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Optimisation of Physical Parameters for Enhanced Antimicrobial Activity of *Acalypha Hispida* Leaf Extract

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ABSTRACT

Utilization of available local resources is essential in primary health care. The Genus *Acalypha* belongs to the family *Eupobiaceae*. It consists of many species of diverse medicinal uses. The plant is found in different geopolitical zones of Nigeria. Extract of *Acalypha hispida* leaves were examined for their antibacterial effect on *Escherichia coli* (ATCC 28923), *Staphylococcus aureus* (ATCC 28923), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* (ATCC 6051). *Staphylococcus aureus* was more susceptible to the effect of *A. hispida* in susceptibility studies while *E. faecalis* which has the lowest ranges of suppression appeared to be more resistant. The minimum inhibitory concentration (MIC) of the extract ranged between 20mg/ml and 35mg/ml. The diameter of zone of inhibition of the extract increased with increase in extraction temperature from 28°C to 80°C when the extract was maintained at these temperatures for 1h. The optimal temperature of extraction for maximum activity of the extract was 80°C while the optimum extraction concentration for maximum activity was 30% (w/v). The extract was thermostable at 45°C temperature. The consequence of the plant extract on the microorganisms suggests that it could be useful in chemotherapy.

Keywords: *Acalypha*, thermostable, inhibitory, antimicrobial, microorganism, chemotherapy

Introduction

Medicinal plants have been known for one thousand years and are considered potential origins of pharmacological agents and/or origins of lead compounds in drug evolution (Alhakmani *et al.*, 2014). The utilization of herbal plants as customary health remedies is the most popular for 80% of the world population in Asia, Latin America and Africa and is reported to have minimal side effects (Krishna *et al.*, 2012).

The use of available local resources is important in primary health care. Traditional medicine and

its practitioners are involved in health care delivery in developing countries. Where communities patronize traditional medicine, it is necessary to embrace safe and useful traditional practices that would enable its incorporation into the design and application of natural health care system. However, that will involve putting folklore medicine on a scientific basis. The use of plants is safe and cost effective in traditional as well as in contemporary medicine (Treasure *et al.*, 2020)

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Many plants from various parts of the world have been known to produce antimicrobial substances (Banso and Mann, 2008). Medicinal flora contains non-toxic phytoconstituents; of which hydrolyse to release phynolic compounds hydrolyse to release phynolic compounds, toxic to microbial pathogens (Dash *et al.*, 2008). It is estimated that only 1% of 265,000 flowering plants on earth have been screened for their active principles and potential as drug sources ((Banso *et al.*, 2020)). Since there are so many naturally occurring substances (Banso, 2009), plant kingdom offers a better opportunity of producing useful medicinal compounds.

Infectious diseases account for approximately one half of all deaths in tropical countries including Nigeria. These challenges have intensified the search for new antimicrobial drugs of plant origin (Dye, 2015). The Genus *Acalypha* is made up of many species used in medicine, the Genus belong to the family *Ephobiaceae*. The plant is found in many parts of Nigeria. Some species of *Acalypha* have been cited either as being used for medication or containing active principle. Different parts of the plant have been used in infectious diarrhea, pulmonary problems and as an expectorant in asthma (Seebaluck *et al.*, 2015). Ellogitannins namely, acalyphidins M₁, M₂ and D₁are present in the plant (Reiersen *et al.*, 2003). Previous phytochemical screening of *A. hispida* leaves extract suggested the presence of reducing sugars, glycosides, steroids, flavonoids and saponins. The leaves of *A. hispida* has been reported to contain cytotoxic, antibacterial (Bokshi *et al.*, 2012) and antifungal properties while the flower extract was reported to contain 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging and cytotoxic activity (Onocha, 2011). This study evaluates the in vitro vulnerability pattern of leaf extract of *Acalypha hispida* against some medically important bacteria. The extract was also optimized for maximal activity.

Materials and Methods

Source of Plant Material and Bacteria

The leaves of the plants were obtained from the school of Medical Laboratory Technology, National Veterinary Research institute, VOM. They were identified based on criteria stipulated by International Committee for Botanical Nomenclature (I.C.B.N) as *Acalypha hispida*

Standard organisms of *Escherichia coli* (ATCC 28923), *Staphylococcus aureus* (ATCC 28923), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* (ATCC 6051) were used in this study. The microorganisms were obtained from National Veterinary Research Institute VOM, Nigeria.

