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Full Length Research Paper

Evaluation of the antibacterial properties of different extracts of beniseed (*Sesamum indicum* linn)

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The active compounds of beniseeds was extracted using n-hexane, methanol, hot water and cold water to know the solvent with the highest percentage yield of the active components. Also, the antibacterial activity of each extract was determined using agar cup plate diffusion method on six diarrhoeagenic bacteria. The result of the analysis showed that n-hexane had the highest percentage yield (8.25%), while cold water had the lowest percentage yield (4.20%). Methanol extract recorded the highest inhibitory effect of 39.33 mm of zone diameter on *Bacillus cereus,* while cold water had the lowest inhibitory effect of 2.67 mm diameter zone on *Enterobacter faecium.* Therefore, n-hexane is the best solvent in terms of high yield of the extract of beniseeds compounds; methanol however should be used as solvent of extraction of beniseed compounds for antibacterial activity.

Keywords: Beniseeds, extracts, yield, antibacterial.

INTRODUCTION

Beniseeds, which serves as food in various parts of the world is known to have medicinal properties (Odugbemi, 2006). The plant belongs to the family *Pedaliaceae* and is an annual crop that grows in tropical areas (Savadogo et al., 2004). The seeds are tiny, flat ovals and measuring about 3mm (Oshodi et al., 2010). The plant roots and leaves are used in treating migraine, hypertension, ulcers, constipation, chicken pox and piles (Odugbemi, 2006). The fermented form of the paste has been reported to have antibacterial activity from previous work. The Ebira people in Kogi State of Nigeria use it for the treatment of intestinal disorder, especially in children, expecting mothers and young adults. They also use it for soup after grinding it into smooth paste with grinding stone and they equally roast it as snacks. The concept of improving intestinal health using cheap and effective nutraceutic agents is presently one of the avenues being exploited for use by medical sciences (Oyetayo, 2009). Also, it has been observed that fermented beniseeds have antibacterial properties. This research is therefore, focused on the evaluation of the antibacterial activities of different extracts of beniseeds and to establish the solvent with the highest percentage yield among the solvents used.

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MATERIALS AND METHODS

Collection of beniseeds

Beniseeds was bought at Okene central market, Kogi State, Nigeria. It's identify was confirmed in the department of crop science of the Federal University of Technology, Akure, Ondo State, Nigeria.

Collection of test organisms

The test organisms (*Bacillus cereus, Escherichia coli, Enterobacter faecium, Salmonella typhi, Shigella dysenteriae and Staphylococcus aureus*) were collected from the Microbiology Department, UCH, Ibadan, Oyo State, Nigeria. Their identities were confirmed using biochemical and morphological characteristics before storing in slants and kept in the refrigerator.

Extraction from seeds

250 g of beniseeds was pounded in mortar and soaked in 750 ml of the extracting solvent inside a transparent

