International Journal of Research and Reviews in Pharmacy and Applied science

www.ijrrpas.com

STUDIES ON THE EFFECT OF AQUEOUS EXTRACT OF DENNETTIA TRIPETALA (MMIMI, PEPPER FRUIT) SEEDS ON ESCHERICHIA COLI.

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ABSTRACT

Studies on the effect of aqueous extract of Dennettia tripetala was carried out on Escherichia coli a gram negative bacterium. The test organisms Escherichia coli were gotten from stock culture and the pepper fruit seeds used were also bought from the market. The extraction was done with ethanol, hot water and cold water. Different concentrations of the extract were made using each solvent, 50µg/ml, 25µg/ml, 12.5µg/ml, and 3.125µg/ml respectively. Escherichia coli were inhibited by the Dennettia tripetala seeds extract. The results showed that Dennettia tripetala (mmimi) seed extract had both bacteriostatic and bacteriocidal effects on the test organism as the extract efficacy increased with an increase in concentration and was suppressed by high temperatures.

Keywords: Dennettia tripetala, Bacteriostatic, Bacteriocidal and Escherichia coli.

INTRODUCTION

Dennettia tripetala is a well known forest fruit and spicy indigenous medicinal plant. It is widely domesticated in the southern, eastern and western parts of Nigeria. Botanically called Dennettia tripetala , the plant belongs to family of annonaceae. Dennettia tripetala is commonly known as pepper fruit by the English, "mmimi" by the Igbo's, "Nkarika" by the Ibibio and Efik "Imako" by the Urhobo tribe of the Niger Delta region, "Ata Igebere" by the Yoruba's; it is a common ethno medicinal plant in west Africa, which appear reddish when ripe and greenish in an unripe form with a pungent spicy taste (Jacob, 2000; Timothy and Okere, 2008 and Nwachukwu et al., 2010).

The test organism (E coli) plays an important role in the pathogenesis of many diseases. Solutions on the modern or alternative treatment of pathogenic organism including Escherichia coli have been the major challenge facing modern science laboratory researchers.
Therefore, it was important that studies on the effect of aqueous extract of Dennettia tripetala on E coli were examined, detected and possibly the experimental procedure determined.

AIM AND OBJECTIVES OF THE STUDY

- To determine the effect of aqueous extract of Dennetia tripetala on E. coli.
- To identify the antimicrobial properties of Dennetia tripetala.

MATERIAL AND METHODS

Apparatus

Auto clave, Weighing balance, Measuring cylinder, Hot air oven, Spirit lamp, Beaker, Conical flask, Cheese cloth, Wire loop, Petri dishes, Cover slip, Refrigerator, Microscopic slide, Microscope, Paper disc and Incubator.

Materials/Reagents

Ethanol, Nutrient agar, Mac Conkey agar, Peptone water broth, Fruits of Dennettia tripetala, Distilled water, Biochemical reagents, Phytochemical reagents, Gram staining reagents, Cotton wool and Marker.

Data Collection
The Dennettia tripetala fruit containing the seeds were bought from Ogbete Main Market Enugu, within Enugu metropolis, Enugu State of Nigeria.

Preparation/Extraction of Dennettia tripetala
Dennettia tripetala seeds were smoke dried for 30 days. It was later ground using a mechanical grinder. A fine powder was obtained.
Different extractions were done with cold water, hot water and ethanol. Three different concentrations of the extracts were made using each solvent.

Firstly

50µg of the extracts were made by dissolving 50g of the powder in 100ml of each solvent.

Secondly

25µg of the extracts were made by suspending 25g of the powder in 100ml of each solvent.

Lastly

12.5µg, 6.25µg, and 3.125µg of the extract were prepared by dissolving 12.5g, 6.25g and 3.125g of the powder in 100mls of each solvents. The suspended solutions were left for 5 days and filtered. The filtrates were labeled as pepper fruit extract seeds with respect to the concentration and the type of solvent used. It was stored at room temperature.

Collection of Test Organism

The test organisms used for the sensitivity test were collected from the isolated routine sample in the Department of Science Laboratory Technology, Institute of Management and Technology, Enugu, Microbiology Laboratory. Stock culture of the identified isolates were made on nutrient agar slant respectively and stored in the refrigerator at 4ºC.

