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Original Research Article

Antimicrobial effects of *Aloe vera* on some human pathogens

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A B S T R A C T

The crude of *Aloe Vera* gel was investigated with the aim of determining the microbial activity, the best solvent to be used for extraction and the organism that is most susceptible to the crude *Aloe vera* gel extract. Ethanol, methanol and aqueous (hot) extracts were used as solvent for extraction. Although aqueous extract had the highest yield (19.0g) after extraction as compared to ethanol (18.40g) and methanol (18.0g), ethanol was still regarded as the best solvent for extraction. The susceptibility of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* to the crude extracts of *Aloe vera* gel was determined by agar well diffusion method. Gentamycin was used as positive control while *dimethylsulphoxide (*DMSO) was used as a negative control. The ethanol extract inhibited the growth of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* with zones of inhibition of 6,5, and 4mm respectively while aqueous extract had zones of inhibition of 6, 4 and 3mm respectively. The methanol extract inhibited the growth of *Escherichia coli* (3mm) only. The ethanol extract gave a better minimum inhibitory concentration (MIC) (0.125, 0.125 and 0.40mg/ml) than aqueous extract t (0.25, 0.25 and 0.25mg/ml) and methanol extract (0.50, 0.00 and 0.00mg/ml) on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* respectively. The study revealed that ethanol and aqueous extracts of aloe vera gel was susceptible to the three pathogens and also lend more weight to general acceptability of these crude extracts for therapeutic purposes.

K e y w o r d s

Aloe vera gel; Zones of inhibition; *Escherichia coli*, *Staphylococcus aureus*; Gentamycin.

Introduction

Aloe vera is a species of succulent plant that probably originated in Northern Africa. The species does not have any naturally occurring populations, although closely related aloes do occur in Northern Africa (Akinyele and Odiyi, 2007). *Aloe barbadensis miller (Aloe vera)* belongs to the lialiaceal family of which there are about 360 species. It is a cactus-like plant that grows readily in hot, dry climates and

currently because of demand; it is cultivated in large quantities (Suleyman and Sema, 2009). The succulent *Aloe vera* plant almost sessile perennial herb, has leaves 30-35cmm long and 10cm broad at the base, colour pea-green (when young), bright yellow tubular flowers 25-35cm in length arranged in a slender loose spike, stamens frequently projected beyond the perianth tube. The gel of *Aloe vera* is contained in the leaves. It was used to treat stomach ailments, gastro-intestinal problems, skin diseases, constipations, radiations injury, inflammatory effect, healing wounds and burns, ulcer and diabetes (Johnson *et al* ., 2012). The gel stimulates cell growth and enhances the restoration of damaged skin. It moisturizes the skin because it has a water holding capacity. As a drink, it protects the mucous membrane of the stomach especially when irritated or damaged.

Aloe barbadensis miller (Aloe vera) posses a number of therapeutic uses viz: anti-inflamatory, immunostimulatory, antibacterial, antifungal and cell growth stimulatory activity (Johnson et al., 2012). This work therefore, examines the antimicrobial activity of *Aloe barbadensis miller (Aloe vera)* on some human pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The main objectives for this study to determine the antimicrobial activity of *Aloe vera* gel. And to determine the most sensitive to the plant extract amongst the selected test microorganisms. Also to determine the best solvent for extraction

Materials and Methods

Materials

All laboratory equipment used for this research project was gotten from the microbiology and biological science laboratories of Michael Okpara university of Agriculture, Umudike, except the *Aloe vera* leaves and the test organisms. The *Aloe vera* leaves were collected from the garden Hon. F.U. Okwandu, located in his home town, Akpahia, Obiohuru, Ohuhu in Umuahia North Local Government Area of Abia State, Nigeria. The Aloe Vera leaves were identified by Dr Garuba Omusun of the Botany department of the Michael Okpara University of Agriculture, Umudike. The test organisms were collected from the pathology laboratory, of the Federal Medical Centre, Umuahia.

Ethanol Extraction Methods

Mature, healthy and freshly collected leaves of *Aloe vera* were washed with clean water, then dissected longitudinally and colourless parenchmatoous tissue (aloe gel)was scrapped out carefully using a sterile knife without the green fibres. The collected plant gel that weighed 790g was grinded and mixed with 100ml of ethanol, then left for 24 hours. The crude extract was then filtered through Whatman filter paper No.1 and evaporated as stated by Thiruppathi *et al.,(* 2010). 18. 40g of the ethanol extract obtained was stored in the refrigerator at 4^0C until when required.

Methanol Extraction Method

Mature, healthy and freshly collected leaves of Al;oe vera were washed with clean water, and then dissected longitudinally and colourless parenchmatous tissue |(aloe gel) was scrapped out carefully using a sterile knife without the green fibres. The collected gel weighing 790g was grinded and mixed with 100 ml of hot water and allowed for

24hours; the extract was then filtered through Whatman filter paper 1 and evaporated. 19.0g of the aqueous extract was obtained and stored in the refrigerator at 4^0C until when required (Joshua *et al.*, 2010).