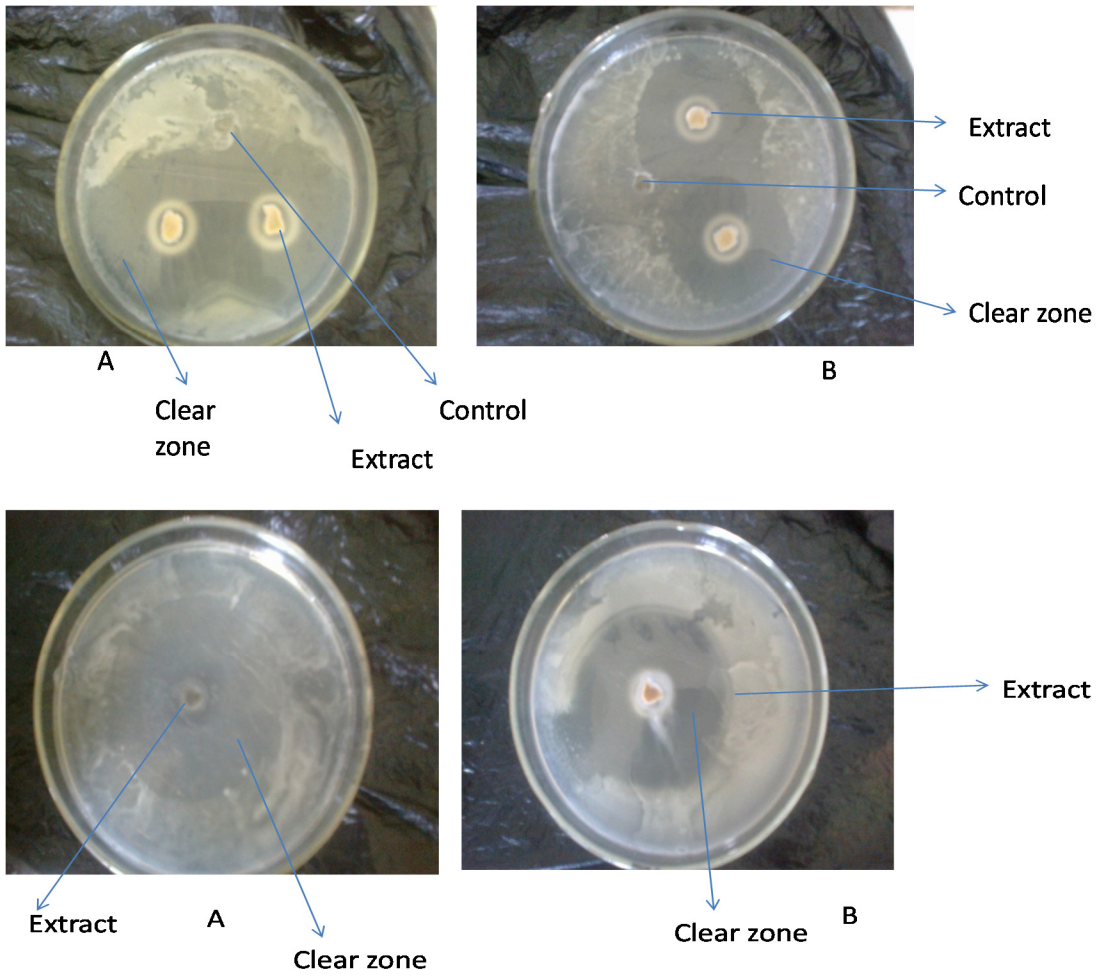

Plate 1. Pictures of zone of inhibition of some beniseed extracts.
Keys: A1=Methanol extract on *B. cereus*, A2=Methanol extract on *S. typhi*
B1= N-hexane extract on *B. cereus*, B2=N-hexane extract on *S. dysentriae*

container for 72 h after grinding it in crucible. It was sieved using a clean muslin bag and allowed to settle before drying by evaporation for methanol and N-hexane extracts respectively. The hot and cold water extract were lyophilized. The percentage yield of each extracting solvent was calculated to know the solvent with the highest yield according to the method of Ogundare (2006).

$$\text{Percentage yield} = \frac{\text{mass of extract} \times 100}{\text{Total mass of seed.}}$$

Standardization of test organisms and antibacterial assay

Each of the test organisms was grown in Nutrient broth at 37°C for 18 h in separate conical flasks. The cells were then harvested and standardized from the stock culture using the method of Fawole and Oso (2004) and Olutiola et al. (2000). The absorbance was measured using a spectrophotometer (Unico 1100 RS series). 1 ml of the harvested cell was pour plated. Two wells were bored using diameter 4 mm of sterilized cork borer and 0.4 ml of the liquor was introduced into one well while the same volume of sterile distilled water was added to the other well to serve as control. The same process was used for the slurry. The plates were carefully incubated at 37°C for 24 h in an incubator and the diameter of zones of inhibition measured. Standard antibiotics were used on the test organisms for the control assay, according to Prescott et al. (2008) (plate 1).

Statistical analysis

The data gathered were processed using descriptive one way analysis of variance, SPSS Version 14 Microsoft Windows 7. The Duncan Multiple Range Test was used

Table 1. Standardized colony forming unit per ml of each organism suspension used.

Organism	Dilution power	cfu/ml	Spectrophotometric reading	Standard cfu/ml
B. cereus	10^6	14	0.050	$1.4x10^7$
E. faecium	10^6	20	0.045	$2.0x10^7$
E. coli	10^6	25	0.043	$2.5x10^7$
Salmonella typhi	10^6	15	0.049	$2.0x10^7$
Shigella dysenteriae	10^6	15	0.051	$1.5x10^7$
Staphylococcus aureus	10^6	22	0.041	$2.2x10^7$

Table 2. Diameter of zone of inhibition of different extract of Beniseeds on test organisms.

Solvent	A	B	C	D	E	F
N-hexane	34.67+ 1.53b	30.67± 1.15a	32.33 ± 0.58a	31.0± 1.0a	38.33± 2.89b	31.33± 4.04a
Methanol	39.33± 3.06c	28.00± 2.0a	27.33±2.31a	28.3± 6.5a	35.33±1.15b	35.33±5.03b
Hot water	33.33±2.31d	10.67±1.15a	26.67±1.15c	30.67± 1.15d	32.67±2.30b	19.33±1.15b
Cold water	18.67±1.15d	2.67±2.31a	4.67±1.15a	12.0±0.0c	13.00±2.65c	8.67±1.15c

Values followed by the same letter in a column are not significantly different at P = 0>0.05.
Keys: A= *Bacillus cereus,* B= *Enterobacter faecium,* C= *Escherichia coli,* D= *Salmonella typhi,* E= *Shigella dysentriae* and F= *Staphylococcus aureus.*

as a follow up test.

RESULTS

Table 1 shows the standardized colony forming unit of each organism used for the antibacterial assay of the extracts and the standard antibiotic discs in this research work.

