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Original Research Article

Phytochemical Screening and Antibacterial activity of Okra extract

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Abstract: Okra (*Abelmoschus esculentus*) belongs to malvaceae family and its widely cultured in the world for its fibrous fruits which contain round white seeds. In this study the bioactive compounds where extracted by using many solvents methanol, ethanol, ethyl acetate and water. The ethanolic extract where used invitro to determine the antibacterial activity of the extracts of dried fruit okra against some selected potential bacterial pathogens, *Klebsiella* and *E. coli*. This research is an experimental study using completely randomized design by using disk diffusion testing method. The object used was the extract of the Okra fruits at concentration of 25%, 50%, 100% and 200%. The zone of inhibition was 3.73mm at 200% with *Klebsiella* and 3.47 mm with E. coli in comparison with the control 4.4 mm. The least concentration of the extract that completely inhibit the growth of the organism MIC is 30%.

Keywords: Okra, klebsiella, E. coli antibacterial activity.

INTRODUCTION

The reduced susceptibility of the bacteria to the antibiotic become a problem worldwide. Also, the increased toxicity of scientific drugs has led to using natural, safe potent antibacterial agents rather than scientific drugs (Gottlieb *et al*., 2002 and Narod *et al*., 2004). Scientists direct to dissolve the problem by use fungi, algae and higher plants to develop new antibiotics. Large number of organic bioactive compounds are produced by these higher plants as secondary metabolites, which used in synthesis of chemotherapeutic, bactericidal, and bacteriostatic agents (Evans *et al*., 1986 and Purohit *et al.*, 1998). In recent years the researchers attention directed toward the identification of antibiotics from plants because, plant derived antibiotics still remain an area of intensive investigation (Cutter, 2000, Jain, *et al*., 2010 and Shirazi *et al*., 2007). In several medicinal applications Okra mucilage has been used (Kumar, 2010). Abelmoschus esculentus is a vegetable crop where the immature pods used in syntehsis of soap and stew. It is called as ladyfingers, gumbo and bhindi. The trop ical and subtropical parts of the world are the main area of its growth such as Nigeria, India, Ethiopia, Turkey, Japan, Malaysia and the south united states (Khomsug, *et al.,* 2010 and Nwangburuka *et al*., 2013). It is rich in vitamins, minerals (iron, potassium, manganese and calcium) and dietary fat. It has been used in genitourinary disorders, in controlling cholesterol and hypertension level, chronic dysentery, ulcer and anti- inflammatory properties (Ansari *et al*., 2005). This study has been conducted to assess the inhibitory activity of ethanolic extract of A. esculentus pods against selected pathogenic bacteria *E. coli* and *Kleibsiella* species.

MATERIAL AND METHODS

Preparation of Extract

150 grams of each selected dried plant powder were weighed and added to a 600 ml of ethanol, in a conical flask of a 1000 ml capacity. The flask was covered and left a side for a 24 hour. The plant mixture was mixed using magnetic mixer and filtered then the plant extract was kept in the refrigerator until use.

Preliminary Phytochemical Screening

Screening of the above selected medicinal plant for various phytochemical constituents were carried out using standard methods (Dibyajyoti *et al*., 2011). Qualitative phytochemical screening of plant extracts was carried out using the following methods to test only the presence of secondary metabolites by using different solvents.

Test for Tannins

0.008 M Potassium ferricyanide was added to 1 ml of the extract in a test tube, 1 ml of 0.02 M Ferric chloride containing 0.1N hydrochloric acid was also added. A blue-black coloration was observed.

Test for Flavonoids

Crude extract was added to 5 ml of diluted ammonia solution and concentrated H2SO4. The presence of flavonoids is indicated yellow coloration which disappeared on standing.

Test for Alkaloids

In 2ml of 1% HCl crude extract was dissolved and gently heated.To the mixture Mayers reagents were added to the mixture. The presence of alkaloids confirmed by the turbidity of the resulting precipitate.

