ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

Phyto-Chemical and Anti-Bacterial Screening of Musabbar Prepared from *Aloe Vera*

M. Zahurul Haque[1*], M. A. Jalil[2], M. Badrul Islam[2]

[1]*BCSIR Dhaka- 1205,* [2]*BCSIR Laboratories, Rajshahi-6206, Bangladesh*

**Corresponding author: mdzahurulhaque@yahoo.com*

ABSTRACT

A quality musabbar has been prepared from the processed leaves juice of *Aloe vera* by a technology developed in BCSIR Laboratories, Rajshahi. The product, musabbar was successively extracted with different solvents on the basis of increasing polarity e.g. n-hexane, ethyl acetate, methanol and water at room temperature. The extracts were studied for their bioactivity against 12 pathogenic bacteria and found most of them highly active. The MIC values of the extracts were also determined. The bioassay indicates cytotoxicity as well as a wide range of pharmacological activities of musabbar.

Keywords: Stabilityconstant, Binarycomplex, Bivalentmetal, Nicotinicacid, Potentiometry.

1. INTRODUCTION

Aloe vera Linn. is a cactus-like plant which is belongs to Liliaceae family. There are over 300 varieties of the Aloe plant, but it is the *Aloe barbadensis* variety that exhibits the best medicinal properties. It is occasionally found to grow wild as planted in gardens in many areas of the country as an ornamental plant [1]. Young and offsets are planted in rows about 50 cm apart just after the rains break. The cutting of leaves is made annually in March and April, during the heat of the day. Each plant continues to yield Aloes for about 12 years, after which the plants are dug up [2]. Drying may be done artificially or directly in sun. *Aloe vera* has a long history as a safe the effective medicine and skin care. Most people in U.K. today know of *Aloe vera* because of its inclusion in many popular cosmetics products. Over the years, the plant has been known by a number of names such as the "Wand of Haven", "Haven's Blessing", "Silent Healer", "First Plant" etc. At present time, folk remedies in Japan, South Africa, China, India and Mexico have used aloe for treatment of gastrointestinal, liver, skin and for the treatment of bronchitis, asthma, burns, hair loss and arthritis [3].

Aloe vera has been found to contain many active components. Some of these are mentioned below.

Anthraquinones (anti-inflammatory and pain killing properties), polysaccharides (antiviral and immuno-potentiating action), enzymes (improves digestion and the absorption of nutrients as well as elimination of waste), salicylic acid (an aspirin like compounding possessing analgesic, anti-inflammatory and anti-bacterial properties), lignin (it endows *Aloe vera* with a penetrative effect, so the other ingredients are absorbed into the skin), saponins (they have cleansing and antiseptic properties, acting against bacteria, viruses, fungi and yeast), fatty acids (important anti-inflammatory agents), hormones (wound healing and anti-inflammatory properties) and amino acids [4, 5]. *Aloe vera* gel provides 20 of 22 necessary amino acids required by the human body and 7 of 8 essential amino acids, which the body cannot synthesize these must be ingested in food. *Aloe vera* contains vitamin A, B_1, B_2, B_6, C, Folic acid and E and minerals (calcium, magnesium, potassium and sodium are all present in significant). Mussabar is prepared from the processed leaves juice of Aloe plant. Huge amount (about 200 M.T.) of musabbar is imported from abroad to meet the home consumption. That is why the plant is very important in traditional uses in Ayurvedic, Unani and pharmaceutical preparations. Hence attempts were made for screening of phyto-chemical and anti-bacterial properties of musabbar prepared from *Aloe vera* leaves juice. The details of the work are described in the present paper.

2. MATERIAL AND METHODS

2.1. Collection of plant materials

For the present study fresh leaves of ghritakumari (*Aloe vera)* plants were collected from the experimental field of BCSIR Laboratories, Rajshahi and washed with water to remove the soil and other adhering.

