactive compounds that would have inhibited the pathogen. The zone of clearance of both the extracts against *Escherichia coli* was noted to be higher than against other pathogens as reported by Musa *et al.* (2000). This could be as a result of inadequate extraction of the bioactive compounds by the solvent.

The minimum inhibitory concentration (MIC) of the extracts was evaluated as seen in Figure 1b, and it was discovered that *E. coli* was inhibited by ethanol extract at concentration of 12.5 mg/ml and by aqueous extract it was inhibited at 25 mg/ml. *Klebsiella pneumoniae* was another pathogen that was inhibited by methanol extract at 25 mg/ml. This indicate that infection cause by *E. coli* can be control by aqueous and methanol extracts of this plant spp. *Klebsiella* infection can be control by methanol extract of the plant. At MIC of 50 mg/ml both extracts inhibited *B. subtilis* and *P. aeruginosa*. The minimum bactericidal concentration of the extracts as seen in Figure 1a indicate that *E. coli* was the only pathogen that was killed at lower concentration at 25 mg/ml followed by *K. pneumoniae* which was inhibited at 50 mg/ml. Other pathogens were killed at concentration of 100 ml.

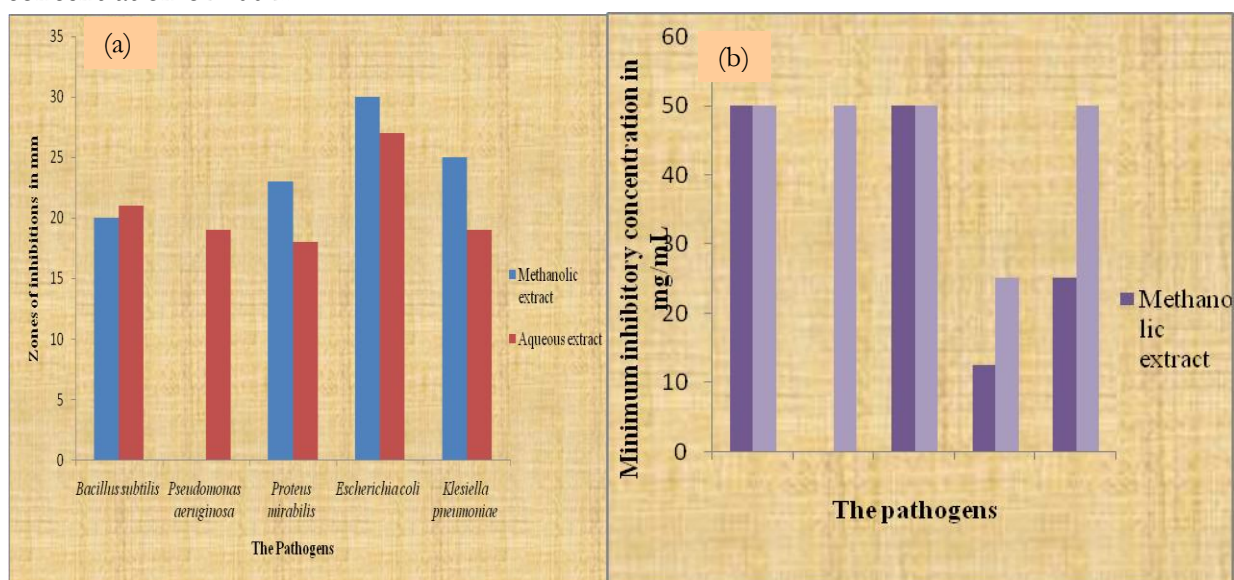


Figure 1. (a) The antibacterial susceptibility of the extracts showing the zones of inhibitions, (b) The minimum inhibitory concentration (MIC) of the extracts against the bacteria

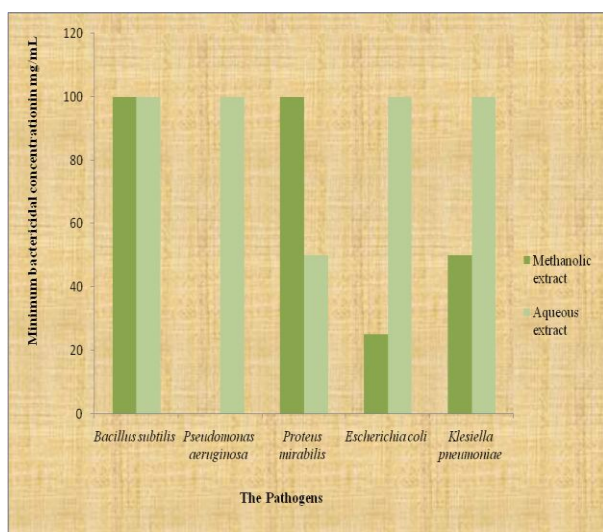


Figure 2. The minimum bactericidal concentration (MBC) of the extracts against the bacteria.

Table 2. The set of controls showing the zones of inhibition

Test organisms	Negative (solvent) control, methanol	Positive(drug) control, Tetracycline (50 mg/ml)
<i>Bacillus subtilis</i>	2	46
<i>Pseudomonas aeruginosa</i>	0	0
<i>Proteus mirabilis</i>	0	39
<i>Escherichia coli</i>	3	41
<i>Klebsiella pneumonia</i>	2	36

Conclusions

The extracts demonstrated presence of saponins, flavonoids, alkaloids and tannins with significant activity against the test pathogenic organisms and this has introduced the plant as a potential candidate for drug development and formulation for the treatment of diseases caused by these pathogens. Of course, there would be the need to ascertain by further studies whether any single or combination of the pure active of the organic compound would be better, safer and more efficient in treating diseases caused by the selected pathogens than the whole plant (crude extracts).

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