Available on line www.eijppr.com

International Journal of Pharmaceutical and Phytopharmacological Research

ISSN (Online) 2249 – 6084

ISSN (Print) 2250 – 1029

Int.J.Pharm.Phytopharmacol.Res. 2011, 1(3): 107-111

(Research Article)

An Evaluation of Antibacterial Activity of *Abelmoschus esculentus* on Clinically Isolated Infectious Disease Causing Bacterial Pathogen from Hospital

Yogesh Chaudhari*[1], Dr. E. P. Kumar[2], Manisha Badhe[3], Hardik R. Mody[1], Vamshikrishna B. Acharya[1]

[1]Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar. India
[2]Karapagam College of Pharmacy, Coimbatore, India
[3] Nandha College of Pharmacy,Kora Palyam Pirivu,Pitchandampalyam,Erode-638052

Received on: *21/11/2011* ***Accepted on:*** *17/12/2011*

ABSTRACT

Six different organic solvents such as n-butanol, petroleum ether, methanol, ethyl acetate and chloroform were used to extract the bioactive compounds from the fruits of Abelmoschus esculentus to screen the antibacterial activity against infectious disease causing bacterial pathogens such as Bacillus subtilis, Streptococcus pyogens, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Proteus mirabillis and Pseudomonas aeruginosa by paper disc method. The butanolic extract of Abelmoschus esculentus was more active against almost 90% of the organism tested. It was followed by Ethyl acetate, Methanol, Petroleum ether, Chloroform in inhibiting the growth of organism tested.

Key Words: *Abelmoschus esculentus*, Pathogens, Antibacterial assay, Malvaceae, Disc diffusion method

INTRODUCTION

Many drug resistant bacterial strains were developed due to the increased use of a number of antibacterial drugs. It also created the problem in controlling the growth of infectious disease causing pathogenic bacteria. Moreover synthetic drugs produce side effect to the users[1].To circumvent this problem, scientists are more interested to develop new antibiotics from unicellular organisms, fungi, algae, and higher plants. Among them, higher plants play an important role, by producing large number of organic compounds as secondary metabolites, which can be used as self-defence. They act as bioactive compounds, chemotherapeutic, bactericidal, and bacteriostatic agents.[2, 3] As a result, anti-microbial substances derived from plants have received considerable attention in recent years. Even though numbers of plant-derived antibiotics were identified, the scientific evaluations of plant derived antibiotics still remain an area of intensive investigation. [4, 5, 6]

Abelmoschus esculentus (Family:Malvaceae) is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits containing round, white seeds. *Abelmoschus* suggesting the musky odour produced by the seeds. This plant is commonly known as okra, gumbo, or lady's finger, and in Southern Asia, usually a variant of "bhindi" or "vendi.". It is a perennial originated in the Ethiopian Hilltops. It grows largely in India, Africa, America, and Brazil. Its fruits are harvested when immature and eaten as a vegetable. Traditionally parts of the plants are assumed to have medicinal properties like antioxidant antispasmodic, demulcent, diaphoretic, diuretic, emollient, and stimulant. [7,8]

Abelmoschus esculentus is a widely cultivated and consumed vegetable in tropical and subtropical countries. It is a source of protein, vitamins C and A, iron, and calcium[9,10] and dietary fiber[11] It contains large quantities of glycans, which are responsible for the viscosity of aqueous suspension[12] and the stringy,

gum-like consistency that is particularly desirable in soups. Nowadays, the most important producing countries are India, Nigeria, Pakistan, Ghana, and Egypt.

METHODS AND MATERIALS

Description of Plant

There are total eighteen accessions of *Abelmoschus esculentus* are observed with genetic variability.[13] It ranges in height from 0.5 to 4 meters, with a main, semi-woody stem, a taproot, and generally five-lobed or five-parted leaves. The conspicuous flower is hermaphroditic, having free petals in yellow colours with a deep red or purple centre. Different varieties in the fruit range in colour from white, green, or purple, depending on the green and red pigmentation. The fruit pod can be as long as 70 cm and straight or curved with four to nine seams. Small spines are found on the mature fruit that often irritate the skin.