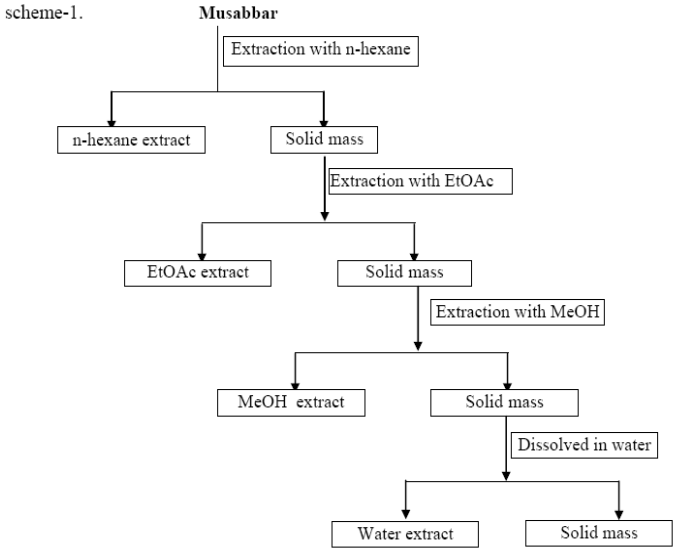
2.2. Production of musabber

Cleaned and washed ghritakumari (*Aloe vera)* leaves (5 kg) were taken and extracted the fillet from the leaves with the help of a chopper. A grinding machine followed by cloth filtration then crushed it. Through this, the suspended particles as well as small pieces of leaves and other impurities were removed from the juice. The clear dense juice obtained was then transferred to a flat metallic vessel and dried in an oven at

35°-40°C for 4 hours. At this stage a semi-solid product was obtained which on triturating with starch (5 gm) yielded the desired musabbar (500 gm). This process for the production of musabbar was found to be economically feasible.

2.3. Fractionation of musabbar

The prepared product, musabbar was then powdered by a crushing machine and sieved (200 mesh). The powder was then successively extracted with n-hexane, ethyl acetate, methanol and water by means of cold extraction. A flow sheet of the extraction and fractionation process of musabbar is given in the following scheme-1



2.4. Phytochemical features of the extracts

For identifying the presence of possible classes of chemical components a phytochemical screening of the extracts was done following the standard methods (International Encyclopaedia of Chemical Science, 1964). A brief description of the methodology is given below.

Test for anthraquinone (Schutzen-Berger): To an alkaline solution of the extract (0.30 gm) was added sodium hydrosulfite. A red colour was produced by anthraquinone. On exposure of the solution to the air, the anthraquinone was regenerated.

Test for carbohydrate (polysaccharides) (Benedict's): To 0.5 ml extract was added 5 ml of benedict's solution and boiled for 5 minutes. Formation of a coloured precipitate indicated the presence of carbohydrates.

Test for lignin (Dahlmann): This test was made by mixing 2 drops of aniline and a few drops of dilute sulfuric acid and applying to the portion of extracts. Formation of yellow colour indicated the presence of lignin.

Test for saponins: The extract gave a greenish coloration with ferric chloride in the presence of alcoholic sulphuric acid indicated the presence of saponins.

Test for amino acids (Deniges): To a small quantity of the extracts was added 0.2 ml aqueous solution of triketohydrinene hydrate and boiled for 1 minute. A characteristic blue-violet colour indicated the presence of amino acids.

2.5. Determination of anti-bacterial activity

The bacterial strains used for this investigation are as follows: **Gram positive:** *Bacillus megaterium (*QL-38), *Bacillus subtilis (*QL-40*), Sarcina lutea (*QL-166), *Staphylococcus aureus (*ATCC-259233), *Streptococcus-β-haemoliticus (*CRL) and *Bacillus megaterium (*QL-38). **Gram negative:** *Shigella sonnei (*AJ-8992), *Pseudomonas aeruginosa (*CRL), *Escherichia coli (*FPFC-1407), *Shigella dysenteriae (*AL-35587), *Shigella shiga (*ATCC-26107), *Klebsiella sp.* and *Salmonella typhi.*

2.6. Preparation of the test plates

The test organism was transferred from the fresh subculture to the test tube containing 15 ml autoclaved medium with the help of an inoculating loop in an aseptic condition. Then the test tube was shaken by rotation to get a uniform suspension of the organism. The bacterial suspensions were immediately transferred to the sterile petridishes in an aseptic area. The petridishes were rotated several times, first clockwise and then anticlockwise to assure homogenous distribution of the test organisms. The media were poured into petridishes in such a way as to give a uniform depth of approximately 4 mm. Finally, after medium was cooled to room temperature in laminar air flow unit, it was stored in a refrigerator (4°C).

