ANTIBACTERIAL ACTIVITY OF ALOE BARBADENSIS RIND EXTRACTS AGAINST SELECTED STANDARD TYPED BACTERIAL ORGANISMS.

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Abstract: The threat posed by resistant bacteria against a wild range of antibiotics is disturbing. Looking inwards for alternatives from medicinal plants have become imperative. Aloe vera has a long history of medicinal use, therefore the research investigated the antibacterial potential of the rind. Macerated Aloe barbadensis rind were soak each in methanol, ethanol, acetone, hot and cold water as raw (100mg/ml) and then in reducing concentration of 50, 20, 10 and 5mg/ml for 24hours. The extracts were filtered with sterile muslin cloth, concentrated by evaporation and stored for use. Susceptibility testing with the extracts was carried out by re-suspending the extracts in dimethyl sulfoxide (DMSO). Twenty milliliters of sterile Mueller Hinton 2 agar was inoculated with 0.5ml of bacterial suspension of Pseudomonas aeruginosa 02, 05, 06 Escherichia coli 0139428 and Staphylococcus aureus held in a 4 hour grown Mueller Hinton broth equivalent to 10^5-10^6 cfu/ml of McFarland standard. Six millimeter holes were bored on the agar after solidification. The wells were inoculated with 0.3ml of the Aloe barbadensis rind extracts and allowed to be completely absorbed into the agar. The plates were incubated at 37 ℃ for 24hours and zones of bacterial inhibition measured in millimeters. The zone of inhibition obtained ranged from 10mm-36mm. higher concentrations of the solvent did not necessarily give rise to higher zones of inhibition. The cold and hot water extract of the rind demonstrated the best of activities against the trio of S. aureus P. aeruginosa and E. coli than the other extracts. Only E.coli demonstrated significant activities at certain concentration of the cold and hot water extracts of the rind ($P<0.05$). The fact that many plant extracts have antibacterial activities against known resistant strains is promising for in vivo trials of standardized extracts against these strains. The susceptibility of the organism to the extracts justifies the use of Aloe Vera in treatment of infections.

Keywords: Antibacterial activity, Aloe barbadensis rind, Bacterial organisms

Introduction

The dangers posed by resistant bacteria against a wide range of antibiotics are greatly alarming and challenging. Today, we have moved from simple antibiotic resistance to a complexity of types.

The ability of bacterial to subvert known targets of antibiotic action has become common. Channeling our search to alternative treatment sources as provided by some medicinal plants should be a wise counsel. There are several reports revealing the effectiveness of traditional herbs against microbes, presenting plants as the bedrock of modern medicine (Evans, Banso and Samuel, 2002). The world health organization (WHO) as a body has seen the need for this paradigm shift.

Aloe Vera has a long history of medicinal use and has been called various names-wonder plants, heaven's blessing, the silent healer, plant of immortality, lily of the desert and lot more. Aloe Vera has stiff gray-green lance-shaped leaves containing clear gel in a central mucilaginous pulp. Phytochemical analysis of the plant has proven the presence of alkaloids, phenols, flavonoids, saponins and vitamins (Beth 2008, Thenmozhi and Shanthi, 2012, Coats 1979), which have antibacterial activity. Of great importance is the lignin component of the plant which facilitates penetration to tissues on administration (coats 1979). Researches on the antibacterial activity of Aloe Vero-rind are limited. We therefore intend to investigate the antibacterial activity of Aloe Vera-rind extracts against selected standard (typed) bacterial organisms.

Materials and Methods

Source of Aloe Vera Plants

Fresh succulent and mature Aloe barbadensis plants were purchased from Aloe Vera farm in Calabar, Nigeria. The plants were identified and confirmed by botanists in the department of Biological Sciences, University of Calabar.

Sources of Bacterial Organisms.

Typed organisms comprising of Pseudomonas aeruginosa 02, 05, 06, Escherichia coli 0139428 and Staphylococcus aureus were obtained from National Institute for Medical Research (NIMR), Yaba Lagos. The organisms were sub-cultured on nutrient agar slants, incubated overnight at 37℃ and then refrigerated until required for use.

Extraction of Aloe Barbadensis Rind

Macerated Aloe barbadensis rind were extracted each with methanol, ethanol, acetone, hot and cold water as raw (100mg/ml) and then in reducing concentrations of 50, 20, 10 and 5mg/ml. The macerated rind was Soak in the above solvents for 24hours. The extracts were filtered with sterile muslin cloth, concentrated by evaporation and store for use. Susceptibility testing with the extracts was carried out by resuspending the extracts in dimethyl sulfoxide (DMSO).

