Borneo Journal of Medical Sciences (2016) volume 10, issue 2, pp: 19-25

Effect of Ethanolic extract of *Aloe vera* gel on certain common clinical pathogens

Halima Begum[1*], Sadia Choudhury Shimmi[2], Mahfuza Mazeda Rowshan[3], Sayeda Khanom[4]

[1] Department of Pharmacology, Enam Medical College, Savar, Dhaka, Bangladesh;

[2] Department of Biomedical Science and therapeutics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia;

[3]Department of Pharmacology, Sir Salimullah Medical College and Mitford Hospital, Dhaka, Bangladesh;

[4]Department of Pharmacology, Central International Medical College, Dhaka, Bangladesh.

[*]Corresponding author's e-mail: halimadnmc@yahoo.com, Tel no: +8801951521350,+8801917964899

(Received: June 10, 2015; Accepted: August 21, 2015)

ABSTRACT

Antimicrobial activity of ethanolic extract of Aloe vera were observed against *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae* and *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* . Aloe vera leaf gel was used for ethanolic extraction.Zones of inhibition in millimeter was used to measure the antimicrobial effect. Antimicrobial susceptibility test showed that ethanolic extract has growth inhibitory effects against the tested pathogens. Ethanolic extract of Aloe vera has growth inhibitory effects against tested pathogen seen by antimicrobial susceptibility testing.

Keywords: *Aloe vera*, drug resistance, antibacterials.

INTRODUCTION

Untimely death due to infectious disease has become a global concern world-wide[1, 2, 3]. Due to the rapid emergence pathogens, the clinical efficacy of many existing antibiotics are being threatened [4, 5]. Throughout the history of herbals are used to treat infectious diseases[6, 7]. Pure compounds or as formulated with measured constituents of plant extracts obtained from natural product, provide tremendous scope for new drug development. Plant origin antimicrobials are very limited side effects converse to the synthetic drugs[8] and have promising therapeutic possibility to treat many infectious diseases. There is need to discover new antimicrobials with novel mechanism of action for new and re-appearing infectious diseases[9, 10]. Present analysis was carried out toward traditional plants identification that are effective against the common

pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa K.pneumonia, S.pyogenes, S. saprophyticus* and *S. pneumonia*.

Even though these are very serious human pathogens and often associated with nosocomial infection, medicinal plants effective against all these pathogens put together and a systematic study thereafter towards purification of bioactive components is still scanty. Pharmaceutical, cosmetic and food industries are mainly used *Aloe vera* products[11]. Aloes grown mainly in the dry regions of North America, Europe and Asia. *Aloe vera* (*Aloe barbadensis Miller*.), in Liliaceae family, has leaves30-50 cm long and 10 cm broad, 25-35 cm in length bright yellow tubular flowers arranged in a slender loose spike; stamens frequently project beyond the perianth tube [12]. Aloe vera contains over 200 active compounds and 75 nutrients like vitamins, minerals, sugars, enzymes, lignin, anthraquinones, salicylic acid, saponins and amino[13]. It has been reported that the antimicrobial constituents of organic extracts of Aloe *vera* gel have diverse activity against several human clinical pathogens either by inhibiting the growth or effective killing[14, 15]. Because of increasing trends of development of antimicrobial resistance, purpose of present study was to observe the effect of ethanolic extract of Aloe vera counter to some common clinical pathogens with emphasis on the acceptability of Aloe vera as a natural medicine against various infections.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology at Enam Medical College Savar, Dhaka, during the period of December 2014 to February 2015(3 months) and a total of 105 different bacterial isolates were tested for antibacterial activity of Ethanolic extract of Aloe vera. Ethical permission (EM22014) was taken.