2.7. Preparation of test sample and discs

The extracts (n-hexane, ethyl acetate, methanol and water) (10 mg each) were dissolved in 1 ml DMSO in separate test tube. Thus the concentrations were 10 mg/ml for each extract. Three types of discs were prepared for antibacterial screening: (a) Sample discs (n-hexane, ethyl acetate, methanol and water extracts), (b) Standard discs (Amoxicillin) and (c) Control discs. Sample discs: Sterilized metrical (BBL, Cocksrvile, U.S.A) filter paper discs (4 mm diameter) were taken in a blank petridish. Sample solution of desired volume (20µl) was applied on the discs with the help of a micropippette in an aseptic condition. The discs were left for a few minutes in an aseptic condition for complete removal of solvent. Standard discs: These were used to compare the antibacterial activity of test materials. In the present study Amoxicillin (30µg/ disc) was used as standard discs. Control discs: it was made by applying solvent of 20µl on a sample discs.

2.8. Placement of the discs and incubation

By means of a pair of sterile forceps, the dried samples discs and standard disc were placed gently on the solidified agar plates seeded with the test organisms to ensure contact with the medium. The plates were then kept in a refrigerator at 4°C for 24 hours in order to provide sufficient time to diffuse the antibiotics into the medium. Finally, the plates were incubated at 37.5°C for 24 hours in an incubator.

2.9. Measurement of the zones of inhibition and MIC values

After incubation, the antibacterial activities of the test samples were determined by measuring the diameter of inhibitory zones in term of mm with a transparent scale. Minimum inhibitory concentration (MIC) values of the compounds were also determined.

3. RESULTS AND DISCUSSION

A quality musabbar is produced by extracting the juice from leaves of *Aloe vera* at low temperature under reduced pressure avoiding heating at higher temperature. Because many of the active ingredients contained in *Aloe vera* leaves juice appear to deteriorate on storage. Rather, the product is sensitivity to enzymatic, oxidative or microbial degradation on heating during extraction [2]. By our developed process, the active ingredients contained in the product remain unchanged. The qualitative chemical examination of various extracts of musabar revealed the presence of anthraquinine, lignin, polysaccharide, amino acids and saponin type of compounds. The results are summarized in Table 1. The antibacterial activity of different extracts (n-hexane, ethylacetate, methanol, and water) and Amoxicillin standard was tested against 12 bacteria at different concentration of 30 μg/disc and 200 μg/disc [6, 7]. Here 30 μg/disc of each extracts showed very weak antibacterial activity against the tested pathogenic bacteria compared to those of standard Amoxcillin (30 μg/disc). However the disc of 200 μg/disc of each compounds exhibited better antibacterial activity against both of gram positive and gram negative bacteria as shown in Table 2. From the results presented in Table 2, it is evident that the all the extracts showed enhanced activity against all the pathogenic bacteria. Only few pathogens such as *Staphylococcus aureus*, *Shigella dysenteriae* and *Klebsiella* sp. did not show any inhibition with water extract. Similarly, the pathogens such as *Streptococcus-β-haemoliticus, Escherichia coli* and *Salmonella typhi* did not show any inhibition with n-hexane, ethyl acetate and methanol respectively. The zone of inhibition was found to vary from 8-22 mm.

The MIC values of the compounds against 2 Gram positive bacteria (*Bacillus subtilus* & *Bacillus megaterium*) and 2 Gram negative bacteria (*Shigella shiga* & *shigella sonnei*) are shown in the Table 3.

