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Full Length Research Paper

Antibacterial properties of the leaf extracts of *Vernonia amygdalina, Ocimum gratissimum, Corchorous olitorius* and *Manihot palmate*

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The antibacterial potency of ethanol, acetone and chloroform leaf extracts of *Vernonia amygdalina*, *Ocimum gratissimum*, *Corchorous olitorius* and *Manihot palmata* were screened against ten bacterial isolates using the agar-well diffusion method. The leaf extracts were screened for antibacterial activities at 25 mg/ml concentration. Antibacterial efficacy of extracts against the bacterial isolates was indicated by the appearance of clear zones of inhibition around the wells. The extracts (except that of *Corchorous olitirious*), showed inhibitory activities against *Bacillus cereus*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella dysenteriae* and *Escherichia coil* with zones of inhibition ranging between 2 and 20mm. Result of the antagonistic activity of the extracts compared favourably with the activity of standard antibiotics. The rate of killing by the extracts was carried out, using 50 mg/ml. It was observed that the number of bacterial cells was decreasing as the time of interaction between the extract and the bacteria increased until all cells were killed. Phytochemcial screening of the extracts revealed the presence of some bioactive components like alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids and cardiac glycosides. These properties determine the antimicrobial potential of the leaf extracts.

Key words: Antibacterial activity, phytochemical components, extracts, zone of inhibition.

INTRODUCTION

Before scientists made inroads into the research of drugs that cure human infections, traditional means of treating diseases involved using concoctions from plants, either in single form or in mixtures. This they did without knowing that these agents were used against some pathogenic microorganisms (Sofowora, 1999). Plants have been found useful to man, not only as food or as sources of raw materials for industrial purposes, but also as sources of medicaments (Azoro, 2004). Limited knowledge about the practices of the use of plants for medication (i.e. herbal medicine and lack of scientific studies of plants) have led to the neglect of novel bioactive components on the field that may bring about remarkable result in the treatment of infectious diseases, with little or no side effects (Slayer and Whiff, 1994).

Medicinal plants are known to contain in one or more of its organ substances that can be used for therapeutic purposes or as precursor for the synthesis of useful drugs (Sofowora, 1999). Many of such plants known to be used primitively to alleviate symptoms of illnesses have been screened to have medicinal importance, some of which include: *Azadirachta indica* (Dogonyaro), *Zingiber officinale* (Ginger), *Piper guineese* (Iyere), *Allium sativum* (Garlic), *Vernonia amygdalina* (Bitter leaf). These plants have been reportedly used in the traditional treatment of ailments such as stomach disorder, fever symptoms and cough (Odugbemi, 2006).

Apart from this efficacy, plants have little or no side effects in the treatment of diseases because they act as food and as medicines. In the treatment of hypertension, for instance, herbs are used first to lower the blood pressure, to clean the arteries, to slow and regulate the heart beating rate, to improve the circulation of blood and to relax the mind (Mann et al., 1983). These are unlike the fundamental conventional drugs that dilate the arteries or the veins until they reach their maximum elastic point which may suddenly burst and cause vascular accident, causing stroke or death (Kafaru, 1994).

There is a renewed interest today in seeking new sources of oils, fibre, drugs and medicine from plants, and modern chemical screening techniques that encourage rapid surveys of potentially valuable species.

The plants used in this study are edible plants. They are boiled and made into soup for food. V. *amygdalina* is used to stimulate the digestive system as well as to reduce fever. The leaf of *Ocimum gratissimum* extracted and taken before meal is a remedy for constipation as well as worms in the gastrointestinal tract; it is also used in the treatment of diabetes mellitus. The syrup of *Manihot palmata* has antiseptic properties and is used for flavouring. The premature roots are used to treat eye problems. *Corchorus olitorius* is made into a common mucilaginous (somey) soup or sauce in some West African cooking traditions. It is also a popular dish in the northern provinces of the Philippines, also known as saluyot. The leaves are rich in betacarotene, iron, calcium and vitamin C. The plant has an antioxidant activity with a significant $\propto$ – tocopherol equivalent Vitamin E. (Ayodele, 2005). The aim of this work is to therefore investigate their inhibitory activities on bacterial pathogens, especially those reportedly implicated in nosocomial infections and community diseases, such as *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Sources of plant samples

V. amygdalina, *O. gratissimum* and *C. olitrorius* were purchased at the King's Market in Akure, Ondo State, Nigeria, while M. palmata was harvested on the grounds of the Federal University of Technology, Akure, Ondo State, Nigeria, where it was found growing naturally. The plants were authenticated at the Department of Crop Science and Pest Management, Federal University of Technology, Akure, Nigeria.

Extraction of samples

The harvested leaves were dried and ground to fine powder. About 350 g of each powdered leaf was soaked in each solvent. Three different solvents were used for extraction namely: acetone, chloroform and ethanol. Each solution was allowed to stand for 72 h, and then filtered by first, using a clean muslin cloth and then, No. 1 Whatman filter paper. The filtrates were concentrated in vacuo using rotary evaporator.

Determination of antibacterial activities of plant extracts

The spread plate method described by Madigan et al. (2002) was employed.

Determination of Minimum Inhibitory Concentration (MIC)

This was carried out using agar dilution method described by Olutiola et al. (1991). Different concentrations (50, 25, and 12.5 mg/ml) of the extracts were used. Plates were incubated at 37°C for 24 h, after which they were observed for clear zones around the wells, indicating inhibition. The concentration below in which there was no zone was noted as minimum inhibitory concentration (MIC).