Preparation of extract

Leaves of Curacao Aloe (*Aloe barbadensis or Aloe vera*) collected from Savar, Dhaka. The plant was verified by taxonomist in Department of Botany, Dhaka University. Fresh leaves of mature and healthy Aloe vera were washed in tap water for 5 minutes and cleaned with sterile distilled water. Then by using a sterile knife, the leaves were dissected longitudinally and the colorless aloe gel (parenchymatous tissue) was scraped out without the fibers. Drained gel was air dried in the oven at 80˚C for 48 hours. Dried gels were ground to obtain powder by using mortar and pastel and 30g of this powder was soaked into 300ml of ethanol for 4 days for proper extraction of the active ingredients at room temperature[16]. This was later filtered by Whatman filter paper No.1 and the filtrates evaporated to dry the extract using a rotatory evaporator .The supernatant was stored in refrigerator at 4˚-C after collection. Before being used for antimicrobial susceptibility testing the extracts were dissolved in sterile water.

Antimicrobial susceptibility testing

The test organisms selected were: *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Staphylococcus saprophyticus*, *Staphylococcus pneumonia* (Gram-positive) and *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Gram-negative).The bacterial isolates were collected from the Department of Microbiology, Enam Medical College. All the isolates were identified by standard biochemical methods[17]. At room temperature seven bacterial cultures were maintained in nutrient agar medium and were sub cultured every two weeks into newly prepared nutrient agar slants. Sterile agar (at 45˚C) was poured into sterile Petri dishes. The plates were allowed to gel for an hour, which had been inoculated with test organisms. Standardization of inoculums and ethanolic extract of Aloe vera were done using McFarland Standards No. 0.5(tube) and corresponding American Type Culture Collection (ATCC) strains. Antimicrobial susceptibility testing of isolates were done by Disc Diffusion Method[18] using sterile filter paper discs (Whatman filter paper No.1) 6 mm in diameter. For bioassay against bacteria ethanolic extracts were used. Sterile disc with 6mm diameter were loaded with 0 .1ml of extract and introduced into sterile medium with the test organisms. The plates were incubated at 37˚C for 24 hours. The results of antibiogram were evaluated by measuring the zone of inhibition around the disc and expressed in millimeter.

RESULTS

Ethanolic extract of Aloe vera gel showed varying degree of response regarding antibacterial property against the tested pathogens. The zone of inhibition with ethanolic extracts ranged from 12.5 – 22 mm. Zone of inhibition was maximum for *S. aureus* and minimum for *E. coli*. Normally the extracts showed less antibacterial activity against Gram- negative in comparison to Gram - positive bacteria .The maximum zone of inhibition was 12.5 mm for *E. coli*, 13.87 mm for *P. aeruginosa*, 13.22 mm for *K .pneumoniae*, 22 mm for *S. aureus*, 20 mm for *S. saprophyticus* and 15 mm for *S. pneumoniae* and 18 mm for *S .pyogenes* (Figure 1).

