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Comparitive antibacterial potential of natural extracts against selected bacteria

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ABSTRACT
The present study was aimed to determine the antibacterial activity of methanolic extracts of azolla and cashew nut shell. The antibacterial activity was measured in terms of their corresponding minimum inhibitory concentration (MIC) by employing broth dilution technique against S.aureus ATTCC 25923, K.pneumoniae ATCC700603 and P.aureginosae ATCC27853.The MIC values of cashew nut shell extract against S.aureus, K.pneumoniae and P.aureginosae were 0.125mg.ml^{-1}, 0.25mg.ml^{-1}and 0.25mg.ml^{-1}respectively. The MIC values of azolla extract against S.aureus, K.pneumoniae and P.aureginosae were 0.625mg.ml^{-1}, 1.25mg.ml^{-1}and 1.25mg.ml^{-1}respectively.The antibacterial activity was compared with the standard drug enrofloxacin against S.aureus, K.pneumoniae and P.aureginosae. Among the tested plant extracts cashew nut shell offered better antibacterial activity when compared to that of azolla.

Key words: antibacterial activity, MIC, azolla , cashew nut shell

I. INTRODUCTION

Bacterial infections are the general and most common complaints among various disease conditions**.** In postoperative conditions and in the treatment of viral infections secondary bacterial infections aggravate the disease scenario which may lead to prolonged recovery from the ailment. Due to the increase in the length of the disease period, the feed intake and productivity is going to be affected in livestock. Several classes of antibiotics are available in the market to reduce the bacterial load. But bacterial infections are not reducing in the same trend as like that of the increased availability of modern drugs. The development of resistance by the bacteria to the antibiotics plays a pivotal role in reduction of the antibiotic efficacy[1].To combat the resistance, the rationale in using the antibiotics has to be checked by the practioner. The sensitivity pattern of the bacteria to antibiotics was also altered due to indiscriminate usage of antibiotics. Another important strategy to overcome the resistance is natural alternatives to antibiotics obtained from plants commonly called as phytobiotics. If the bacteria are exposed to natural products the mechanism of exposure is altered thereby resistance development can be reduced. By keeping these things in view the present study was designed to determine the antibacterial potential of azolla and cashewnut shell extracts against S.aureus ATTCC 25923, K.pneumoniae ATCC700603 and P.aureginosae ATCC27853.

Cashewnut shell consists of 60-65% anacardic acid ,15-20% cardol,10% of cardonol[2]. Cashew nut shell liquid possess various pharmacological properties such as antioxidant, antibacterial, antifungal, antiparasitic, antitumor, antiulcerogenic activities. The derivative of cashew nut shell liquid 2-hydroxy6-pentadecylbenzamide possess significant antibacterial activity against S. aureus and E. coli[3]. Cashew pulp i.e cajuína protected S. typhimurium from damage induced by aflatoxin B1[4]. The methanolic extract of cashew bark at the rate of 500-2000 µg/ml prevented the doxorubicin induced damage in chineese hamster fibroblasts[5]. In addition cashew pulp posseses anticlastogenic and antimutagenic activity[6].. The protection against genotoxicity may be attributed due to its tannic acid. The antibacterial activity against S.

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mutans and the inhihition of its biofilm formation makes its clininal usage in dental caries [7]. Cashew shells also contain a significant amount of gallic acid[8]. The phytochemicals in cashew responsible for cholinesterase activity are cardol, cardanol, carbachol and anacardic acid. [9,10].

Azolla ,blue green algae commonly used as feed supplement in livestock practice to improve the body weight gain and feed conversion efficiency[11]. Azolla also contains probiotics and biopolymers. Azolla is the first fern whose nuclear genome has been entirely sequenced [12]. Flavonoids, hormones, alkaloids, phenols, triterpenoid derivatives, amino acid and fatty acid types are among the valuable phytochemicals present in Azolla. These bioactive components have antioxidant, anticarcinogenic, anti-inflammatory, antidiabetic, hepato- and gastroprotective, antiviral, neuroprotective, cardio-protective, and anti-hypertensive activities[13]. Tannins, phenolic content, and flavonoids are among the phytoconstituents found in Azolla extracts, which are responsible for antioxidant action[14].

Materials and Methods

The experiment was carried out in the Department of Veterinary Pharmacology &Toxicology,NTR College of Veterinary Science,Gannavaram.

Drugs and Chemicals

Methanol, DMSO,Mueller Hilton Broth,calcium chloride, Magnesium chloride, Iodonitrotetrazolium salt

Preparation of plant extracts

The extracts were prepared by using standard procedures. The cashew nut shells were collected from local agriculture market, Nuzivid, Krishna dt. Azolla was procured from ILFC,NTR College of Veterinary Science, Gannavaram. The samples collected were shade dried and pulverized to fine powder and kept for solvent extaction using methanol. The extract yield was 34% for cashewnut shell and 3.12% for azolla The extacts were further converted to stock solution using 40%DMSO.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

Preparation of 0.5 McFarland turbidity standards

Stock solutions of 0.18 M (0.36 N) H_2SO_4 (1% v/v) and 0.048 M $BaCl_2$ (1.175% w/v $BaCl_2$•$2H_2O$) were prepared. With a constant stirring to maintain a suspension, 0.5 mL of the $BaCl_2$ solution was added to 99.5 mL of the H_2SO_4 stock solution. The correct density of the turbidity standard was verified by measuring absorbance using a spectrophotometer with a 1 cm light path and matched cuvettes. The absorbance at 625 nm was 0.08 to 0.13 for the 0.5 McFarland standard. 5 mL aliquots of $BaSO_4$were transferred into screw cap tubes of the same size as those used for standardizing the bacterial inoculum (CLSI, 2012).