Collection of Plant Material

The material in present study is fruit of *Abelmoschus esculentus.* The fruits were collected from Thane district of Maharashtra (India). They were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface. The fruits were then air dried. They were cut in small pieces and dried in shade and made into fine powder. The powder was used for extraction of bioactive compounds.The Herbarium specimen of the *Abelmoschus esculentus* was prepared and preserved in Dr.L.H.Hiranandani College of Pharmacy,Ulhasnagar.

Chemicals and Reagents

Organic solvents such as Ethyl acetate, n-Butaol, Methanol, Petroleum ether and Chloroform used for extraction were of LR grade.

Microbial Culture

Bacteria using infectious diseases both in animals and humans were used in present study. They were both gram positive and gram negative. The stock cultures of *Bacillus subtilis, Streptococcus pyogens, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Proteus mirabillis* and *Pseudomonas aeruginosa* were collected from Dr. L. H. Hiranadani Hospital, Powai, Mumbai. They were subcultured in nutrient media from stock culture 24 hrs. prior to the experiment and used for the bioassay. The slants were prepared from the pure cultures and stored in refrigerator at 4^0 C for further use.

Extraction Procedure:

The fruits were cut into small pieces and dried under shade. The dried fruits were then powdered using a mortar pestle and passed through 40 # mesh. The powdered samples were mixed with different solvents like methanol, n-butanol, chloroform, ethereal, ethyl acetate individually in the ratio of 1:6. They were kept at room temperature for 72 hrs. Each mixture was stirred every 24 h using sterile glass rod. These mixtures were then filtered through Whatman No. 1 filter paper. Extraction procedure was done further twice for complete extraction of the bioactive compounds. The filtrate was then collected in a separate beaker and concentrated by evaporating the solvents. The extracts were then resuspended in the respective solvents before testing it for antibacterial evaluation. They were kept in refrigerator until they use [14].

Preparation of Antibiotic Disc

Sterile empty antibiotic disc were purchased from Hi-media Company. 50 mcg of dried crude extract was dissolved in 1ml of respective solvent. From this stock solution, 10 µl of respective solvent of extract of *Abelmoschus esculentus* was added to the disc (0.5 mg/disc) individually and aseptically. Each disc contained 0.5mg of extract. Then the disc allowed drying at room temperature. After drying they were used for screening the antibacterial activity.

Culture Medium

Muller-Hinton agar medium was used to study the antibacterial activity of the crude extract of fruit of *Abelmoschus esculentus.*

Inoculums Preparation

Pure cultures of bacterial pathogens were removed from nutrient agar slant and transferred to fresh broth and incubated at 37^0 C for 24 h. The turbidity was adjusted to that of standard level by adding sterile broth.

Antibacterial Assay

Antibacterial activity of the fruit extracts were evaluated using disc diffusion method. [15] The discs of Whatman® paper were prepared of the different extracts which were used for the antibacterial assay along with negative discs with respective solvents. Standard antibiotic discs were used as a positive control to compare the antibacterial activity. Muller-Hinton agar medium was prepared and sterilised in an autoclave at 121^0C for 30 minutes at 15 psi then it was transferred into previously sterilised glass petri plates. 0.1 ml of 24h old culture of bacterial pathogens were placed on Muller-Hinton agar medium and spread throughout the plate by spread plate technique.The discs loaded with test extracts, their corresponding solvents and the standard antibiotic were placed with help of sterile forceps carefully with adequate spacing between each other. The plates were kept at room temperature for 30 min, which helps to diffuse the extract on the medium. Later the plates were then incubated at 37^0 C for 24 hrs in an incubator to determine the antibacterial activity of the respective solvent extraction of *Abelmoschus esculentus*. Chloramphenicol antibiotic discs (30 mcg/disc) were used as positive control and the disc with respective solvent (10 ul) was used as negative control. After incubation, zone of inhibition in diameter was measured and recorded. [16,17]

RESULTS AND DISCUSSION:

The efficacy of different extracts of *Abelmoschus esculentus* on antibacterial activity is shown in the Table-1. Ethanolic, butanolic and methanolic extracts of the fruit of *Abelmoschus esculentus* exhibited broad spectrum of antibacterial activity. It was observed in the present study that the butanolic, ethanolic and methanolic extracts inhibited the growth of pathogenic bacteria approximately 90, 70 and 70% respectively. The broad spectrum of antibacterial activity of these extracts was due to the presence of active principle in the extracts. The active principle may be polar compounds like saponins[18] responsible for broad spectrum of antibacterial activity than other extracts[19]. Chloroform extracts of the fruit of *Abelmoschus esculentus* exhibited least antibacterial activity against the bacterial pathogens tested in Table 1, whereas petroleum ether extract did not inhibit the growth of the bacterial pathogens tested in the present study.

In the present study, it was also observed that gram negative bacteria were more sensitive to most of the extracts tested compared to gram-positive bacteria. Among gram positive bacteria *B. subtilis* and *S. pyogens* were sensitive to butanolic and methanolic extract of fruit of *Abelmoschus esculentus* while *S. aureus* and *S. pyogens* were sensitive to ethanolic extract. The resistance were observed by *B. subtilis*, *S. pyogens* and *S. pyogens* to petroleum ether and chloroform extract in gram positive bacteria while in case of gram negative, *E. coli* and *P. aeruginosa*, also showed resistance to petroleum ether and chloroform extract.

In general, gram-negative bacteria were more resistant to antibiotics than gram-positive bacteria.[20,21] The resistance is due to the differences in their cell wall composition. In gram-negative bacteria the outer membrane acts as a great barrier to many environmental substances including antibiotics.[22] Presence of thick murine layer in the cell wall prevents the entry of the entry of the inhibitors.[23] But the present study revealed a controversy report that gram-negative bacteria were more susceptible to the crude extracts than gram-positive bacteria. It may be due to the presence of broad spectrum of antibiotic compounds present in the fruit of *Abelmoschus esculentus*

CONCLUSION

From this study it can be concluded that butanolic, ethanolic and methanolic extract of the fruit of *Abelmoschus esculentus* exhibits antibacterial activity. These extracts can be used to develop new herbal antibacterial formulations in the ethnomedical practice. Further research is need to be carried out to explore the medicinal importance of this plant.

ACKNOWLEDGEMENT

Authors thankfully acknowledge Hyderabad (Sind) National Collegiate Board and Dr. (Mrs.) S. S. Bhalerao Principal, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar for their constant support and guidance.

Table-1: Inhibitory properties of fruit extract of *Abelmoschus esculentus*

Test Organism	Zone of Inhibition in diameter (mm)					
	n-Butanol	Methanol	Petroleum Ether	Chloroform	Ethyl Acetate	Positive control
Gram Positive Bacteria						
B. subtilis	09	07	ND	ND	ND	10
S. aureus	09	ND	ND	ND	08	10
S. pyogens	08	07	ND	ND	07	10
Gram Negative Bacteria						
E. coli	09	10	ND	ND	08	12
K. pneumoniae	08	08	ND	07	07	09
P. aeruginosa	08	07	ND	ND	07	10
P. mirabilis	ND	ND	ND	ND	ND	15

Crude extracts were used for study
Inhibition zone diameter includes the diameter of the disc (06mm)
ND: Antibacterial activity not detected