Antibacterial Screening

Twenty milliliters of sterile Mueller Hinton 2 agar was inoculated with 0.5ml of bacterial suspension grown in a 4hour grown Mueller Hinton broth equivalent to 10^5-10^6CFU/ML of McFarland's standard. This was further confirmed with the Lovibond comparator. The agar was allowed to solidify after which 6mm holes were bored with a sterile borer in each plate (agar well diffusion method).

The wells were then inoculated with 0.3ml of the Aloe barbadensis rind extracts and allowed to be completely absorbed into the agar. The plates were inverted and incubated at 37°C for 24hours. Some selected antibiotics were used as control. A zone of clearance around each well meant bacterial growth inhibition and the average zone diameter were measured in mm as the plates were in duplicate. Susceptibility was based on <8-16mm, resistant, 8-16mm/intermediate and >16mm sensitive (Medha et al; 2011).

Results

Figures 1-4 show the antibacterial activity of the rind extracts on the selected typed bacteria.

In Fig 1. Only E. coli was susceptible to the ethanol extracts of the rind.

In Fig 2&3. E.coli and P.aeruginosa were susceptible to both methanol and acetone extract of the rind.

In Fig 4. The three bacterial organisms, E.coli, P.aeruginosa and S.aureus were susceptible to the hot and cold water extract of the rind.

Only E.coli demonstrated significant activities at certain concentrations of the cold and hot water extract of the rind ($P<0.05$)

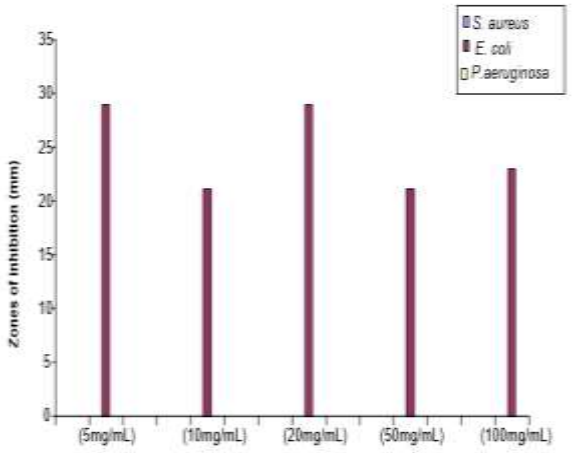

Figure.1. Antibacterial activity of ethanol extracts of A. barbadensis rind against the typed organisms

Fig 2&3. E.coli and P.aeruginosa were susceptible to both methanol and acetone extract of the rind.

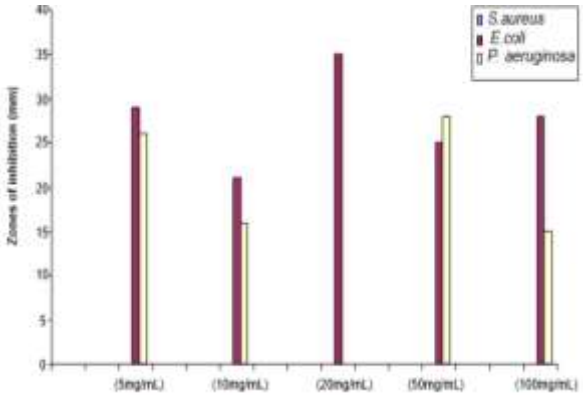

Figure 2. Antibacterial activity of methanol extracts of A. barbadensis rind against the typed organisms

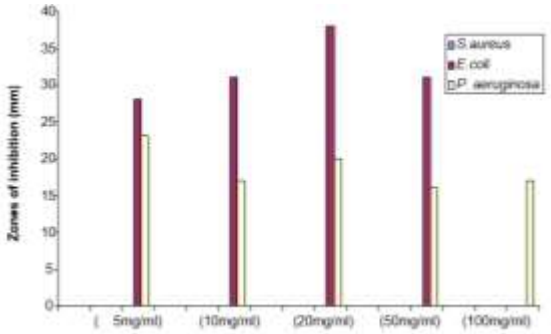

Figure 3. Antibacterial activity of acetone extracts of A. barbadensis rind against the typed organisms.

Fig 4. The three bacterial organisms, E.coli, P.aeruginosa and S.aureus were susceptible to the hot and cold water extract of the rind.