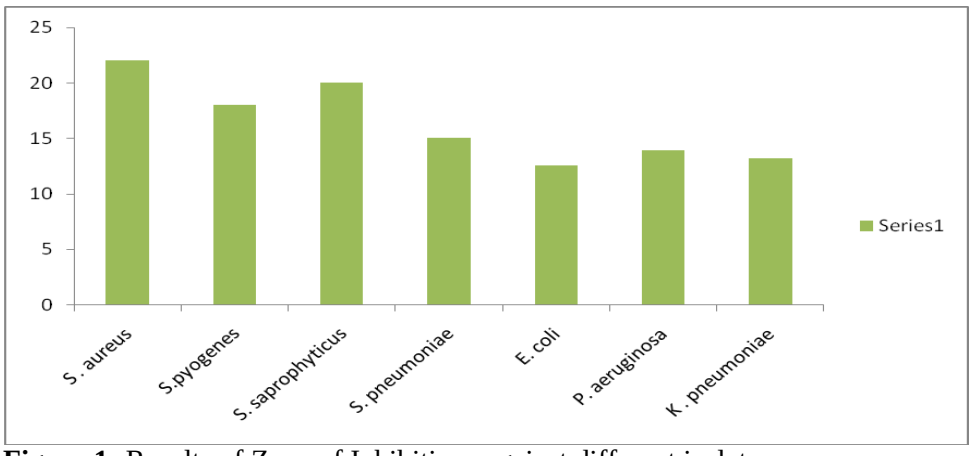

Figure 1: Results of Zone of Inhibitions against different isolates

DISCUSSION

Antibacterial activity of ethanolic extract of Aloe vera was evaluated on the basis of zone of inhibition (ZIs) by disc diffusion method in the present study. This ethanolic extract showed different degree of inhibitory effects against the test pathogens. Among the Gram- positive and Gram- negative pathogens ,ethanolic extract of Aloe vera showed good inhibitory effect on *S.aureus* (22 mm) and the findings were similar with the findings of Agarry Oo(18mm), Lawrence R et al. (15.66 mm) and Ibrahim et al[16, 19,20]. The extracts also inhibited the growth of *S. saprophyticus* 20 mm and 15 mm for *S.pneumoniae*, *S. pyogens* (18mm) and the findings are almost consistent with the findings of another study conducted by Lawrence R et a l(16 mm)[19]. In the present study of ethanolic extract of Aloe vera exhibited growth inhibitory action against E. coli (12.5 mm), 13.87mm for *P .aeruginosa*, 13.22mm for *K.pneumoniae*. The findings of Zone of Inhibitions (ZIs) are similar with findings of Lawrence for E coli but less for P. aeruginosa and K. pneumonia[19]. Lawless and Allan and Pugh et al screened antimicrobial activity against different pathogens and observed minimum inhibitory activity against *E.coli*[14,15] where as Alemdar and Agaoglu in their study found no inhibitory effect against *E.coli*[21], due to presence of additional lipopolysaccharide layer a lower antibacterial activity against Gram-negative microorganism may be seen. Furthermore, maximum zone of inhibition against *E.coli* was 12.5 mm in this study. The finding correlates with the antimicrobial activity of ethanol observed in this study. Lawrence R et al in their study isolated the antibacterial compounds from *Aloe vera* gel extracts and found Pyrocatechol, cinnamic acid, p-coumaric acid and ascorbic acid. Possible mechanism of antibacterial actions of that compounds are as - Hydroxylated phenol- pyrocatechol, which is toxic to microorganisms and its presence in Aloe vera extract[22, 23].The sites and hydroxyl group's number on the phenol are thought to be relative toxicity to microorganism and increased in hydroxylation results in more toxicity. Cinnamic acid in Aloe vera also proved by Dukes phytochemical databases acts by inhibiting resting bacterial glucose uptake and ATP production[24, 25].

CONCLUSION

Globally bacterial resistance of antimicrobial agents is an important human health concern. Hence, there is need to find out new antimicrobial agents to combat this situation. Thus having auspicious antibacterial activity and less adverse effects Aloe vera could be used as an effective antibiotic substitute herbal remedy. Hence, we concluded that Aloe vera gel extract has opposing effect on the growth of different bacterial pathogens. In vivo study is recommended to determine its mechanism of action, doses and toxicity.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Iftikhar Ahmed, Head Dept of Microbiology, EMC and all the other teachers and staffs from Department of microbiology for their technical support.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. Mahady GB. (2005). Medicinal plants for the prevention and treatment of bacterial infections. *Curr. Pharm. Des* 11: 2405-27.

2. Kumar MS, Kirubanandan S, Sripriya R, Sehgal PK.Triphala. (2008). Promotes healing of infected full-thickness dermal wound. *J. Surg. Res* 144: 94-101.

3. Sakata H, Toyonaga Y, Sato Y, Hanaki H, Nonoyama M, Oishi T , Sunakawa K. (2009). Nationwide survey of the development of drug Resistance in the pediatric field: drug sensitivity of Haemophilus influenzae in Japan. *J. Infect. Chemother* 15: 402-9.

4. Westh H, Zinn CS, Rosdahl VT. (2004). An international multicenter study of antimicrobial consumption and resistance in Staphylococcus aureus isolates from 15 hospitals in 14 countries. *Microb. Drug Resist* 10: 169-76.

5. Penner R, Fedorak RN, Madsen KL. (2005). Probiotics and nutraceuticals: non-medicinal treatments of gastrointestinal diseases. *Curr. Opin. Pharmacol* 5: 596-603.

6. Mitscher LA, Drake S, Gollapudi SR, Okwute SK. (1987). A modern look at folkloric use of anti-infective agents. *J Nat Prod* 50: 1025-40.