REFERENCES

1. Tomin E, Tomasz A., Beta-lactam specific resistant mutants of Staphylococcus aureus. Antimicrobiol. Agents Chemother. 1986, 30: 377-383.
2. Evans JS, Pattison E, Moris P., Antimicrobial agents from plant cell culture, in secondary metabolites in plant cell culture (edited by Moris PA, Scraggs A, Stafford A, Flower M) Cambridge University, London. 1986
3. Purohit P, Bohra A., Effect of some plants extracts on conidial germination of some important phytopathogenic fungi. Geobios New Report. 1998, 17:183-184.
4. Cutter CN, Antimicrobial effect of herb extracts against E. coli 0157.H.7. Listeria monocytogensis and Salmonella typhimurium associated with beef. J. Food. Protect. 2000, 63: 601–607
5. Jain P, Bansal D, Bhasin P, Anjali, Antimicrobial activity and phytochemical screening of five wild plants against Escherichia coli, Bacillus subtilis and Staphylococcus aureus, Journal of Pharmacy Research, 2010, 3(6), 1260-1262.
6. Shirazi M.H., Ranjbar R, Eshraghi S, Sadeghi G, Jonaidi N, Bazzaz N, Izadi M, Sadeghifard N, An Evaluation of Antibacterial Activity of Glycyrrhiza galbra Extract on The Growth of Salmonella , Shigella and ETEC E.coli, Journal of Biological Sciences, 2007, 7 (5), 827-829.
7. Ansari NM, Houlihan L, Hussain B, Pieroni A, Antioxidant activity of five vegetables traditionally consumed by south-Asian migrants in Bradford, Yorkshire, UK, Phytotherapy Research, 2005, 19(10), 907–911.
8. Oladimeji A, Kannike MA, Comparative studies on the efficacy of neem, basil leaf extracts and synthetic insecticide, lambda-cyhalothrin, against Podagrica spp. on okra, African Journal of Microbiology Research, 2010, 4 (1), 033-037.
9. Aworh, O. C., Olorunda, A. O., and Akibo, O., Quality attributes of frozen okra as influenced by processing and storage. Journal of Food Technology, 1980, 15, 429–433.
10. Ihekoronye, A. I., and Ngoddy, P. O., Integrated food science and technology for the tropics London: Macmillan. 1985, 205–206, 293.
11. Adom, K. K., Dzogbefia, V. P., Ellis, W. O., and Simpson, B. K., Solar drying of okra-effects of selected package materials on storage stability. Food Research International, 1996, 29(7), 589–593.
12. Owoeye, A. L., Laurie, M. C., Allagheny, N. N., and Onyezili, F. N., Chemical and physical parameters affecting the viscosity of mixed okra and tomato homogenate. Journal of the Science of Food and Agriculture, 1990, 53, 283–286.
13. Ariyo, O. J., Measurement and classification of genetic diversity in okra (Abelmoschus esculentus). Annals of Applied Biology, 1990, 116: 335–341
14. Khan NH, Kamal MSA, Rahman M, Antibacterial activity of Euphorbia thymifolia Linn., Indian J Med Res., 1988, 87, 395-397.

15. Bauer AW, Sherris TM Kirbay WMM, Turk M., Antibiotic susceptibility of testing by a standard single disc method. Amer. J.Clinical Pathol. 1966, 45: 493-496.
16. Murray PR, Baron EJ, Pfallar MA, Tenover FC, Yolke RH, Manual of clinical microbiology, 6ed, ASM, Washington, DC, 1995.
17. SathishR, Ahmed MH, Natarajan K, LalithaKG, Evaluation of wound healing and antimicrobial activity of Lannea coromandelica (Houtt) Merr, Journal of Pharmacy Research, 2010, 3(6), 1225-1228.
18. Singh R, Gupta AK., Antimicrobial and antitumor activity of the fractionated extracts of Kulimusli (Curculgo orchioides). Intl. J. Green Pharamcy. 2008, 2(1): 34-36.
19. Kafaru E. Immense Help from Nature's Workshop. Elikat Health Services, Lagos. 1994, 31-210.
20. Paz EA, Lacy RN, Bakhtiar M. The betalactum antibiotics penicillin and Cephalosporin in Prespective Hodder Stongton, London, 1995, 227.
21. Chowdhury AA, Islam MS. Antibacterial activity of Trema orientalis. Dhaka University J. Pharamaceutical Sci. 2004, 3(1-2): 115-117.
22. Tortora GJ, Funke BR, Case CL. Microbiology: An Introduction. Benjamin Cummings, San Francisco. 2001, 88.
23. Martin GJ. Ethnanobotany, A Methods Manual. Chapman and Hall, London. 1995

***Corresponding Author:** *Mr. Yogesh S. Chaudhari*
Dr. L. H. Hiranandani College of Pharmacy,
Ulhasnagar -421003, Maharashtra, India
Tel. No.: +91-0251-2561461
Email ID: replytoyog@gmail.com