INDO GLOBAL JOURNAL OF PHARMACEUTICAL SCIENCES

Screening of Antimicrobial Activity of Alcoholic & Aqueous Extract of Some Indigenous Plants

D Benito Johnson[a*], B N Shringi[b], Dinesh Kumar Patidar[a], Nehru Sai Suresh Chalichem[a], Ashok Kumar Javvadi[a]

[a] *Department of Pharmacology, R V S College of Pharmaceutical Sciences, Sulur, Coimbatore-641 402, India*

[b]*Department of Pharmacology, College of Vet. & Animal Sciences, Rajasthan Agricultural University,Rajasthan, India*

Received: 11th Mar. 2011; Accepted: 10th May, 2011

Address for Correspondance: benitojohnsond@gmail.com

Abstract— Plants known to possess several antimicrobial compounds are used in all traditional medicine. Our study was to screen the leaf extracts of *Aloe vera, Datura stromonium, pongamia pinnata, Lantona camara , Calotropis procera.* These plants were collected and aqueous and alcoholic extracts were prepared by decoction and hot percolation process. Microbes for study are staphylococcus, *E.coli* and *Aspergillus* species. They were isolated from different sources and identified by morphological and chemical tests. Different dilutions of the test drugs were prepared with saline and by using turbidity method MIC was found and anti fungal activity by spore germination method. Alcoholic extracts displayed higher antibacterial and anti fungal activity than aqueous extract. Results concluded that *Aloevera* had the highest and strong activity against *S.aureus* and *E.coli. Lanata camara* does not show anti bacterial and anti fungal activity. *Pongamia pinnata* aqueous extract showed better anti *E.coli* activity than its alcoholic extract. *Datura stramonium* showed better activity against staphylococcus aureus & showed little anti *Aspergillus* activity. *Calotropis procera* showed antibacterial activity against *Staphyloccus aureus* & *E.coli* and does not showed anti *Aspergillus* activity.

Keywords : *Aloe vera, Datura stramonium, Pongamia pinnata, Lantona camara, Calotropis proceram, Staphylococcus aureus, E Coli.*

INTRODUCTION

Many crude preparations of herbal drugs are in clinical use in medical and veterinary practice. Ethano pharmacologist, botanists, microbiologists and natural product chemists are combing the earth for phytochemicals which could be developed for treatment of infectious diseases. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types microorganisms *invitro.* More of these compounds are being subjected to animal and human studies to determine their potential to restrict the growth /multiplication of pathogenic organism as well as examination of their effects on beneficial normal micro biota. Traditional healers have long used plants to prevent or cure the infectious condition. Plants are rich in a wide variety of secondary metabolite such as tannins, terpenoids, alkaloids, and flavanoids which have been invitro to have anti microbial properties.

The advent and continuous use of antibiotics in previous century led to success in limiting most of the prevalent bacterial diseases which affected man and animals in epidemic proportions. At the same time inadvertent and over use of antibiotics resulted into emergence of resistance in organism against the commonly used antibiotics and urgency in developing newer antibiotic to check the prevailing infection. The emergence of multi drug resistant organisms necessitates the search for alternative source of antimicrobial agents. Indian traditional system of medicine i.e., Ayurveda has successfully employed plants derived products in the treatment of almost all types of ailments in human and animals.

Major part of the Rajasthan state covers unique ecosystem i.e., Thar desert , that is rich in several unique plants and shrubs advocated to this climate. Traditional healers are known to employ many applications in the treatment of infectious and non infectious diseases which are derived from locally available in local literature it thus become important to explore the therapeutic potential variety to this area in a systematic manner.

The present study was undertaken- i. To determine anti bacterial activity some plant extracts

ii. To determine MLC and MIC of plant extracts.

iii. To determine antifungal activity of the plant extracts.