Standard antibiotics sensitivity assay

The agar dilution method described by Olutiola et al. (1991) was employed; however, instead of wells borne inside the agar, antibiotic discs were applied. Standard antibiotic discs were placed aseptically on seeded agar plates using sterile forceps. The inoculated plates were incubated at 37°C for 24 h. Zones of inhibition around the antibiotic disc were measured in millimeters.

Determination of the rate of killing of the extracts

This was done to determine the rate of killing of the test organisms by the extracts. Exactly 5 ml of an 18 h broth culture was introduced into 5mls of 50mg/ml concentration of each of the extracts. The suspension was mixed and then plated using pour plate method at 0, 10, 20, 30 min; and 1, 2, 4, 6 and 8 h. The plates were incubated at 37°C for 24 h after which observation was made for microbial growth. The numbers of colonies were counted using the digital colony counter. Control experiment was carried out by introducing sterile water into 1m of the 18 h broth culture.

Phytochemical screening

The extracts obtained were subjected to phytochemical screening (Harbone, 1984) to determine the presence of bioactive agents such as alkaloids, saponins,tannin,phlobatannins, anthraquinones and cardiac glycosides

RESULTS AND DISCUSSION

Leaf extracts used in this study inhibited both Gram positive and Gram negative bacteria (Table 1). This is an indication that it is of a broad spectrum activity. On the average, the chloroform extracts exhibited the strongest antibacterial activity.

The chloroform extract of *V. amygdalina* inhibited *Shigella dysenteriae*, *Bacillus cereus*, *S. aureus*, and *E. coli* with zones of inhibition ranging from 2 to 20 mm (Table 1). However, *O. gratissimum* extract recorded zones of inhibition ranging between 2 and 15 mm with *B. cereus*, *Salmonella typhi*, *S. aureus* and *S. dysenteriae*. The activity of the chloroform extract of *O. gratissimum* against *S. typhi* is a pointer to the fact that it can be used in the treatment of typhoid fever. Moreover, *S. aureus* and *E. coli* are organisms implicated in nosocomial infection (Prescott et al., 2008); hence, the antagonistic activity of these extracts to such organisms may be vital in the clinical management of nosocomial infections. In the same trend, chloroform extract of *M. palmate* exhibited the highest antagonistic potential against the bacterial isolates with zone of inhibition values ranging between 3 and 11 mm against *B. cereus*, *E. coli* and *S. aureus* (Table 1). *C. olitrorius* exhibited no inhibition on any of the organisms.

Table 1. The antibacterial activities of ethanol, acetone and chloroform extracts of *O. gratissimmum, V. amygdalina, M. palmata, C. olitorius.*

Organism	Zone of inhibition (mm)/ solvent											
	O. gratissimmum			***V. amygdalina***			***M. palmata***			***C. olitrorius***		
	E	**A**	**C**	**E**	**A**	**C**	**E**	**A**	**C**	**E**	**A**	**C**
Bacillus cereus	7	10	12	3	10	13	3	3	11	-	-	-
Escherichia coli	-	-	-	-	-	20	-	-	10	-	-	-
Salmonella typhi	-	-	14		-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-		-
Staphylococcus aureus	7	8	15	8	10	14	-	10	-	-	-	-
Shigella dysenteriae	2	2	2	2	2	2	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-	-	-	-	-	-	-
Enterobacter aerogenes	-	-	-	-	-	-	-	-	-	-	-	-
Clostridium sporogenes	-	-	-	-	-	-	-	-	-	-	-	-

- = No zone of inhibition.

Table 2. The Minimum Inhibitory Concentration of *Ocimum gratissimum*, V*ernonia* a*mygdalina and Manihot palmata* extracts.

Test organism	Concentration (mg/ml)								
	O. gratissimum			***V. amygdalina***			***M. palmata***		
	E	**A**	**C**	**E**	**A**	**C**	**E**	**A**	**C**
Bacillus cereus	25	25	25	25	25	12.5	25	25	12.5
Salmonella typhi	ND	ND	12.5	ND	ND	12.5	ND	ND	ND
Staphylococcus aureus	25	25	12.5	25	25	25	ND	25	ND
Shigella dysenteriae	25	25	25	25	25	25	ND	ND	ND
Escherichia coli	ND	ND	ND	ND	ND	ND	ND	ND	12.5

ND = Not determined.

The extracts used in this work showed varying antimicrobial activities among the plant extracts, with *V. amygdalina*, and *O. gratissimum* appearing to exhibit the highest antibacterial activity. Varying degrees of susceptibility of organisms to ethanol, water and chloroform extracts of garlic have also been reported by El-mahmood (2009).

The outcome of this study has shown that leaf extracts of *V. amygdalina*, *O. gratissimum* and *M. palmata* possess inhibitory potentials against *B. cereus, E. coli, S. typhi, S. aureus,* and *S. dysenteriae*, while *P. aeruginosa*, *B. subtilis*, *Proteus vulgaris*, *Enterobacter aerogenes* and *Cl. sporogenes* were resistant to their activities. The extract of *C. olitorius* exhibited no antagonism against any of the test organisms as shown in Table 1. Studies have implicated *S. aureus*, *E. coli*, and *P. aeruginosa* as leading causative agents of community infections (Branger et al., 2005; Oteo et al., 2005), hence the possible use of these plants in the treatment of infections caused by such organism. It appears that the chloroform extracts of these plants possess stronger antibacterial compounds in comparison to the others, as they exhibited higher antimicrobial values.