Thin Layer Chromatography (TLC) Analysis

The Ethanolic, Methanolic, Ethyl acetate and Water Okra extracts were loaded on silica plate (Merck Aluminium sheet—silica gel 60 F 254). A mixture of H:C:M (1:1:1), P: E: W (1:2:1), M: E: W (1:1:2) and P:M: W (3:1:1) were used as the solvent system. The TLC plate was kept in iodine chamber for one minute and under UV light (254 nm) to visualize bands on chromatogram (Asha *et al.*, 2013 and Das *et al*., 2010).

Preparation of Bacterial Isolates

Two different types of bacterial strains were obtained from the medical laboratories which are Escherichia coli and Klebsiella.

Screening of antimicrobial activity

Media for test organisms

36 g of Muller Hinton Agar was added to 1000 ml of sterile distilled water and autoclaved at 121^{o} C for 30 minutes at 1.5 lbs. After cooling both the agar was poured into sterile Petri plates approximately 4mm and allowed to set at ambient temperature and used. Sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The culture plate then had at most 4 holes of 7 mm diameter and 5 mm depth made into it using a sterile agar glass borer. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough, 2002).

Inhibition Activity of Different Concentration of Okra Extracts

This was carried out using agar well diffusion method. 200 µl of different concentration of the aqueous and ethanoic extracts (25mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) of Okra pods were dispensed separately in wells already seeded with the test isolates and incubated at 37°C for 24 h. After incubation, the inhibitory activity of the minimum concentration of the extracts against the test organisms was determined by measuring the clear zones around the wells in diameter. Standard antibiotic discs were used as a positive control to compare the antibacterial activity. The discs loaded with test extracts, and the standard antibiotic were placed with help of sterile forceps carefully with adequate spacing between each other. After incubation, the antibacterial activity of the extracts against the test organisms was determined by measuring the clear zones around the wells in diameter.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of the crude leave extract of Okra was determined by using the method of Greenwood (1989) as described by (Geidamet *et al*., 2007). Serial dilution of the extract at the concentrations of 25, 30,35, 40, 45,50,100 and 200 mg/ml. Where 18 mg of the Muller Hinton Agar media was prepared in 500 ml of distilled water and autoclaved at 121º C and 5Ib for 30 minutes then cooled, the media filled in tubes each tube contain 17 ml. Astandarazed inoculum for each bacterial strain was prepared to give an inoculum size approximately 10^{-5} in 5 tubes each tube contain 10 ml of distilled water. Put each extract concentration in the tube containing 3ml of distilled water and mixed properly then taken off by a sterile syringe and filtered by filter paper and add the prepared M.H.A broth and mixed properly then add 100 micro of bacterial isolate and mixed again then put them in autoclaved petri dishes and move the dishes in different directions to homogenize the plant extract. The control sample containing only the bacteria without extract. Then kept at 37 C for 24 hrs. in incubator. Then determine minimum inhibitory concentration and recorded as the least concentration of the extract that completely inhibit the growth of the organism.

Determination of Minimum Bactericidal Concentration (MBC):

Two nutrient agar plates were prepared. The bacterial isolate of kliebsiella incubated for 24 hr. then 10^{-5} serial dilution were prepared. The diluted bacterial isolate spread on one plate in different direction. The other plate cultured by non-diluted bacterial isolate. 200mg/ml of ethanol extract mixed with 3ml of distilled water. 5 mm size discs from filter paper were cut and filled with the extract and then put them on the agar as well as antibiotic discs and then kept in the incubator for 24 hr at 37º C with a control plate. The lowest concentration with no visible growth was defined as MBC, indicating 99.5 % killing of the original inoculum.