Preparation of Media

The media used were MacConkey agar, Nutrient agar and Sabouraud Dextrose agar. The required amount was measured and prepared according to manufacturer's instruction and poured into conical flasks and covered with non-absorbent cotton wool and wrapped with aluminum foil respectively then sterilized by autoclaving at 121^0C for 15 minutes as stated by Chessbrough, 2002.

Test for Sterility and Purity of Materials

The glass wares wre washed caredfully and packed into the autoclave for sterilization at 121^0C for 15minutes (Chessbroughh, 2000). The gel was exposed to ultraviolet rays for 24 hours and checked for sterility by streaking on a freshly prepared sterile nutrient agar and incubated for 24 hours at 37^0C.

Confirmation of the Viability of organisms

Clinical strains of microorganisms used were *Escherichia coli*, *Staphylococcus aureus* and *candida albicans* and were obtained from the pathology laboratory of the Federal Medical Centre (FMC) Umuahia, Abia State.

Escherichia coli was sub-cultured into MacConkey agar, *Candida albicans* was sub-cultured on Sabouraud dextrose agar and Staphyloccus was sub-cultured on nutrient agar plate and incubated for 24 hours respectively to check if there will be growth.

Determination of the Antimicrobial, Activities of Aloe Vera Gel Extracts using Agar Well Diffusion Method

The bench used was cleaned with cotton wool soaked in ethanol The respective media (MacConkey agar, nutrient agar and Sabourand dextrose agar) were prepared according to their manufacter's instruction into conical flasks and autoclaved at 121 ^{0}C for 15 minutes; cooled and poured into labeled Peti dishes using pour plate method of dispensing agar as stated by Chessbrough, 2000, and allowed to solidify. A sterile wire loop was used to pick a loopful of the test organisms and streaked on the appropriate solidified medium respectively. A sterile cork borer was used to make wells and surface kof the solidified agar media in the Petri dishes used for the sub-dishes for the sub-culturing of the test micro-organisms. The pour plate method was used.

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is the concentration giving the least inhibitory activity and below which there is no further inhibition. It is therefore regareded as the concentration giving the lowest possible zones of inhibition. The MIC of the ethanol and methanol extracts were obtained by dissolving a constant volume of each of the extracts in various volume of distilled water. 0.2g of the various extracts was dissolved in 2ml, 4ml, 8ml, 16ml 32ml of distilled water to obtain 1mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml and 0.0625mg/ml respectively. 0.2ml of the dilutions obtained 1mg/ml-0.0625mg/ml was

introduced into different wells made. After 24 hours of incubation, the results of each concentration were measured.

Control Experiment Using Gentamycin and Dimethylsulphoxide (DMSO)

Gentamycin wa s used as the positive control in order to compare the diameter of zone of inhibition or clearance from the extracts and already standardized antibiotic (Gentamycin) and it was carried out aseptically (Oyagede *et al.*, 1993).

Gentamycin (280mg/ml) bottle with stock solution 80mg/ml was used by diluting 1ml of the Gentamycin in 19ml of distilled water that is 1.20 dilutions (1ml+ 19mls) giving a final concentration of 2mg/ml. DMSO was used as a negative control.

Results and Discussion

Preparation of the Aloe Vera Extracts

790g of the aloe vera gel was scrapped out from the aloe vera leaf samples collected. The gel was colourless, smooth in texture after grinding. The grinded gel was mixed with 100ml of each of the solvent respectively (Ethanol, Aqueous and Methanol extraction) in a separate conical flask with a rubber stopper corks and left for 72 hours. They were filtered through Whatman No.2 filter paper into clean conical flasks and filterate was obtained. The standard ethanol extracts obtained weighed 18.40g, the standard methanol extracts obtained weighed 18.2g and that of aqueous extract weighed 19.0g.

Determination of Antimicrobial activity of Aloe Vera

The antimicrobial activity of aloe vera gel extracts were assayed in-vitro by agar well diffusion method against three pathogenic microorganisms. A Gram positive organism, a Gram negative organism and fungus (*Staphylococcus aureus, Esherichia coli* and *Candida albicans* respectively) respectively as shown in plate 1,2 and 3. The microbial growth inhibition of ethanol, methanol and aqueous extracts of aloe vera gel was summarized in tables 3 and 4. All the extracts of aloe vera gel had clear zones of inhibition on *Escherichia coli;* ethanol and aqueous extracts inhibited *Staphylococcus* respectively (*Staphylococcus aureus and Candida albicans).*ethanol extract had the highest zone of inhibition on all the test microorganisms, 6mm, 5mm, and 4mm (*Escherichia coli Staphylococcus aureus albicans* respectively). Methanol extract inhibited only *Escherichia coli* with of inhibition of 3mm and none on the other organism (*Staphylococcus aureus and candida albicans).*

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the aloe vera gel extract which is the concentration giving the least inhibitory activity and below which there is no further inhibition. The aloe vera gel minimum inhibitory concentration of ethanol extract on *Escherichia coli, Staphylococcus aureus* and *Candida albicans* was 0.125, 0.1 and 0.50 as shown in table 5 and the aloe vera gel minimum inhibitory concentration of aqueous extract on E. coli *Staphylococcus aureus* and *Candida albicans* was 0.25, 0.25 and 0.5 respectively as shown in table 6. The minimum inhibitory concentration of methanol extract on *E. coli* was 0.50 as shown in table 7.