Antimicrobial activities in plants have been reported to be as a result of bioactive components present in the plants, such as alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids etc (Harbone, 1984; Odugbemi, 2006). The minimum inhibitory concentration (MIC) values of the extracts ranged from 12.5 to 25 mg/ml (Table 2). This corroborated with the antagonistic activities, as the chloroform extracts recorded the least MIC across board.

Table 3 shows the presence of various bioactive components in the extracts. It is of vital note that saponin was absent in *C. olitorius*, while it was present in all the other extracts (Table 3). This may be singularly responsible for its inactivity on the test isolates (Table 1).

The extracts compared favourably with commercial antibiotics in their bacterial inhibitory potentials. This was seen as recorded in Table 4. This result is a pointer to the fact that, if the crude extracts were subjected to purification, the active components will record same (if not higher) zones of inhibition than what is obtained for the commercial discs. It is possible that the presence of

Table 3. Phytochemical components of *Ocimum gratissimum*, *Vernonia amygdalina* and *Manihot palmata* extracts.

Micro-organism	*O. gratissimum*			*V. amygdalina*			*M. palmata*			*C. olitorius*		
Bioactive component	**E**	**A**	**C**	**E**	**A**	**C**	**E**	**A**	**C**	**E**	**A**	**C**
Alkaloids	+	+	+	–	–	–	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	–	–	–
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinones	–	–	–	–	–	–	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	–	–	–
Flavonoids	+	+	+	+	+	+	–	+	+	+	+	+
Phlobatannins	–	–	–	–	–	–	–	–	–	–	–	–
Cardiac glycosides												
Legal test	+	+	+	+	+	+	–	–	–	–	–	–
Lieberman test	–	–	+	–	–	+	–	–	–	–	–	–
Salkowski test	+	+	+	+	+	+	+	+	+	+	+	+
Keller kiliani test	–	–	–	–	–	–	–	–	–	–	–	–

– Absent; +Present.

Table 4. Standard antibiotics sensitivity test.

Test organism	CHL	AUG	AMX	ERY	GN	CD	NIT	E	OFX	TET	CX	CRO	GEN	COT	PFX	AP	FX	AU
Bacillus cereus	-	-	-	-	-	15	14	11	17	-	-	-	-	-	-	-	-	-
Escherichia coli	11	-	-	12	-	-	-	-	-	14	-	-	12	15	-	-	-	-
Salmonella typhi	-	-	-	-	-	-	-	-	-	-	-	-	16	-	14	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	19	-	-	21	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	19	-	17	-	-	-	-	-	-	-	-	-
Shigella dysentriae	-	-	-	-	-	-	-	-	-	12	-	-	12	-	14	-	-	-
Bacillus subtilis	-	-	-	11	10	13	10	15	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	19	-	-	11	-	-	-	-	-	-	8	-	-	10	-	-	-	-
Enterobacter aerogenes	19	-	-	14	-	-	-	-	-	-	6	-	-	21	-	-	-	-
Clostridium sporogenes	-	-	-	-	-	19	-	17	-	-	-	-	-	-	-	-	-	-

CHL - Chloramphenicol 20 mg; E - erythromycin 10 mcg; AP - cloxacillin 30 mcg; AUG - augmentin 30 mg; OFX - contromoxazole 25 mg; FX - floxapen 30 mcg; AMX –amoxycillin 25 mg; TET - tetracycline 30 µg; AU - augumentin 30 mcg; ERY - erythromycin 30 mg; CX - cephaplexin 30 mcg; GN - gentamicin 10 mcg; CRO – ceftilazone 30 µg; CD - clindamycin 10 mcg; GEN - gentamycin 10 mg; NIT - nitro furuntoin 200 µg; COT - cotrimoxazole 25 mg; PFX - penfloxacin 5 mg.

some impurities has lowered the potency of the crude extracts, which when removed will exhibit higher potency (Slayer and Whiff, 1994). The result rate of killing of the organisms by the extracts at 50 mg/ml showed that the number of the cells decreased as the time of interaction between the extract and bacteria increased. It can be inferred from Figures 1 to 10 that the effect of the extracts is cidal because, the graph of the