7. Wadud A, Prasad PV, Rao MM, Narayana. (2007). A Evolution of drug: a historical perspective. Bull Indian Inst. *Hist. Med. Hyderabad* 37: 69-80.

8. Mukherjee PK, Wahile A. (2006). Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *J Ethno pharmacol* 103: 25-35.

9. Hazni H, Ahmad N, Hitotsuyanagi Y, Takeya K, Choo CY. (2008). Phytochemical constituents from Cassia alata with inhibition against methicillin-resistant Staphylococcus aureus (MRSA). *Planta Med* 74: 1802-5.

10. Barbosa LN, Rall VL, Fernandes AA, Ushimaru PI, da Silva Probst I, Fernandes A. (2009). Essential oils against food borne pathogens and spoilage bacteria in minced meat. *Food borne Pathog. Dis* 6:725-8.

11. Eshun K and Q He. (2004). Aloe versa: A Valuable ingredient for food, pharmaceutical and cosmetics industries: A review. *Crit.Rev.Food, sci.Nutr* 44(2): 91-6.

12. WHO Monographs on Selected Medicinal Plants, World Health Organization, Geneva 1999; 1. ISBN:9241545178. http://whqlibdoc.who.int/publications/1999/9241545178.pdf.

13. Park, Y.I. and T.H.Jo. (2006). Perspective of industrial Application of Aloe vera.In: park, Y.I. and S.K.Lee (Eds.). New Perspective on Aloe. Springer Verlag, New York, USA : 191-200. ISBN:0387317996. http://rapidshare.com/files/46675452/New_perspectives on aloe.pdf.

14. Lawless J, Allan J. (2000). The clinical Composition of Aloe vera, In: Aloe vera natural wonder cure. Thorsons, Publishing Ltd., London,United Kingdom : 161-71.

15. Pugh N, Ross SA, ElSohly MA,Pasco DS. (2001). Characterization of Aloeride, a new high molecular weight polysaccharide from aloevera with potent immunostimulatory activity. *J. Agri.Food.Chem* 49(2): 1030-4.

16. Agarry OO, Olaleye MT, Bello-Michael Co. (2005). Comparative antimicrobial activities of Aloe vera gel and leaf. *African Journal of Biotechnology* 4(12): 1413-4.

17. Cheesbrough M. (2000). Summary of the clinical and laboratory features of microorganism in District Laboratory Practice in Tropical Countries. Part-2, Cambridge University press, UK: 105-94.

18. Bauer AW, Kirby WM, Sherris JC,and Turck M. (1966). Antibiotic susceptibility testing by a standardized simple disk method. *American Journal of Clinical Pathology* 45:493-6.

19. Lawrence R, Tripathi P, Jeyakumar E. (2009). Isolation, Purification and evaluation of antibacterial agents from *Aloe vera*. *Brazilian journal of Microbiology* 40: 906-15.

20. Ibrahim M, Srinivas M, Lakshmi Narassu M. (2011). Phytochemical analysis and antimicrobial evaluation of *Aloe vera* gel against some human and plant pathogens. *Asian Journal of Current Chemistry* 1(1):1-11.

21. Alemdar S, Agaoul S. (2009). Investigation of in vitro Antimicrobial Activity of Aloe vera juice. *J.Amin.Vert.Adv* 8(1): 99-102.

22. Kametani S,Yuasa Ak,Kikuzaki H, Kennedy DO,Honzawa M, Yuasa M. (2007). Chemical constituents of Cape Aloe and their synergistic growth inhibiting effect on Ehrlich Ascites Tumor Cells. *Biosci.Biotechnol.Biochem* 71(5): 1220-9.

23. Cown MM. (1999). Plant products as Antimicrobial Agents. *Clin. Microbiol. Rev* 12(4): 564-82.

24. Duke JA. (1992). Handbook of Phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL. CRC Press: 320-40.

25. Kouassi Y, Shelef LA. (1998). Inhibition of Lysteria monocytogenes by cinnamic acid: possible interection of the acid with cysteinyl residues. *J. Food Safety* 18(3): 231-42.