PLANT SELECTED	PLANT PART USED
Aloe vera	Leaves
Datura stramonium	Leaves
Pongamia pinnata	Leaves
Lanata camara	Leaves
Calotropis procera	Leaves

MATERIALS AND METHODS

All the mentioned plants were collected and required parts were separated, washed and cleaned by muslin cloth and kept for drying for 7 days.

Preparation of aqueous extract

Aqueous extraction was carried out by decoction process (Davis, 1956). This was carried out by boiling in hot water, in this process one part of dried powder plant and 5 part of sterilized water were taken in a boiling water flask and boiled for 15 min. after boiling the extract was filtered through a whatmann filter paper no.1, autoclaved at 121˚c for 15 min and kept in clean and sterilized test tubes and stored at 4˚c till further use.

Preparation of alcoholic extract (Davis 1956) :The alcoholic extract was prepared by soxhelet extraction. In this process the dried powder form of plant material extracted by using ethyl alcohol. After completion the process the concentrated active constituents from plant material were kept in sterilized test tubes stored in refrigerator till further use. The traces of ethanol were removed by keeping the tubes at 50˚c for 1 hr.

Isolation and identification of test bacteria: Milk samples from 4 mastitis cattle and one fecal sample from calf diarrhea were screened for the presence of bacteria by cultivation, isolation, and identification (Cowan and steel 1975).

The samples were withdrawn with an inoculating loop aseptically and streaked on blood agar, nutrient agar and mac-conkey agar culture media plates in primary, secondary, and tertiary fashion in order to obtain isolated colonies of bacteria. These petri plates were incubated for 24 hrs at 37˚c. following incubation, the plates were observed for colonial characteristics and hemolytic zones on blood agar plates. If more than one type of colony appeared on agar plates, the different colonies were selected out and subculture separately for obtaining the pure culture of the bacterial isolates.

The pure isolates were taken on nutrient agar slants and preserved in a refrigerator at 4˚c until subjected to further biochemical characterization.

Determination of concentration of test organism: The concentration(total count) of test bacteria was determined by nephlometry using McFarland scale (McFarland, 1907). The standard tubes were prepared by mixing varying amount of 1% $Bacl_2$ and 1% H_2SO_4 in stoppered tubes as follows:

Scale	1% $Bacl_2$ (ml)	1%H_2SO_4 (ml)	No.of bacteria value listed * 10^6 Approx.
1.	0.1	9.9	300
2.	0.2	9.8	600
3.	0.3	9.7	900
4.	0.4	9.6	1200
5.	0.5	9.5	1500
6.	0.6	9.4	1800
7.	0.7	9.3	2100
8.	0.8	9.2	2400
9.	0.9	9.1	2700
10.	1.0	9.0	3000

The turbidity of overnight broth culture of test organism was compared with that of McFarland scale tubes against while background and concentration was approximated to the table.

Antibiogram of the culture isolates were based on the Bauer et al.(1966) disc method.

Preparation of dilution of plant extract:

Two fold serial dilution of aqueous and alcoholic plant extracts were prepared in sterilized test tubes with sterile normal saline solution beginning from undiluted and 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512. The total amount of each dilution was kept 2ml.

Inoculation of test organism

Each dilution of plant extract was added with equal volume of double strength(2x) of nutrient broth so as to make normal concentration of nutrients after the addition of medium. Along with the desired plant extracts two sets of control tubes were simultaneously taken one of which lacked plant extract and the other one was kept for uninoculated control.

All the dilutions of plant extracts and control tubes (except un inoculated control tubes) were inoculated 0.1 ml of broth culture of test organism having turbidity comparative to McFarland tubes no.1 with app.no.of organism as $300*10^6$ /ml. following inoculation all the tubes were measured after 2, 4, 6, 8 hrs. The highest dilution of plant extract that showed inhibited growth of test organism as compared with control was considered as MIC (Tsuchiya et al., 1996).

Determination of MLC: After 8 hrs of incubation a series of 10 fold dilutions of each inoculated tubes was prepared in sterile normal solution and were spread evenly on nutrient agar petri plates with the help of sterile spreader. The plates were incubated at 37˚c over night and on following the number of colonies were counted on colony counter to utilize live number of bacteria present. The highest dilution of the plant extract that was to kill the test organism was considered as MLC (Sato et al., 1997).