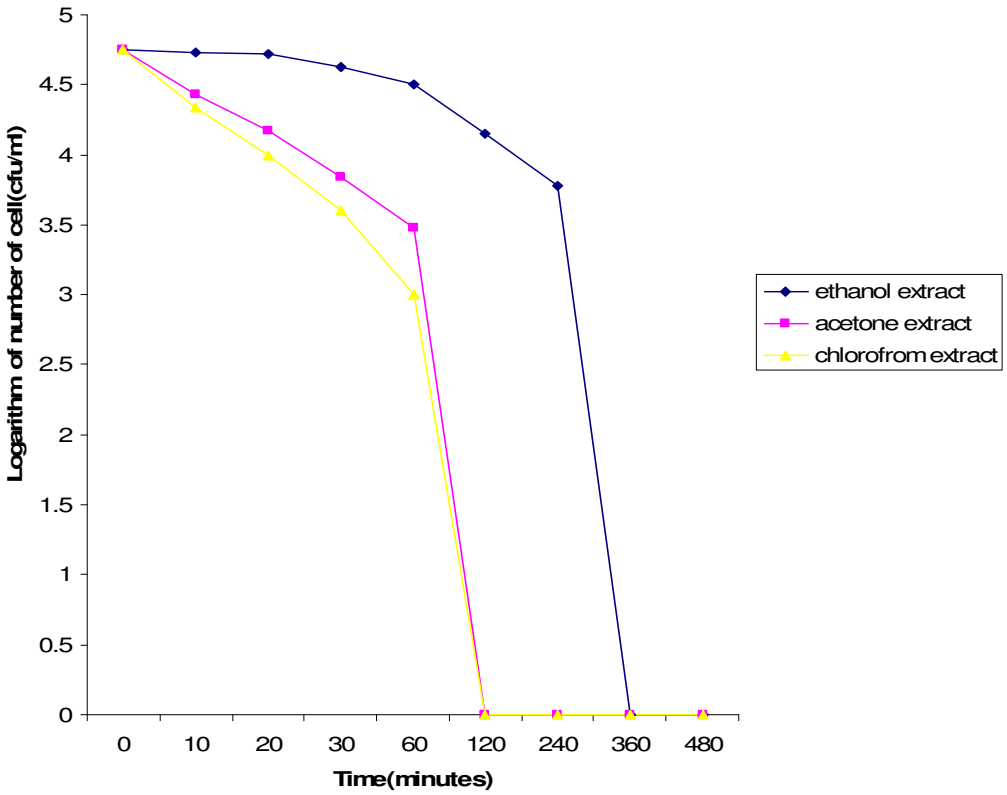

Figure 1. Rate of killing of *Bacillus cereus* by *V. amygdalina* extracts.

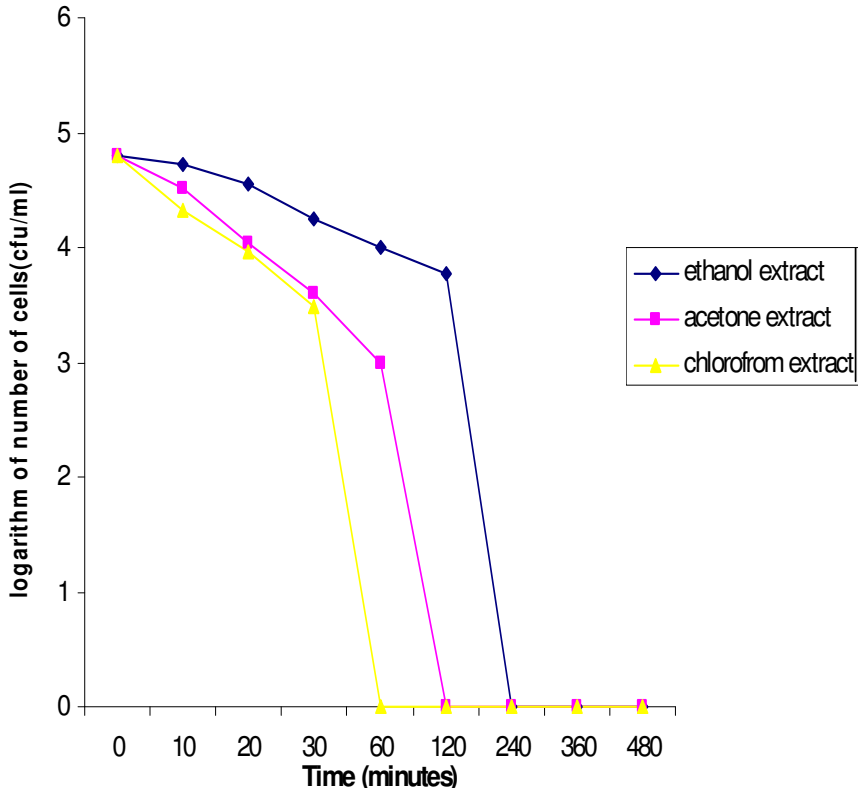

Figure 2. Rate of killing of *Staphylococcus aureus* by *V. amygdalina* extracts.