The result of the solvent extraction shows that the highest percentage yield was obtained from n-hexane, while the solvent with the lowest percentage yield was cold water. The colour description and the percentage yield of each extract from the various solvents are below:

i.) N-hexane extract: The extract was light brown; it turned dark brown with oily liquid afloat upon evaporation of the n-hexane. The percentage yield was 8.25

ii.) Methanol extract: The extract had a dark brown colour with little oily semi-solid sediment after evaporation of the solvent. It had 5.82% yields.

iii.) Hot water extract: The hot water extract had a milky white colour with strong fermentative smell. It turned dark brown after lyophilization. The percentage yield was 6.50.

iv.) Cold water extract: It had a light brown even after lyophilization and a percentage yield of 4.20.

Although, none of the test organism resisted any of the extract, *E. coli* and *E. faecium* had the least zones of inhibition to the extract, especially the cold water extract. It had a value of 2.67 mm diameter on *E. faecium* and 4.67 mm diameter on *E. coli* which were the least values recorded in this research. All the extracts had their highest zone of inhibition on *B. cereus*, making it the most susceptible organism to the extracts. The next susceptible organism to the extracts was *S. dysentriae.* Methanol extract had the highestinhibitory effect on B. cereus and S. aureus while n-hexane had the highest inhibitory effect on all the other test organisms. Table 2 shows the mean values and standard deviation of the diameter of zones of inhibition of the different extracts on the test organisms. Comparison of the extracts with standard antibiotics showed that the former was more effective with the exception of the cold water extracts. Norfloxacin recorded the highest inhibitory value against all the test organisms, except *Staph.aureus* which Tarivid recorded the highest value for. Many of the test organisms resisted the standard antibiotics. Though, some of the antibiotics showed high antibacterial activity against the test organisms, none of the antibiotics inhibited all the organisms used. Table 3 shows the values obtainedfor the standard antibiotics used.

DISCUSSION

In this study, different extracts of beniseeds used had growth inhibitory effect on all the test organisms. This inhibition was superior to most of the antibiotics used. This shows that beniseeds contains bioactive components that had greater activity than that of the antibiotics in inhibiting growth of the test organisms. Olorunfemi et al. (2006) got similar result when she used whey for antimicrobial assay The superior effect may also be as a result of the fact that most the bacteria have become resistant to the antibiotics. The higher inhibitory effect displayed by the extracts gives green light to a type alternative ways of treating infections caused by this organisms. More so that most bacteria are resistant to antibiotics and most of the antibiotics may not readily is available in some rural communities. Moreover, some of these antibiotics induce

Table 3. Diameter of zone of inhibition of standard antibiotics on test organisms.

Day	B. cereus	E. Faecium	E. Coli	S. typhi	S. dysentriae	S. aureus
CIP	0.00±0.00a	7.33±1.15b	16.0±0.0e	11.33±1.15d	12.0±0.0d	10.00±0.0c
TET	10.33±0.58c	6.00±0.0c	11.67±1.53c	8.0±1.0b	11.33±2.30c	0.0±0.0a
NB	0.0±0.0a	15.33±3.0b	19.67±0.58c	19.33±2.31c	25.67±1.15d	0.0±0.0a
AX	0.0±0.0a	11.0±1.0d	10.0±0.0b	9.33±1.15b	19.0±1.0c	0.0±0.0a
OF	10.67±1.15a	0.0±0.0a	0.0±0.0a	0.0±0.0a	9.3±1.15c	7.0±1.0b
C	0.0±0.0a	3.67±0.58b	0.0±0.0a	0.0±0.0a	6.0±0.0c	0.0±0.0a
CF	13.67±0.58e	7.67±0.58c	10.0±0.0d	2.0±0.0b	9.0±1.73c	0.0±0.0a
AM	0.0±0.0a	1.67±1.53a	8.0±1.73b	0.0±0.0a	0.0±0.0a	0.0±0.0a
GN	10.33±0.58b	9.33±1.15b	9.0±1.0b	9.33±1.15a	2.0±0.0a	0.0±0.0a
N	3.67±0.57c	2.33±2.01a	2.0±0.0b	0.0±0.0a	0.0±0.0a	0.0±0.0a
S	0.0±0.0a	0.0±0.0a	10.0±0.0b	10.0±0.0b	0.0±0.0a	0.0±0.0a

Values followed by the same letter in a column are not significantly different at P = 0 > 0.05.
KEYS: CIP = Ciprofloacin, TET = Tetracyclin, NB = Norfloxacin, AX = Augmentin, OF = Tarivid, C = Cephalexin, Am = Ampicilin, GN = Gentamycin, N = Nitrofurantoin, S= Streptomycin.

diarrhoea. The extract with the least growth inhibitory effect was cold water extract while the most susceptible organism to these treatments among the test organisms was *B. cereus*. It may be that there are compounds present in beniseeds extracts that inhibit the production of spore in this organism. The least susceptible organism to all the treatments was *Enterobacter faecium*, except to methanol extract in which *E. coli* was least susceptible. Poole (2001) and Oladunmoye (2006) reported that these organisms have complex cell wall that gave them ability to resist antimicrobial agents. The results obtained so far in this work show that beniseeds extracts have high antibacterial properties against common diarrhoeagenic bacteria. These properties could therefore be harnessed in development of antimicrobial agents for the treatment of diarrhea.

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