Isolation and identification of test fungi

Sheep nasal swab was taken and cultured on Sabouraud's dextrose agar and the plates were incubated at room temp., for 2 days. Grayish brown mycelia were seen which turned later to black colour. Smear was prepared and stained with lacto phenol cotton blue stain and observed under high power microscope.

Preparation of spores: 10 ml of sterile normal solution was added to the petri plate containing fungal growth i.e., showing fungal mycelium and was shaken gently. The fluid containing fungal spores was collected with the help of a sterile pipette into centrifuge tube and centrifuged at 1500rpm for 10 min. Finally the spore sediment was suspended in normal saline at a conc.,of about 100-200 spores per high power yield.

Germination of spores: To a glass cavity (normally used for hanging drop preparation), 30µl of sabourad's dextrose agar was placed in cavity while in molten state. It was then added with 10 µl of test fungal spores preparation under possible aseptic conditions. The cavity portion of the slide was covered with a sterile and clean cover slip and its margins were sealed with sterile paraffin wax. It was then incubated for 2days in moist chambers at room temperature.

Inhibition of spore germination activity: For determination of antifungal activity of plant extracts, two fold serial dilutions(1:1 to 1:512) of aqueous and alcoholic extracts of test plants were prepared and 40µl of each dilution were added along with inoculation of spores as described above.

The micro culture slides were incubated at room temp., in moist condition and observed for inhibition spore germination at 24hrs and 48hrs of inoculation.

RESULTS & DISCUSSION

Isolation and identification of test bacteria:

Table1: Biochemical and metabolic reaction of S.aureus

S.NO.	**Primary Identification**		**Secondary Identification**	
1.	Gram reaction (Hucker&cohn,1923)	+	Growth in MSA	+
2.	Morphology	Cocci	Coagulase	+
3.	Motility	-	Growth in EMB	-
4.	Spore	-	Indole Test	-
5.	Growth in McConkey agar	-	MR test	+
6.	Catalase (Thomas 1963)	+	VP test	+
7.	Oxidase	-	Citrate utilisation	-
8.	O-F test, (Hugh & leifsons 1953)	F	Nitrate reduction test	+

Table2: Biochemical and metabolic reaction of E.Coli

S.NO.	**Primary Identification**		**Secondary Identification**	
1.	Gram reaction (Hucker&cohn,1923)	-	Growth in MSA	+
2.	Morphology	Rods	Coagulase	+
3.	Motility	-	Growth in EMB	Metallic sheen
4.	Spore	-	Indole Test	-
5.	Growth in McConkey agar	-	MR test	+
6.	Catalase (Thomas 1963)	+	VP test	+
7.	Oxidase	-	Citrate utilization	-
8.	O-F test, (Hugh&leif sons 1953)	F	Nitrate reduction test	+

Table3: Antibiotic sensitivity and resistance of different test organism in sensidisc diffusion test

S.NO.	Test organism	Resistant	Sensitive
1.	Staphylococcus aureus	Amoxicillin, Ampicillin, Ciprofloxacin, Doxycycline, Methicillin, Penicillin, Sulfadizine	Bacitracin, Chloramphenicil, Ciprofloxacin, Gentamycin, Kanamycin, Neomycin, Vancomycin
2.	Escherichia coli	Amoxicillin, Bacitracin, Penicillin, Vancomycin, Ciprofloxacin, Sulfadizine	Amoxicillin, Chloramphenicil, Ciprofloxacin, , Gentamycin, Kanamycin, Neomycin, Doxycycline, Methicillin