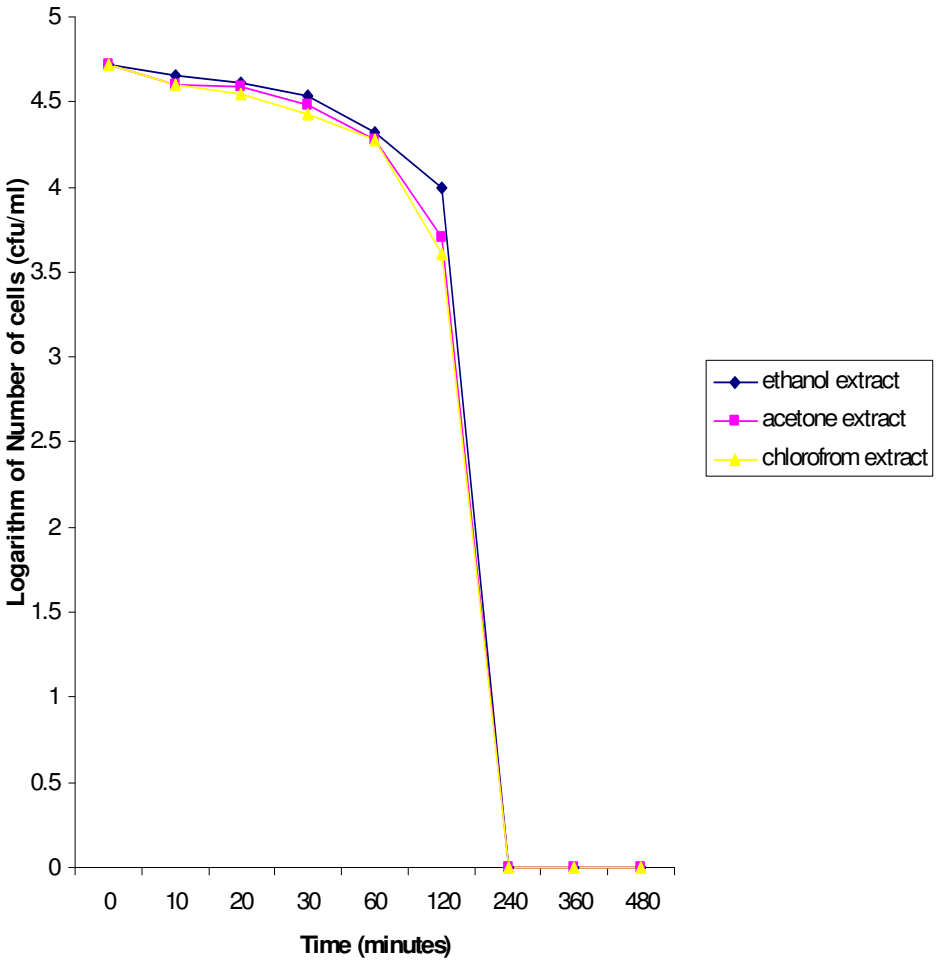

Figure 3. Rate of killing of *Shigella dysenteriae* by V. *amygdalina* extracts.

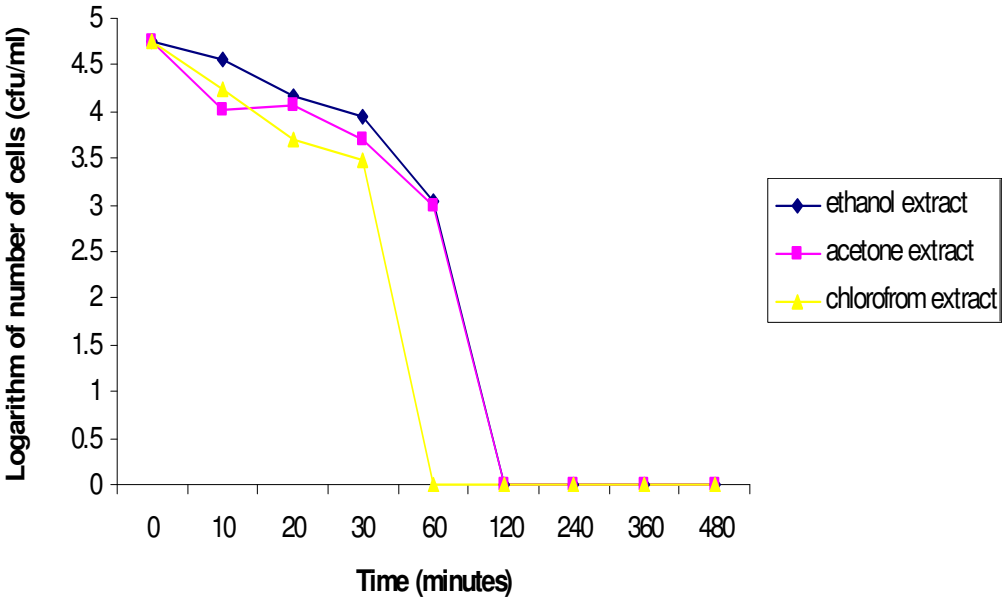

Figure 4. Rate of killing of *Bacillus cereus* by *O. gratissimum* extracts.