Media Preparation

The culture media used during the research work were prepared as directed by the manufacturer's specification. 28g of nutrient agar was dissolved in 1 litre of distilled water, 50g of MacConkey agar was also dissolved in 1 litre of distilled water and autoclaved at 1ºC for 15 minutes. Then, the remaining media in flasks were stored at 4ºC.

Planting Techniques (Spread Method)

0.1ml of the sample was spread on sterilized media in Petri dishes. These were spread using hockey stick and thereafter incubated at temperature of 27ºC at 24 h.

Pour Planting Method

The original sample was diluted several times to reduce the microbial population sufficient to obtain separate colonies when planting. Then small volume of several diluted samples were mixed with liquid agar that has been cooled at about 45ºC, the mixture was poured immediately into sterile culture dishes. Most bacteria were not killed by a brief exposure to the warm agar. After the agar hardens, each cell was fixed in the plate and later on formed individual colonies.

Phytochemical analysis of the Seed Extract

Phytochemical analyses were carried out according to the methods of Harborne (1973) and Trease and Evans (1989).

Biochemical Tests

Gram Staining

Smear was made on clean slide and heat fixed using bursen flame. The heat fixed smear were stained for one minute with crystal violet, rinsed under running water, created with lugos iodine for 1 minute and rinsed again. Discoloration was then carried out by treating the smear with ethanol (95%) for 2 seconds. The resultant smears were raised immediately in running water and counter stained for 60 seconds with safranin. After rising with water, the smear was allowed to air dry and examine under oil immersion objective lens of microscope.

Methyl Red Test

The sterile glucose-phosphate peptone water medium was slightly incubated from a young agar slant culture and incubated at 37ºC for 48 h. Then, 5 drops of the methyl red reagent were mixed and read immediately. Positive and negative results were bright red and yellow respectively.

Indole Test

The peptone water medium was incubated with the test organism and incubated for 48 h at 37ºC. The tubes were further allowed to stay for more 48 h in the incubator for the accumulation of indole. After this period, 0.5ml Kovac's reagent was added separately to each tube and swirled gently. Then appearance of a red colour in the alcohol-layer indicated a positive reaction.

Motility Test

About 2-3 drops of peptone water with growth of the organism was placed on a clean slide with a loop. The cover slip was placed over the slide. Then, the slide was left for sometimes and then examined microscopically with the high power objectives. Motile organisms would be seen swimming around.

Sugar Fermentation Test

Fermentation tests were carried using the following sugars – glucose, lactose, maltose and sucrose. To each 10ml of peptone water in test tube 15g of each sugar was separately dissolved into and labeled in 3 drops of 0.01% phenol red added. Durham tubes were in an inverted position into the tubes for detection of gas production.

The tubes were plugged with non-absorbent cotton wool and sealed with aluminum foil before being sterile in the autoclave at 121ºC in 151 pound pressure for 15 minutes. After sterilization, any trace of air in the Durham tubes were removed by inverting the test tubes. The tubes were then aseptically inoculated with small bacterial culture using sterile wire loop. The tubes were incubated for hours at 37ºC and incubated tubes serves as control. Acid production was indicated by a change in colours from orange (alkaline) to yellow (acid) in the fluid. And gas production was indicated by the presence of air space at the bottom or sealed and inverted Durham tubes.

Catalase Test

Mix a colony in 1ml of 1% solution to Tween 80 in a screw cap bottle. Add to it 0.5ml of 20 volume hydrogen peroxide and replace the cap of bottle. Appearance of bubbles or effervescence indicates presence of catalase or positive.

Coagulase Test

Mix one or two colonies in a drop of saline on a clean slide. If no clumps-appear dip straight wire loop in rabbit plasma and stir the mixture with straight wire loop. In positive test, clumps appear within 10 seconds.

Antimicrobial Potency Test

Use of Paper Disc Impregnated with Extract

Filter paper was cut into circular pieces of 9mm in diameter. These circular pieces were impregnated with the extract from different solvents. The impregnation was done by dipping these pieces of circular papers into a beaker bearing the extract with reference to their extraction and allowing the solution to be absorbed by the paper discs, the papers were allowed to dry overnight.