Preparation of Supplements and Media Cation stock solutions

Stock solution of 10 mg of Mg^{++}/ml was prepared by dissolving 8.36 g of $MgCl_2$•$6H_2O$ in 100 ml of deionized distilled water and stock solution of 10 mg of Ca^{++}/ml was prepared by dissolving 3.68 g of $CaCl_2$• $2H_2O$ in 100 ml of deionized distilled water. They were sterilized by membrane filtration and stored at 2 to 8°C (CLSI, 2012).

Preparation of Cation-Adjusted Muller-Hilton Broth (CAMHB)

Two hundred ml of Muller-Hilton Broth was prepared according to manufacturer's recommendations, autoclaved and chilled overnight at 2 to 8°C. To this chilled broth, 0.2 ml of $MgCl_2$ stock solution was added with constant stirring followed by addition of 0.4 ml of $CaCl_2$ stock solution so that the final concentration of Mg and Ca ions in the broth was 10 and 20 mg/l, respectively. The pH of the broth after addition of cations was 7.2 to 7.4.

MIC by broth microdilution method

The broth microdilution method was used to determine the MIC of enrofloxacin against S.aureus ATCC 25923, K. pneumoniae ATCC700603 and P.aureginosa ATCC27853. Working standard of 1 mg/ml enrofloxacin,10mg/ml azolla and 4mg/ml cashew nut shell extracts were prepared by diluting the stock solution with normal saline. Two-fold serial dilution of test compounds in CAMHB was prepared in 96 well microtiter plate, so that final volume in each well was 100 µl. The bacterial culture incubated in CAMHB at 37±1°C for 16 to 18 h was taken and its turbidity was adjusted to 0.5 McFarland turbidity standard (1 X 10^8 CFU/ml) which was then diluted 1:20 in CAMHB. When 0.01 ml of this suspension was inoculated into the broth, the final concentration of bacteria was

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approximately 5 X 10^5 CFU/ml (range 2 - 8X 10^5 CFU/ml or 5 X 10^4 CFU/well). Each plate was sealed properly to prevent drying during incubation. Inoculated microdilution trays were then incubated at 35±2°C for 16 to 20 h in an ambient air incubator.

MIC End Point

The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the microdilution wells as detected by the unaided eye or microplate reader (Multiskan^{TM} GO, Thermofisherscientific^{TM}) to discern growth in the wells. The amount of growth in the wells containing antimicrobial agent was compared with that of growth-control wells (no antimicrobial agent) used in each set of tests. Alternatively, bacterial growth and inhibition was detected by adding 25 µl of INT to each well and incubation for 30 min at 35±2°C. INT is reduced to a red formazan compound by biologically active organisms; in this case the dividing bacteria. Bacterial growth was considered to be inhibited when the solution in the well remained clear. This concentration was considered as MIC. Solvent controls and growth controls were included in each experiment [15].

II. RESULTS AND DISCUSSION

The antibacterial activity was assessed by determining the MIC of methanolic extracts of azolla and cashew nut shell extract against ATCC cultures of S.aureus, K,pneumoniae and P.aureginosae. The MIC values of the plant extracts against selected bacteria were presented in tableno.1.The test was repeated for six times and the mean values of MIC were calculated for each organism and the units of measurement were mg/ml.The results of the present study revealed that among the bacterial species tested S.aureus was more sensitive to azolla and cashew nut shell extracts when compared to K.pneumoniae and P.aureginosae.The antibacterial agent enrofloxacin offered better antibacterial efficacy against S.aureus in comparision to K.pneumoniae and P.aureginosae It is also evident that cashew nut shell extract offered better antibacterial activity relative to azolla extract against the selected bacterial cultures.

Table:1 **MIC (mg/ml)values of natural extracts against selected bacteria(n=6)**

Test compound	S.aureus	K.pneumoniae	P.aureginosae
Azolla extract	0.625	1.25	1.25
Cashew nut shell extract	0.125	0.25	0.25
Enrofloxacin	0.0002	0.00165	0.003

The results of the present study revealed that cashew nut shell extract and azolla extract showed comparatively better antibacterial activity against Gram positive organism S.aureus when compared to K.pneumoniae and P.aureginosae, which may be due to the alterations in bacterial cell wall structure. The antibacterial action of azolla is effective against S.aureus. Azolla produced same level of efficacy against K.pneumoniae and P.aureginosae.

Cashew nut shell extract offered better antibacterial activity against all the three bacterial species[16]. selected when compared to azolla because of the presence of flavonoids in cashew nut shells and due to its antioxidant activity.

Based upon the results obtained the natural extracts can be used to treat bacterial infections as an alternative to classical antibiotics to combat antimicrobial resistance.

Further studies has to be conducted to determine the components of the extract responsible for antibacterial potential and safety pharmacology of the extracts in different species.

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