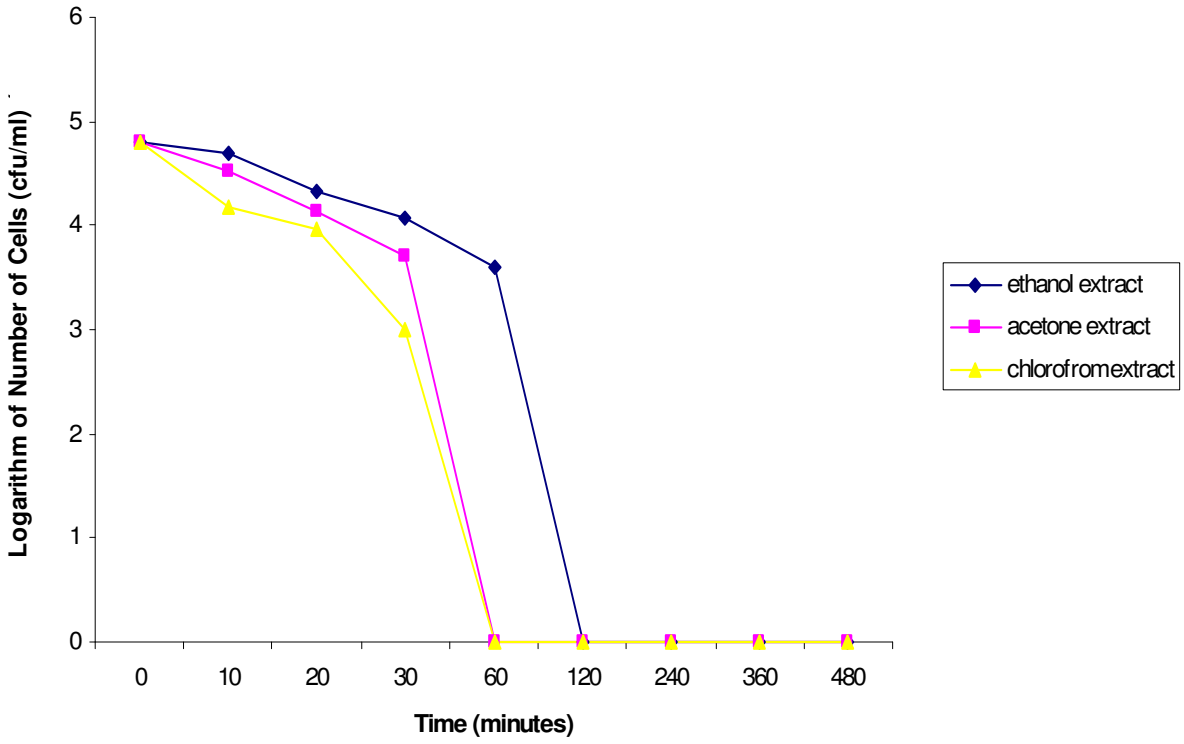

Figure 5. Rate of killing of *Staphylococcus aureus* by *O. gratissimum* extracts.

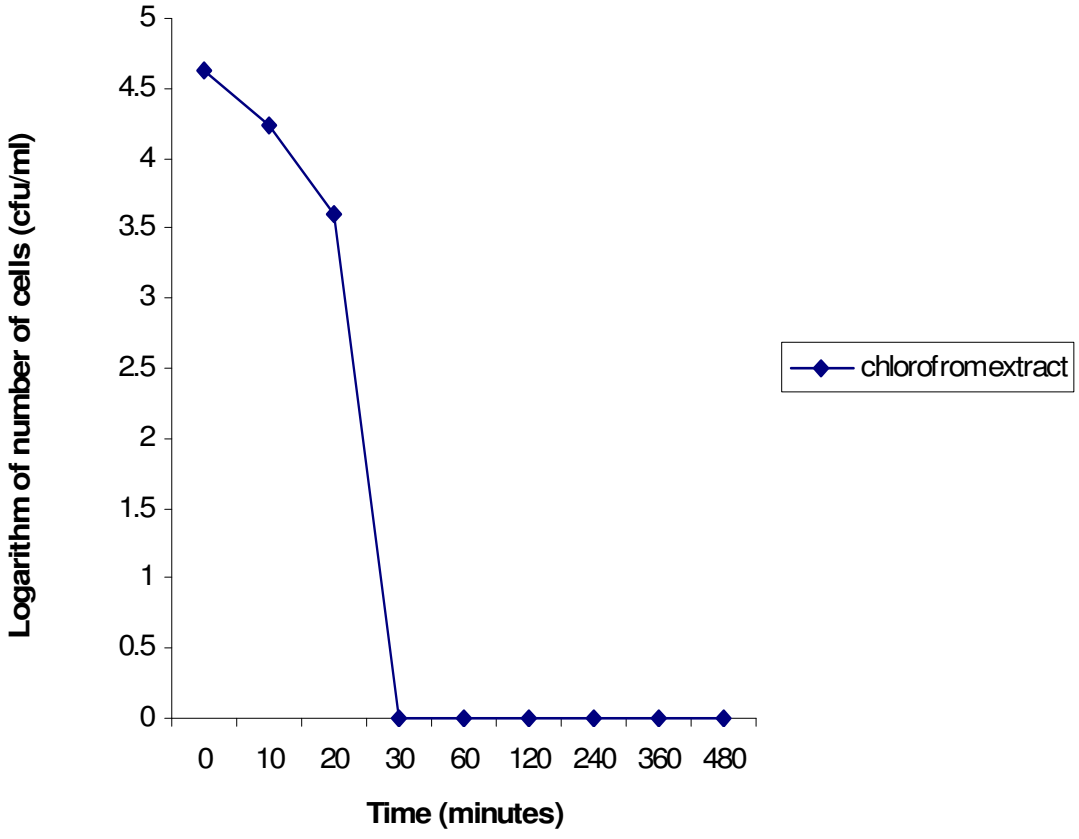

Figure 6. Rate of killing of *Salmonella typhi* by *O. gratissimum* extracts.