From Table 3, it is observed that for n-hexane extract the MIC value was 96 μg/ml against 2 pathogenic bacteria *Bacillus subtilus* & *shigella sonnei* and 32 μg/ml against another 2 bacteria *Bacillus megaterium* & *Shigella shiga*. The MIC value of ethyl acetate extract was 72μg/ml against 3 pathogenic bacteria *Bacillus subtilus, Bacillus megaterium* & *Shigella shiga* but 132 μg/ml against *shigella sonnei*. The MIC value of water extract was 120μg/ml against bacteria *Bacillus subtilus* & *shigella sonnei* and 56 μg/ml against *Bacillus megaterium* & *Shigella shiga*. In case of methanol extract, the MIC value was 128μg/ml against bacteria *Bacillus subtilus* & *Shigella shiga* and 156 μg/ml against *Bacillus megaterium* & *shigella sonnei*, which was relatively higher. The MIC value for Amoxicillin standard was 18μg/ml, 16μg/ml, 12μg/ml and 22μg/ml against bacteria *Bacillus subtilus, Bacillus megaterium, Shigella shiga* and *shigella sonnei* respectively. A good MIC value of these extracts obtained from musabbar indicates its justification as a potent anti-bacterial compound. Further investigation is needed to elucidate whether it is possible to use it as an antibiotic against both gram positive and gram-negative pathogenic bacteria.

Table 1: Phytochemical screening of musabbar

Class of compounds indicated	Extracts			
	n-Hexane	Ethyl acetate	Methanol	Water
Anthraquinie	+	+	+	+
Polysaccharides	-	-	+	+
Lignin	-	+	-	-
Saponins	-	-	+	+
Amino acids	+	+	-	-

Table 2. Antibacterial activity of different extracts of musabbar and Amoxicillin standard

Test organisms	Zone of inhibition (mm)				
	n-hexane ext. (200µg/dsic)	Ethyl acetate ext. (200µg/dsic)	Methanol ext. (200µg/dsic)	Water ext. (200µg/dsic)	Amoxicillin (30 µg/disc)
Bacillus megaterium	18	15	11	9	40
Bacillus subtilis	15	10	20	15	45
Sarcina lutea	14	8	13	17	45
Staphylococcus aureus	17	19	14	-	34
Streptococcus-β-haemoliticus	-	12	9	10	36
Shigella sonnei	10	13	18	8	36
Pseudomonas aeruginosa	20	9	22	12	35
Escherichia coli	19	-	12	10	30
Shigella dysenteriae	9	20	12	-	40
Shigela shiga	13	8	10	15	30
Klebsiella sp.	22	9	11	-	33
Salmonella typhi	11	16	-	8	45

Table 3. MIC values of different extracts of musabbar and Amoxicillin standard

Test Bacteria	Compounds				
	n-hexane ext. (µg/ml)	Ethyl acetate ext. (µg/ml)	Methanol ext. (µg/ml)	Water ext. (µg/ml)	Amoxicillin (µg/ml)
Shigella sonnei	96	132	156	120	22
Shigela shiga	32	72	128	56	12
Bacillus megaterium	32	72	156	56	16
Bacillus subtilis	96	72	128	120	18

4. REFERENCES

1. Ghani A. *Medicinal Plants of Bangladesh Chemical Constituents and Uses.* Published by Asiatic Society of Bangladesh, 1998, 72.
2. Grindlay D, Reynolds T. *J ethnopharmacol*, 1986; **16:**117-151.
3. Coats BC. *The Silent Healing of Modern Study of Aloe vera.* Texas, 1979, Garland,
4. Shelton MS. *International Journal of dermatology,* 1991; **30:** 679-683.
5. Hirat T, Suga T. *Cosmetics and toiletries,* 1983; **98:**105-108.
6. Bauer AW, Kirby WMM, Sherries JC, Turck M. *Amer J Clin Pathol,* 1966; 44: 493-496.
7. Reiner R. *Detection of antibiotic activity. In antibiotic an introduction.* Roche Scientific Service, Switzerland, 1982; **1:** 21.