Extraction of plant material

Aqueous extract of the fresh leaf of *A. hispid* was prepared according to the method described by Banso (2009). A 50g sample of the plant leaf was ground in a porcelain mortar. The thick slurry obtained was transferred into a beaker and 10ml of distilled water was added at room temperature. The mixture was extracted by agitation on a rotary shaker at 20rpm for 30minutes. The extract obtained was filtered through four layers of muslin cloth and then through sterile Millipore filter. The procedure was repeated for the air-dried leaf sample of the plant (Banso, 2009)

Optimisation of extraction temperature

Five gramme thick slurry of *A. hispida* leaf sample was taken into a beaker and 10ml of distilled water was added. The mixture was divided into five portions in test tubes. One portion was maintained at ambient temperature ($28 \pm 2^{\circ}\text{C}$) and extracted by agitation on a rotary shaker for 30minutes. The remaining four portions were placed in a water bath maintained at 40°C , 60°C , 80°C , and 100°C for 30 minutes respectively. The extracts obtained were filtered as earlier described and then assayed for antimicrobial activity.

Thermo stability of extract

Aqueous leaf extract (35%w/v) of *A. hispida* was prepared according to optimum conditions. One set was kept as control, and the other sets were exposed to 40°C, 45°C, 50°C, 55°C and 60°C in a water bath for 1h. Antimicrobial assay of the extract was performed and the results were compared with the untreated control to determine the loss of activity.

Optimisation of period of extraction

A five gramme sample of fresh leaves of *A. hispida* was mixed with 10ml of distilled water in 100ml flasks. Samples were extracted in a water bath at 50°C for periods ranging from 10 to 120 minutes. The extracts were assayed for antibacterial activity (Banso *et al.*, 2020).

Optimisation of extraction concentration for maximal activity

Nutrient agar in Petri dishes was seeded with inoculum of *Escherichia coli* (ATCC 28923), *Staphylococcus aureus* (ATCC 28923), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* (ATCC 6051) using sterile swabs. Wells of 5mm in diameter were made on the seeded plates using sterile cork borer. Each well was filled with 0.1ml of various concentrations (5, 10, 15, 20, 25, 30, 35w/v) of aqueous leaf extract. Control experiments were performed with sterile water. Plates were incubated at 28±2°C and the zones of inhibition measured after 24hours.

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts

Different concentrations of the plant extract were introduced into different test tubes; each tube was inoculated with an overnight culture of the test organisms diluted to give a final concentration of one million cells per ml. The organisms were incubated at 37°C for 24 hours. The least concentration of the plant extract that did not permit any visible growth (turbidity) of the

inoculated test organism in broth culture was regarded as the MIC in each case (Banso, 2009).

Results

The effect of extraction temperature of *A. hispida* on its antimicrobial activity against *Escherichia coli* (ATCC 28923), *Staphylococcus aureus* (ATCC 28923), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* (ATCC 6051) is shown in Table 1. Generally, the diameter of zone of inhibition in all the test organisms increased when the temperature of extraction of the leaves was increased from 28°C to 80°C (Table 1). Thereafter a constant growth was recorded with increase in temperature of extraction from 80°C to 100°C. When aqueous leaf extract of *A. hispida* was subjected to 40°C and 45°C for 30 minutes and assayed against *E. faecalis*, it was found to be thermal stable. However, when the extract was subjected to 60°C for 30 minutes there was 52.5% and 56.0% loss of activity against *E. coli* and *S. aureus* respectively (Table 2). Percent losses of activity (23.2%, 0.5% and 18.9%) were recorded against *S. aureus*, *E. faecalis* and *B. subtilis* respectively when the extract was subjected to 40°C.

In an assay to investigate the effect of period of extraction of extract of leaves of *A. hispida* on its antimicrobial activity, it was discovered that antimicrobial activity of the extract increased with increase in period of extraction up to 90minutes (Table 3). It was discovered that the higher the concentration of the plant extract the more effective the extract against the test organisms (Table 4). The zone of inhibition of *E. faecalis* increased from 14.5± 0.3mm to 21.5± 0.1mm with increase in the concentration of the extract from 5%(w/v) to 35%(w/v). The effect of different concentration of aqueous extracts of the plant leaf on *Escherichia coli* (ATCC 28923), *Staphylococcus aureus* (ATCC 28923), *Enterococcus faecalis* (ATCC 29212) and *Bacillus subtilis* (ATCC 6051) were similar (Table 3). When aqueous extract of the plant material was

subjected to 40°C and 50°C and assayed against *E. faecalis*, zones of inhibition of 21.5±0.2 and 15.5±0.1 were recorded against the organism (Table 4). A loss of activity of 17% was recorded against *E. coli* at 40°C (Figure 1).

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The aqueous leaf extract of *A. hispida* produced inhibitory effect against all the test organisms. The MIC of the extract ranged between 20mg/ml and 35 mg/ml (Figure 2).