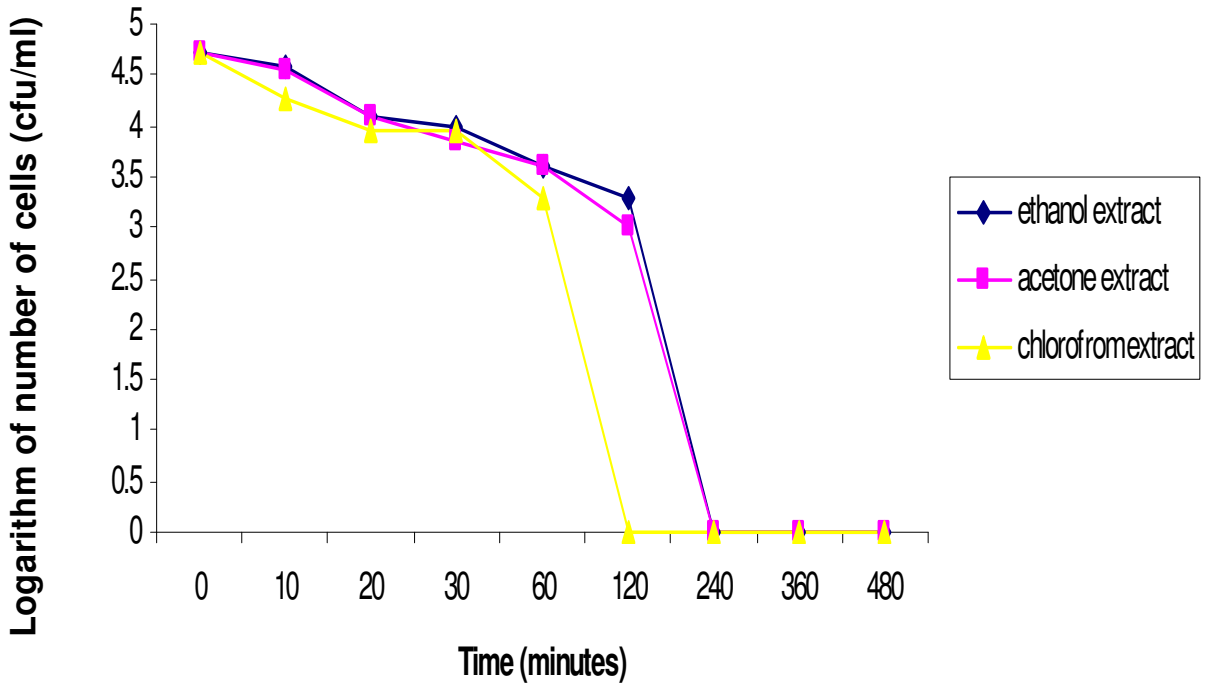

Figure 7. Rate of killing of *Shigella dysenteriae* by *O. gratissimum* extracts.

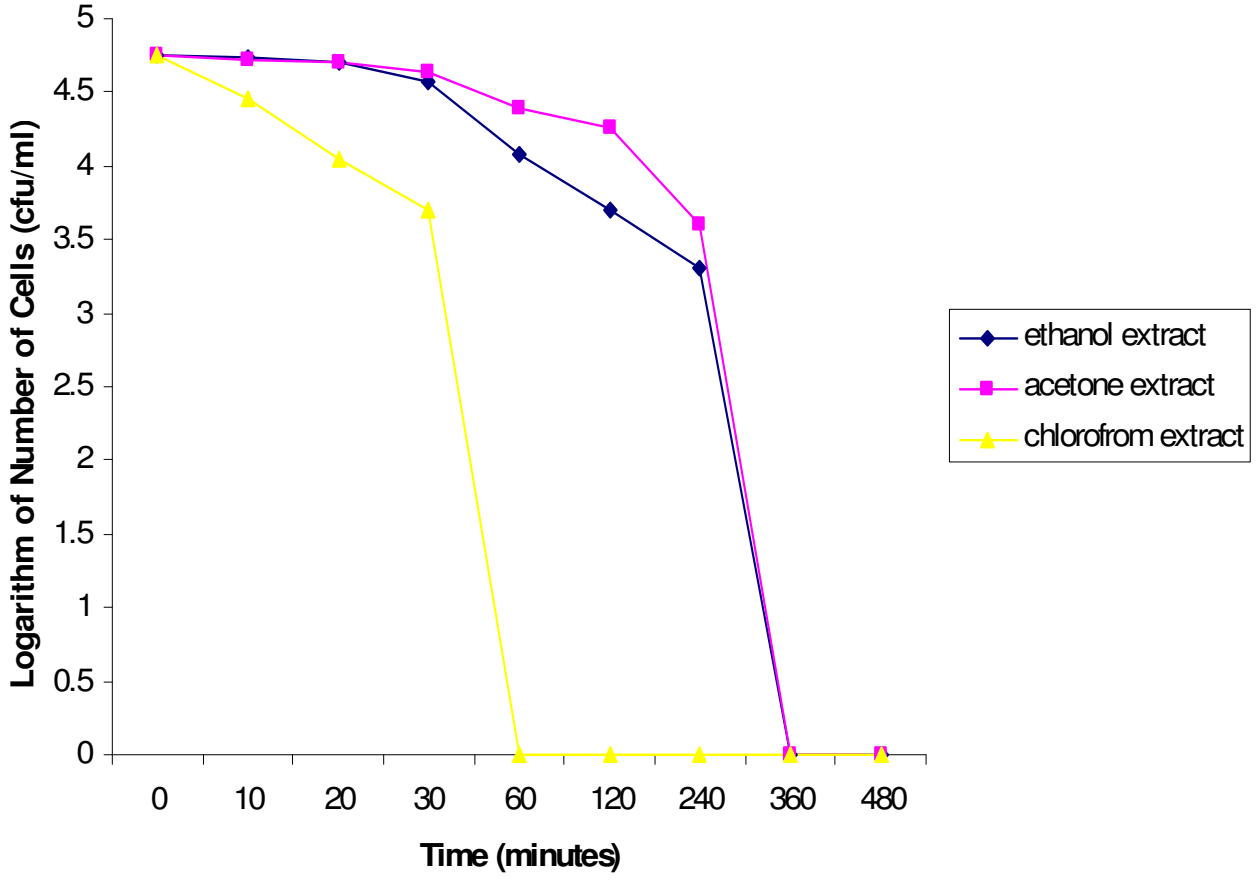

Figure 8. Rate of killing of *Bacillus cereus* by *M. palmata* extracts.