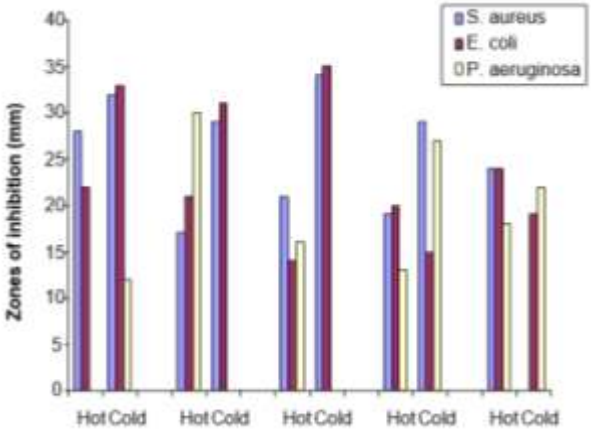

Figure 4: Antibacterial activity of hot and cold water extracts of A. barbadensis rind against the typed organism.

Table I: Shows the mean inhibition zone diameters (mm) of the variable concentrations of the solvent extracts of the rind against the typed bacterial organisms.

Extracts of the rind	Standard (typed) bacteria	Mean inhibition zones of the variable concentrations
Ethanol	S. aureus E. coli P. aeruginosa	00.0 20.6 00.0
Methanol	S. aureus E. coli P. aeruginosa	00.0 27.6 00.0
Acetone	S. aureus E. coli P. aeruginosa	00.0 25.6 18.6
Cold water	S. aureus E. coli P. aeruginosa	24.8 22.6 12.5
Hot water	S. aureus E. coli P. aeruginosa	21.8 20.2 15.4

Table II Antibiogram showing inhibition zone diameters (mm) of selected antibiotics against the typed bacterial organisms.

Antibiotics (conc.ug/ml)	CPX	S	CN	AU	OFX	SXT	AMI
Standard bacteria	*5	20	10	30	5	30	20
P. aeruginosa	30	14	20	15	10	18	10
S. aureus	34	24	30	12	15	12	15
E. coli	36	20	24	32	34	34	22

*Zones of inhibition (mm)

Discussion

Studies have shown that ‘’plants extracted in organic solvents provide more consistent antimicrobial activity compared to those of water extracts’’ (Parek et al, 2005).However it was only the water extracts of the Aloe barbadensis rind that demonstrated activities against The three bacterial organism compared to the organic solvents of ethanol, methanol and acetone. This indicates that water could also be of a great advantage to those whose religious beliefs prohibit the use of alcohol.

All the solvents proved to have better extractive potentials for the rind against E. coli. On the average, E. coli exhibited the highest susceptibility pattern (27.6mm zone diameter from methanol extract, Table 1). This is a pointer to the fact that the rind extract could be effective and useful in the treatment of E. coli infection. The same pattern was replicated when compared with the control antibiotics used (Table II). Agarry et al, (2005) have stated the antibacterial potential of Aloe vera which is supported by this research. The inability to obtain better susceptibilities with P. aeruginosa and S. aureus could probably be attributed to the complexity of both organism which makes both ‘’stubborn’’ to drug administration. The order of susceptibility indicated E. coli >P. aeruginosa >S. aureus.

Considering the problem of resistance posed by both organisms, enhanced treatment could be achieved through Herboallopathy. A combination of a high amount of herbal plant like Aloe Vera and a low amount of allopathy drugs like roxythromyain, cefixime and levofloxacin enhance effectiveness in treatment (Medha et ali, 2011). Aloe Vera gel has also been noted to have enhanced hydrocortisone penetration thereby adding to its biological activity.

One would have expected that higher concentrations of the solvents would give more zones of inhibition but that was not the case. A similar work by (Cleidson et al., 2007), showed that higher concentration of the substance or solvent did not necessarily produce higher zones of inhibitions. For instance, at concentrations of 0.2mg/ml, 1.0mg/ml and 5.0mg/ml, of p-iodonitrotetralium violet salt solution, the zones of inhibition against S.aureus were 12mm, 10mm and 11mm respectively. The extracts were probably more soluble at lower than higher concentrations of the solvents.

Conclusion

Cold water, hot water, methanol, acetone and ethanol extracts of Aloe barbadensis rind have different antibacterial activities. The cold and hot water extracts of the rind demonstrated the best activities against the trio of S.aureus, P.aeruginosa and E.coli than the ethanol, methanol and acetone extracts. The other of susceptibility in this research remains E. coli > P. aeruginosa > S. aureus. The presence of resistance in non-pathogenic environmental bacterial strains should not be taken for granted. The ability to undergo transduction, transformation or conjugation remains normal reproductive mechanisms in bacteria. The fact that many plant extracts have activities against known resistant strains of bacteria give hope for in vivo trials of standardized extract against these strains.

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