Table 1: Effect of different temperature (°C) of extraction on antimicrobial activity

Temperature of extraction (°C)	Mean diameter of inhibition (mm) ± SD			
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>B. subtilis</i>
28	16.1±0.1	23.1±0.1	13.0±0.1	12.0±0.2
40	18.5±0.1	26.1±0.2	15.1±0.2	14.0±0.1
60	24.0±0.1	30.2±0.1	20.0±0.1	25.0±0.2
80	36.0±0.2	45.2±0.1	33.0±0.1	35.0±0.1
100	36.0±0.2	45.0±0.1	33.0±0.1	35.5±0.2

Table 2: Thermo stability study of leaf extract of *A. hispida* leaf

Temp° C	Ec	% loss of A		% loss of A		Ef	% loss of ABs	%loss of A
		Sa	Sa	Ef	Ef			
AMT (28±2°C)	34.1±0.1	0	39.1±0.1	0	21.6±0.1	0	36.4±0.1	0
40	28.2±0.2	17	30.0±0.2	23.2	21.5±0.2	0.5	29.5±0.1	18.9
45	28.0±0.3	17.8	32.1±0.1	17.9	15.5±0.1	28.2	29.0±0.2	20.3
50	21.1±0.1	38	24.0±0.2	38.6	14.5±0.3	32.8	20.5±0.3	43.9
55	21.2±0.1	37	20.2±0.2	48.3	14.0±0.2	35.2	20.5±0.1	43.7
60	16.2±0.2	52.5	17.2±0.1	56.0	14.0±0.1	35.2	19.0±0.2	47.8

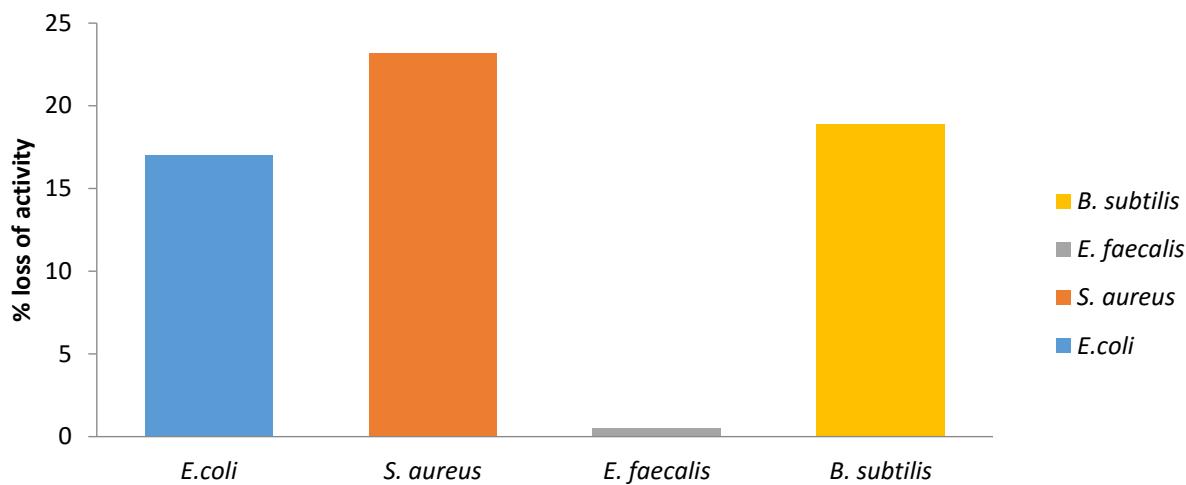
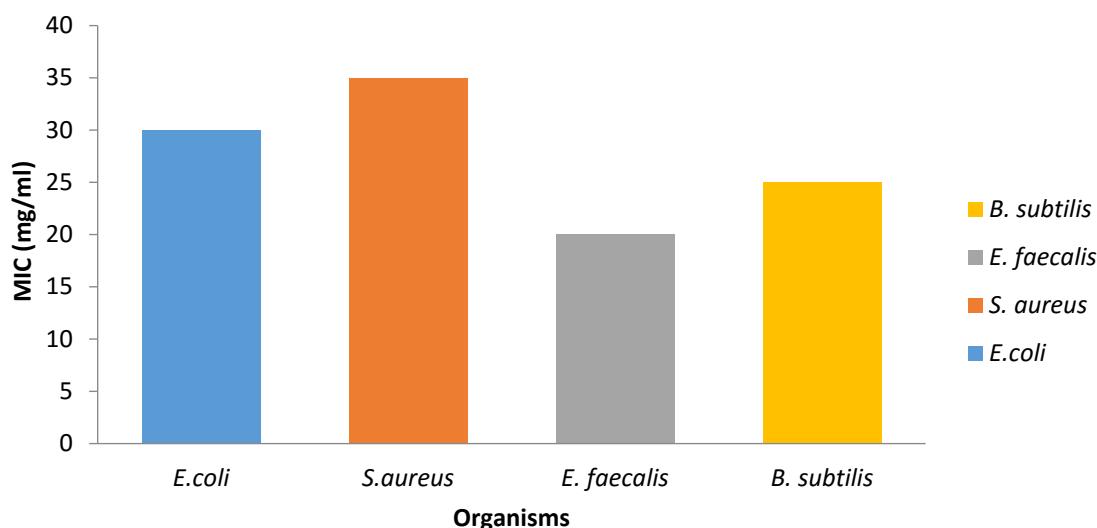
AMT = Ambient temperature, Ec = *Escherichia coli*, *Enterococcus faecalis*, Sa = *Staphylococcus aureus*, Bs = *Bacillus subtilis*, A = Activity, SD = Standard deviation