Prepared culture plates were seeded with Escherichia coli, microorganism of choice for the research. After inoculation, the small pieces of filter paper disc impregnated with the pepper fruits seeds extract were placed at fairly spaced position on each of the place bearing the micro-organism. Five different concentrations with three different solvents of the extracts were used to perform the antimicrobial activity using the above method. The plates were incubated at 37ºC for 24 h. After incubation, the one of inhibition were measured and recorded, the antimicrobial potency was performed using the above stated experimental procedures. Different concentrations were tested against the test organism to evaluate the antimicrobial activity of the pepper fruit seeds extract. One method of antimicrobial screening of the extract was adopted. The results obtained were recorded based on the activity of the extract on the test organism. The method used to determine the antimicrobial potency of the seed extracts gave a positive result and they were recorded depending on the concentration of the extract.

RESULTS

Organisms	**Morphological characteristics**	**Cell morphology**	**Gram reaction**	**Identified organism**
A	Pinkish red colonies on MacConkey agar media	Rod	-ve	Escherichia coli

Table1: Morphological Characteristics and Confirmatory Test for Bacterium Isolates.

Organism code	Test	Result	Identified Organism
A	Methyl red	+ve	Escherichia coli
	Indole	+ve	
	Motility	+ve	
	Catalase	-ve	
	Coagulase	-ve	
	Glucose	AG	
	Lactose	AG	
	Maltose	AG	
	Sucrose	AG	

Table: 2 Biochemical Test of the Isolated Bacterium

Keys:

A = Acid

Ag = Acid and gas

+ve = Positive

-ve = Negative

± = Variable

Extract	**50µg/ml**	**25 µg/ml**	**12.5 µg/ml**	**6.25 µg/ml**	**3.12 µg/ml**	**MIC**
Cold H_2O	5.00	2.00	0.00	0.00	0.00	25
Hot H_2O	7.50	5.00	2.05	0.00	0.00	12.5
Ethanol	11.0	8.50	5.05	3.00	0.00	6.25

Table:3 Effect of Dennettia tripetala Seed Extract On Escherichia coli Zone of Inhibition (MM).

Tests	**Observation**	**Result**	**Interpretation**
Tannin	Blueblack colouration	+	Positive
Saponin	Persistence frothing	+	Positive
Flavonoid	Green colouration	+	Positive
Glycoside	Blueblack colouration	+	Positive
Anthraquinone	Pinkish colouration	+	Positive
Alkaloid	Black colouration	+	Positive

Table: 4 Phytochemical Results

DISCUSSION

Many plants have different medicinal values (Loronzani, 2003). An investigation unto the microbial activities of Dennettia tripetala pepper fruit (mmimi) using water and ethanol extracts against Escherichia coli, revealed that the extract from pepper fruit seed had bacteriostatic effects and sometimes bactericidal depending on the concentration of the extracts used.

The method used to carry out the investigation was paper disc method and different concentration of the pepper fruit seeds extract were made using solvents such as ethanol, cold water and hot water at a concentration of 50µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml and 3.12 µg/ml respectively. The inhibition zone found on the culture media after 24 h at 37ºC incubation shows that the anti-microbial activities of pepper fruit seed extracts on these test organism increased with an increase in the concentration and was suppressed by high temperatures.

From the results of this research, it was observed that the ethanol extract was more effective than hot and cold water extracts in clearance of zone of inhibition of E.coli. These were shown from the large zone of inhibition exhibited when tested on the organism. It could be due to the active nature of the ethanol extract as shown in table 3.

However, the extracts from other solvents (hot and cold water) also gave positive results. This shows that Dennettia tripetala pepper fruit extract had an antimicrobial properties which was in line with the results of research work conducted by an international research team where some strains of bacteria was found sensitive to Dennettia tripetala seed extract (Melian, 2003). It was found that oil extracted from the Dennetia tripetala pepper fruit seed extract inhibited the growth of the test micro-organisms (Melian, 2003). The crude extract of pepper fruit seed used in this research was not tested on man or animal infected by the micro-organism. But by virtues of its activity on the organism, it is hoped that the extract could be therapeutic against diseases caused by E.coli.

CONCLUSION

The results obtained in this research would form a base for further research work by the natural production chemists and pharmacologists on the pharmacological effects of Dennettia tripetala on pathogenic micro-organisms.

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