RESULT AND DISCUSSION

Phytochemical Screening of Sequential Extracts of Okra

The results of plant extract under investigation are shown Table 1. leaves extract showed positive result for the presence of medicinally active constituents. In the Water extract; tannins, phenolic compounds, flavonoids, alkaloids, were the most common present in the tested plants. While phenolic compounds are absent in methanolic, ethanoic and Ethyl acetate extract. Plants which rich in a wide variety of secondary metabolites, such as terpenoids, alkaloids, tannins, flavonoids appear biological and pharmacological activities and may have potential to be used as chemotherapeutic agents or serve as starting material in the developing of new antibiotics.

Table 1: Preliminary phytochemical screening of Okra extract

leaves methanolic extract	phytochemical compounds of Okra			
	Phenolic compound	Flavonoids	Alkaloids	Tannins
Methanolic extract	**-ve**	**+**	**-ve**	**+**
Ethanolic extract	**-ve**	**-ve**	**+++**	**+++**
Ethyl acetate extract	**-ve**	**+**		**-ve**
Water extract	**+++**	**+**	**+++**	**+++**

(+++) high (++) medium (+) poor (-) no found

Thin layer chromatography profiling several bands or spots were observed during partitioning of extract components with mobile phases systems indicating separation of bioactive compounds depending on polarity (10). The RF values are shown in table 2 of methanolic, ethanolic, ethyl acetate and water extracts.

Table 2: Thin Layer Chromatography

	R_f values			
	Methanolic Extract	**Ethanolic**	**Ethyl acetate**	**water extracts**
H:C:M(1:1:1)	**0.25, 0.29**	**0.085, 0.148**	**0.106, 0.212, 0.97**	**0.063, 0.074, 0.106**
P:E:W(1:2:1)	**0.71, 0.77, 0.82, 0.91**	**-**	**0.68, 0.77**	**0.77**
M:E:W(1:1:2)	**0.028, 0.074, 0.048**	**0.029, 0.0102, 0.42**	**0.075, 0.45, 0.54**	**-**
P:M:W(3:1:1)	**0.45, 0.72**	**0.5, 0.67, 0.85**	**0.45, 0.82**	**-**

Antibacterial activity of the pods extract

The present study was on the determining antibacterial activity using agar well diffusion method by measuring the inhibition zone in mm against two bacterial strain E. coli and Kleibsiella species and phytochemical screening in Leaves of Okra with different solvents water, 70% ethanol, 80% methanol and petroleum ether. The extract used in this study was the ethanolic extract. The potency of the ethanolic extract A. esculentus pods against Ecoli and Klebsiella was examined based on the presence and absence of of zone of inhibition measured in diameters as shown in table 3. In the search, plant parts play important role because their huge production of organic compounds for medicinal use. The ethanolic extract of the Okra pods exerted inhibitory properties against the test bacterial isolates (E. coli and Klebsiella). This could be due to presence of bioactive compounds in most plant parts which show antibacterial activity (Pereira JA *et al.*, 2007). Results of research on the growth of E. coli and Klebsiella, by using the disk diffusion disk and measuring the inhibitory zone, have revealed that the Okra extract can inhibit the growth of the two microorganisms. The optimum concentration to inhibit the growth of E. coli at 100 mg/ml and 200 mg/ml with zone 2.74 mm and 3mm respectively, where for the Klebsiella the zone was 2.37 mm and 3 mm respectively in comparison with the control inhibitory zone was 4.4 mm. However, there is no inhibition at concentration of 25 and 50 mg/ml. This indicate that Okra fruit has optimum concentration to suppress the growth of E. coli and Klebsiella bacteria which can be seen from the inhibition zone diameter Fig-1 and Fig-2 for E. coli and Klebsiella respectively.