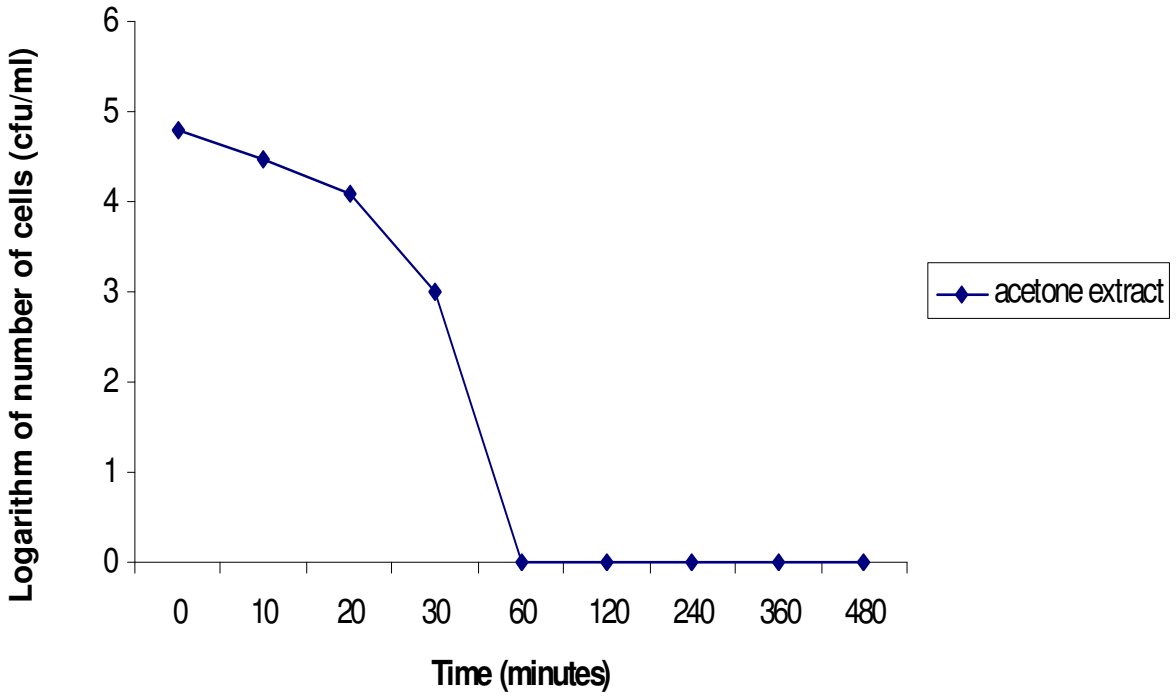

Figure 9. Rate of killing of *Staphylococcus aureus* by *M. palmata* extract.

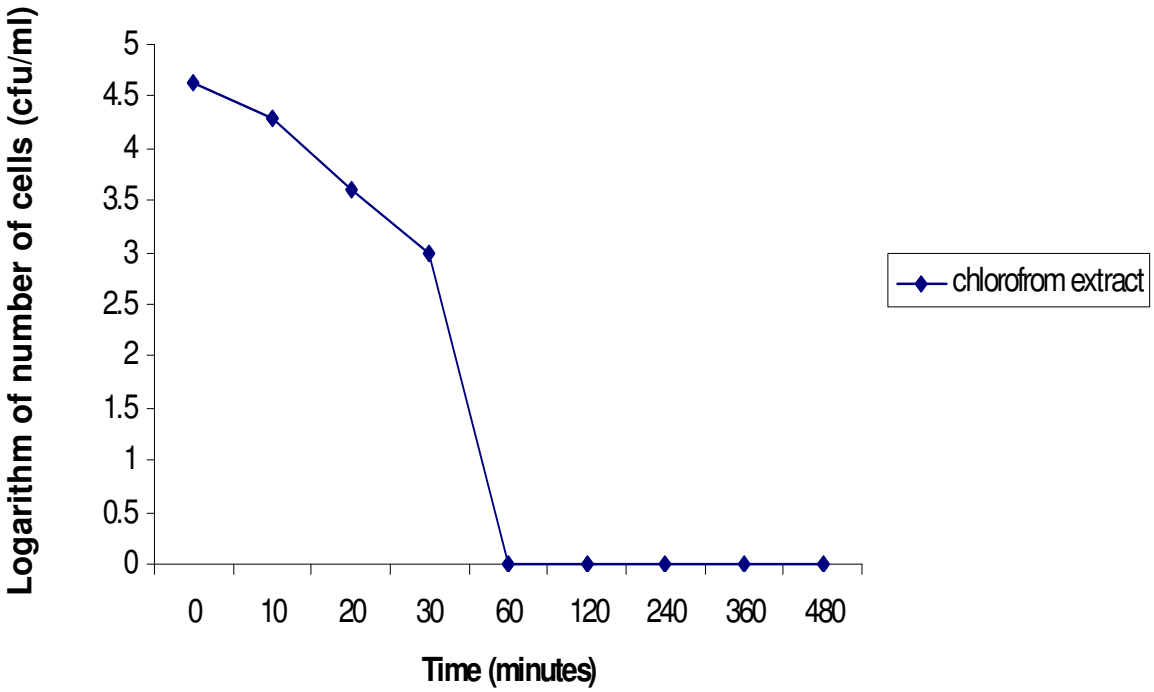

Figure 10. Rate of killing of *Escherichia coli* by *M. palmata* extract.

number of organisms dropped to zero, some after 1 h and some after 2 h. This indicates that there was total annihilation of the bacteria. *S. aureus* was totally killed by all the active extracts at the 60th min. The rate of killing by the extracts of *C. olitorius* was not investigated because it had no inhibition on any of the organisms. It is

possible that if other solvents were employed in the extraction of *C. olitorius* leaf, it may display antimicrobial potentials.

The scope of this work did not extend to purification of the crude extracts. If the extracts were purified, an increase in their antimicrobial activity is likely to be recorded. The structure of the bioactive components can further be investigated with the view to using it, in the production of synthetic drugs.

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