The antimicrobial activity of aloe vera gel

extracts against test organisms with varying zones of inhibition has revealed the antimicrobial potency of the gel of this plant. The results showed that aloe vera gel inhibited the growth of both Gram positive and Gram negative organism and little inhibition on fungi. This is presumed to be due to the active compound present in theis plant (Yebpella *et al.*,2011).

The crude extracts of aloe vera gel showed conspicuous degrees of antibacterial activity. All bacteria species were susceptible to the crude extract of aloe vera gel but variations may occur depending on the type of extraction method used. For instance, methanol extraction method inhibited the growth of *Escherichia coli* but did not inhibit *Staphylococcus aureus and Candida albicans.* This result conformed with the result of Cock, 2008 on similar study. The ethanol and aqueous extracts were active in inhibiting the growth of *Escherichia coli Staphylococcus aureus and Candida albicans* though *Candida albicans* had the least zone of inhibition. This result conformed with the result of investigators on similar studies such as (Johnson *et al*., 2012; Joshua *et al.,* 2010). The study carried out by Suleyman and Sema, 2009 on similar study conformed to the result of this present investigation.

In the face of ever increasing microbial antibiotic resistance, it is becoming more imperative for studies which seek to, identify natural antimicrobial compounds and the future development of this compound.

The results presented above showed that ethanol and aqueous extracts of Aloe vera gel had appreciable antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus and Candida albicans*. Methanol extract had lowest activity on *Escherichia coi* but did not inhibit *Staphylococcus aureus* and *Candida albicans.*

Ethanol extracts had a better minimum inhibitory concentration MIC) compared to others used in this investigation . the gel e xtraccts were more susceptible to *Escherichia coli* (Gram negative) followed by *Staphylococcus aureus* (Gram positive) and the least on *Candida albicans.* This therefore indicates higher antibacterial activities of the extracts on Gram negative bacteria. The minimum inhibitory concentration (MIC) of each extract of the gel revealed the best solvent for extraction

It was determined that aloe vera gel hafd inhibitory effects against pathogenic bacteria, causing different diseases in humans, especially Escherichia coli and Staphylococcus aureus. Aloe vera can be alternative to chemicals used in medication, food and cosmectics. It is hoped that this study would lead to the establishment kof some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

Recommendation

Aloe vera could be used as alternative to chemicals used in medication. This weil help to reduce the toxicity of the chemicals used in medications. It is recommended that ethanol extraction is most preferable to other extraction methods.

Table.2 percentage Yield of Crude Extract of Aloe Vera Gel

Plant species	Extract type	Weight of gel	Weight of extract	%yield of extract
Aloe vera	Ethanol	790	19.20G	2.40
	Methanol	790g	18.40g	2.30
	Aqueous	790g	19.0g	2.41

Table.3 Antimicrobial Activity of Aloe vera gel Extract

Microorganism	Ethanol extract	Aqueous extract	Methanol
Escherichia coli	+	+	+
Staphylococcus aureus	+	+	-
Candida albicans	+	+	-

Key:+ = inhibition;no inhibition

Table.4 Antimicrobial Activity of Aloe vera gel Extract

Microorganism	Ethanol extract	Aqueous extract	Gent	methanol	DMSO
Escherichia coli	6.0	6.0	13	3.0	0.0
Staphylococcus aureus	5.0	4.0	12	0.0	0.0
Candida albicans	4.0	3.0	9	0.0	0.0

Table.5 minimum Inhibition of Ethanol Extract of aloe vera gel on the test microorganisms

Test microorganism	Concentration in mg/ml					
	1.0	0.50	0.25	0.125	0.0625	MIC
Escherichia coli	+	+	+	+	-	0.125
Staphylococcus aureus	+	+	+	+	+	0.125
Candida albicans	+	+	-	-	-	0.5

Key:+ = inhibition;= no inhibition

Table.6 minimum Inhibition Concentration of Aqueous Extract of aloe vera gel on the test microorganisms

Test microorganism	Concentration in mg/ml					
	1.0	0.50	0.25	0.125	0.0625	MIC
Escherichia coli	+	+	+	+	-	0.25
Staphylococcus aureus	+	+	+	+	+	0.25
Candida albicans	+	+	-	-	-	0.50

Key: + = inhibition; = n
o inhibition

Table.7 MIC of Ethanol extract of aloe vera gel on the test organisms

Microorganism	1.5	0.5	0.25	0.125	0.0625	MIC
E. coli	+	+	-	-	-	0.50
S. aureus	-	-	-	-	-	0.0
C. albicans	-	-	-	-	-	0.0

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