Table 4: Antifungal activity of plant extracts

Name of plants	Control	Alcoholic extract					Aqueous extract				
		Undiluted	1:2	1:4	1:8	1:16	Undiluted	1:2	1:4	1:8	1:16
Aloe vera	Anti aspergillus spore germination activity(-)	-	-	-	-	-	-	-	-	-	-
Datura stramonium		+	-	-	-	-	-	-	-	-	-
Pongamia pinnata		+	+	-	-	-	-	-	-	-	-
Lanata camara		-	-	-	-	-	-	-	-	-	-
Calotropis procera		-	-	-	-	-	-	-	-	-	-

Table 5: MIC of aqueous plant extracts by turbidity method

S.NO.	Name of plants	Control	Against E.coli						Against S.aureus					
			0	1:2	1:4	1:8	1:16	1:32	0	1:2	1:4	1:8	1:16	1:32
1.	Aloe vera	Mcfarland reading 2 approximated $600*10^6$ no.of bacteria/ml	-	2	2	2	2	2	-	1	1	1	1	1
2.	Datura stramonium		-	2	2	2	2	2	-	1	1	2	2	2
3.	Pongamia pinnata		-	-	1	2	2	2	-	-	1	1	2	2
4.	Lanata camara		2	2	2	2	2	2	2	2	1	2	2	2
5.	Calotropis procera		-	1	1	2	2	2	-	1	1	1	2	2

1= $300*10^6$ 2 = $600*10^6$ - = not measured

Table 6: Anti bacterial activity of different plant extracts

Name of the plant	Against E. coli		Against S. aureus	
	Alcoholic (MLC)	Aqueous(MLC)	Alcoholic (MLC)	Aqueous(MLC)
Aloe vera	1:64	Undiluted	1:512	1:32
Datura stramonium	undiluted	Undiluted	1:2	1:2
Pongamia pinnata	undiluted	1:4	1:2	Nil
Lanata camara	Nil	Nil	Nil	Nil
Calotropis procera	1:4	1:2	1:8	1:2

In the present investigation boiling water and ethanol were used as solvents for aqueous and alcoholic extraction of phyto chemicals. A conc. Of 3:1(w/v) and 5:1(w/v) could be achieved in aqueous and alcoholic extraction from dried plant parts, respectively. Following removal of traces of ethanol, alcoholic extract were found inconvenient and inaccurate in determining the turbidity. As such alcoholic extracts could not be used to determine MLC in turbidimetric method, however, aqueous extract could be used for this purpose.

Among acetone, methanol, ethanol-acetone is considered superior (Eloff 1998). Considering the biohazard of traces left and ease of removal of the solvent from the fraction, ethanol was preferred in the present investigation.

Water soluble polysaccharides, polypeptide, including fabatin and lectins of plant origin act as inhibitors of microbial pathogens(Zhang &Lewis,1997). The test organisms were also studied for their antibiotic sensitivity resistance pattern. These results are in conformity to earlier findings of Mittal (1997) and Chatterjee(2004) who found similar resistance pattern of S.aureus and E.coli isolates respectively.

Anti bacterial and antifungal activity of Aloe vera leaf extract-

It is clear from the results that aqueous and alcoholic extracts of Aloe vera leaf did not show any antifungal activity in spore germination assy. Our results regarding antibacterial activity are in conformity to earlier findings of Agars et al.,(2005) who found anti S.aureus activity of alcoholic extract of Aloe vera. Similarly, Tian et al.,(2003) found anti E.coli activity of aloe species extract. Our results are not in agreement with many other reports regarding antifungal activity of Aloe vera extracts namely Keisuke et al.,(1978), Klein and Penney's(1988), Ali et al.,(1999), Agars et al.,(2005). It might have been due to difference in test fungi susceptibility, the assay condition or due to difference in extraction process. Keisuke et al.,(1978), reported that fungicidal activity of Aloe vera extracts was lost by heating 100˚c for 30 min. In our procedure, for aqueous extraction the leaves were boiled for 15 min at 100˚c and in alcoholic extraction traces of ethanol were removed by heating at 50˚c for 1hr which might have resulted into loss of anti fungal activity.