Fig-1: Zone of inhibition for E. coli, positive control of chloramphenicol (Middle), (1) for 25 mg/ml, (2) for 50 mg/ml (3) for 100 mg/ml and (4) for concentration of 200 mg/ml

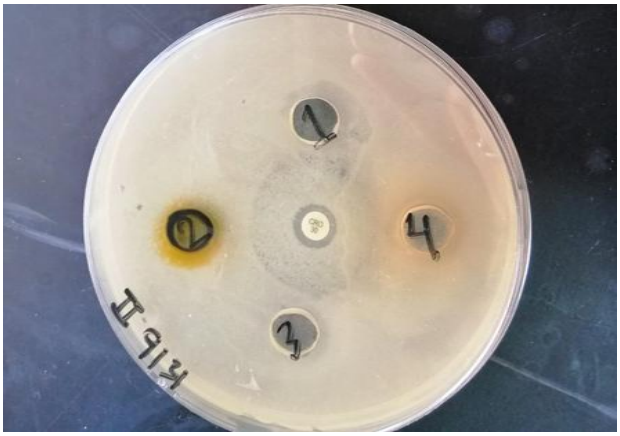

Fig-2: Zone of inhibition for Klebsiella, positive control of chloramphenicol (Middle), (1) for 25 mg/ml, (2) for 50 mg/ml (3) for 100 mg/ml and (4) for concentration of 200 mg/ml

In addition to the factor of concentration, the ability to inhibit bacterial growth also determined by antimicrobial material substance which produced by the plant (Rastina *et al*., 2015). In this research, the antibacterial was due to the presence of bioactive compounds such as flavonoids, tannins. Saponins, in okra fruit (Septianingrum *et al*., 2018). Due to the interaction between flavonoids and bacterial DNA the flavonoids cause damage to bacterial cell wall, microsomes and lysosomes (Nagappan *et al.*, 2011). In addition, flavonoids have lipophilic characteristics therefor they have ability to damage the cell membrane of bacteria (Rianto *et al*., 2015). Moreover, flavonoids are also important as a powerful antioxidant in decreasing the risk of chronic diseases, the cancer process, anti-inflammatory, antibacterial, and antiallergic. The antibacterial action of flavonoid substances thought to degradation of bacterial cell proteins and damage cell membrane beyond repair (Sudoyo, 2009). In this study tests one way Anova showed the calculated p value for the Ecoli bacteria was 0.000 at the concentration 200%. Whereas the p value for the Klebsiella was 0.001 at same concentration so that the okra pud extraction can inhibit the growth of both gram negative bacteria (E. coli and Klebsiella). Positive control showed more antibacterial activity against test bacteria compared with tested samples.

Table 3: Antibacterial Activity of Okra pods Extract against bacteria (E.coli and Klebsiela) concentration (µg /mL)/ Zone of inhibition (mm)

Organism	**25**	**50**	**100**	**200**	**Control**
E.coli	no	no	2.47 ± 0.06	3.00 ± 0.00	4.40 ± 0.00
P-value	no	no	0.000	0.000	
Organism	25	50	100	200	Control
Klebsiella	no	no	2.37 ± 0.16	3.00 ± 0.00	4.40 ± 0.00
P-value	no	no	0.000	0.001	

The minimum inhibitory concentration (MIC) value of ethanolic extract of Okra pods against Kleibsilla. According to Table the MIC value of ethanolic extract treated on Kleisilla was found to be 30 mg /ml.

MIC of ethanoic okra leaf extract:

Extract concentration	**MIC**
45	no
40	no
35	10
30	no
25	20
20	23
15	30
5	90

The Minimum Bactericidal Concentration (MBC) Klebsiella was 30 mg/ml. This was the lowest concentration, from which there was no bacterial growth during MIC determination. The plates were examined after 24 hours incubation of the test organisms. The result revealed that MBC equals to MIC.

MBC of ethanolic okra leaf extract:

Concentration of extract	**No of colonies**
45	no
40	no
35	10
30	no
25	20
20	23
15	30
5	90

CONCLUSION

The phytochemical compounds present in the okra pods extract exhibits antibacterial activity. That could prove the plant extract as potential natural antibacterial agent. More research work can be carried out on the isolation and characterization of bioactive compounds present in A. esculentus pods for better therapeutic use against pathogenic bacteria.

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