Table 3: Effect of period of extraction of *A. hispida* leaves at 50°C on its antifungal activity

Period of extraction (min)	Mean diameter of zone of inhibition(mm)±SD			
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>B. subtilis</i>
Control	0	0	0	0
10	18.2±0.1	25.1±0.1	14.5±0.1	19.2±0.1
20	19.5±0.1	28.4±0.1	15.5±0.1	19.5±0.2
30	21.5±0.1	29.8±0.2	16.0±0.1	20.5±0.1
40	22.5±0.2	30.2±0.1	17.5±0.1	20.3±0.2
50	24.0±0.1	30.6±0.1	16.6±0.2	25.5±0.3
60	28.0±0.2	33.2±0.2	17.5±0.2	28.5±0.2
70	29.2±0.1	38.1±0.2	21.2±0.1	30.5±0.2
80	31.1±0.1	35.1±0.1	18.2±0.1	30.5±0.2
90	33.1±0.1	38.2±0.1	19.5±0.1	34.5±0.1
100	34.1±0.2	39.1±0.2	21.1±0.1	36.4±0.2
120	34.1±0.2	39.1±0.1	21.1±0.1	36.4±0.1

Table 4: Effect of different concentrations of *A. hispida* leaves at 50 °C on its antifungal activity

Concentration%(w/v)	Mean diameter of zone of inhibition (mm)±SD			
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>B. subtilis</i>
5	18.0±0.2	19.0±0.2	14.5±0.3	19.0± 0.2
10	18.0±0.3	19.5±0.1	14.5±0.1	19.0±0.1
15	19.0±0.1	25.1±0.2	15.0±0.2	20.2±0.3
20	24.5±0.2	28.0±0.2	15.0±0.1	25.0±0.1
25	28.5±0.1	30.5±0.2	16.5±0.3	29.5±0.2
30	32.0±0.2	35.1±0.1	17.5±0.2	33.5±0.3
35	35.1±0.1	38.0±0.2	21.5±0.1	36.5±0.1

**Figure 1:** Effect of temperature (40°C) on the antimicrobial activity of *A. hispida***Figure 2:** Minimum inhibitory concentration (mg/ml) of aqueous extract of *A. hispida*

Discussion

Non-selective use of drugs contributes to the rising prevalence of resistance among pathogenic microorganisms. The enhanced effect of extract of *A. hispida* when extracted at temperature as high as 80°C suggest the occurrence of bioactive chemical constituents which are thermo stable at 80°C in the leaf extract of the plant. Increase in antimicrobial activity with increase in temperature of extraction may be as a result of increased solubility of bioactive constituents accountable for antimicrobial activity (Arora and Sood, 2017). The enhanced effect of the plant leaf extract obtained within 90 minutes of extraction temperature agrees with the report of Buckley (1985) which indicated that when plant materials used for drug are boiled in water the active compounds are extracted and when the extracts are left in pot with continuous warming the concentration of the extracts increased to a point and then remained constant. The optimal period of aqueous extraction of *A. hispida* leaf extract for maximal antibacterial activity is therefore probably 90°C.

The increase in antibacterial potency observed with increase in the concentration of the agrees with the work of Onsare *et al.* (2013) who arrived at a conclusion that higher concentrations of antibacterial substances showed appreciable growth inhibition of microorganisms. When aqueous leaf extract of *A. hispida* was subjected to 40°C and 45°C for 30 minutes and assayed against *E. faecalis*, it was found to be thermal stable. This could be as a result of the resistance of the plant secondary metabolites to heat at 40°C and 45°C. The heat stability exhibited by the extract could be prospective in purification purposes for the isolation of compounds responsible for antibacterial activity (Arora and Hmadri, 2017).

The MIC of the extract ranged between 20mg/ml and 35 mg/ml. This shows that the extracts of the plant material may be used against any of the test

bacteria to prevent infections caused by the bacteria. This observation confirms the validity of its folkloric use for the treatment of diseases.

Conclusions

The results of this study suggest that the leaf extract of *A. hispida* possess antibacterial active metabolites that are capable of inhibiting the growth of common pathogenic bacteria. Aqueous extracts of *A. hispida* leaf exhibit antibacterial properties thus justifying scientifically their traditional uses as medicinal agents.

Competing interests

Authors have declared that no competing interests exist.

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