Our results clearly indicate that alcoholic and aqueous extract of Aloe vera leaves had very high antibacterial activity specially against S.aureus.

Anti bacterial and antifungal activity of Datura stramonium leaf extract-

Our results regarding antibacterial activity of Datura stramonium are in conformity to earlier finding of Uzun et al.,(2004), who found antibacterial activity against both S.aureus and E.coli. our results regained antifungal activity in confirmation of Rajesh and Sharma(2002)., Dabur et al(2004), who have reported antifungal activity of Dature metel phytochemicals. It was also reported that ant fungal activity was due to a pyrrole derivative.

Anti bacterial and antifungal activity of Calotropis procera leaf extract-

It is evident from the results that alcoholic extract shows antibacterial activity against E.coli with MLC 1:4 and S.aureus with MLC 1:8. The aqueous extract showed antibacterial activity against E.coli with MLC 1:2 and S.aureus with MLC 1:2 dilutions. Extract did not show any anti fungal activity in spore germination assay.

Anti bacterial and antifungal activity of Pongamia pinnata leaf extract-

Not many references are available regarding antimicrobial activity of Pongamia pinnata. Basawa et al(2001) found anti microbial of karanj oil probably due to inhibition of cell membrane synthesis. One observation regarding antifungal activity in our investigation need to be mentioned that the activity was retained up to only 48hrs there after, the spores started germination.

Anti bacterial and antifungal activity of Lantana camara leaf extract-

In present study the alcoholic and aqueous extract does not show any antibacterial and antifungal activity against E.coli and S.aureus. No reference could be found in literature regarding antibacterial activity of alcoholic and aqueous extract of plant of this plant and thus our result could be confirm with earlier finding.

REFERENCES

1. Agarry O, and Bello-Michel C.O. Comparative antimicrobial activities of Aloe vera gel and leaf. African journal of biotechnology 2005;4: 1413-1414.

2. Ali M, Elgamal M.H, and Mousa A.S. Anifungal effect of different plant extracts and their major comprehends of selected Aloe species. 1999;13: 401-407.
3. Baswa M, Rath C, Mishra R.K. Antibacterial activity of karanj and neem seed oil. A preliminary report. Microbios. 2001; 105:183-189.
4. Bauer A.W., Kirby W.M. and Turck M. Antibodies susceptibility testing by a standardized single disc method. American journal of clinical pathology 1966;45: 493-496.
5. Brander G.C, Pugh D.M., Sherries J.C., and Jenkins W.L. Veterinary applied pharmacology and therapeutics,(5th edition), 1991:1-624.
6. Cowman S.T, and Steel K.J. Manual for identification of Medical bacteria, Cambridge university, Cambridge, 1975.
7. Dabur R, Ali M, Singh H, Gupta J, and Sharma G.L. Antifungal potential of Indian medicinal plants, Fitoterapoa, 2004b;75: 389-391.
8. Davis H. Bentley's text book of pharmaceutics (6th edition). 1956:272-30.
9. Eloff J.N. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J.Ethnopharmacol 1998;60: 1-8.
10. Hucker G.J and Cohn H.J. Methods of Gram's staining. Technical bulletin, Agriculture Experiment station, New York. 1923; No:93.
11. Hugh R, and Leifson E. The Taxonomic significance of fermentative versus oxidative metabolism of carbohydrate by various gram negative bacteria. Journal of bacteriology. 1953;6:24.
12. Johne S, Mc Donald. Streptococcal and Staphylococcus mastitis. Journal of American Veterinary Medical Association, 1977;170:1157-1159.
13. Keisukae et al.,. Effect of leaf extracts of Aloe barbodences on growth of Trichophyton mentagrophytes. Antimicrobial agents and chemotherapy, 1978: 132-136.
14. Klein A.D, and Penneys N.S. Antimicrobial effect of Aloe vera, J.Am.Acad.Dermatol, 1988;18:714-720.
15. McFarland J, The Nephelometer: An Instrument for Estimating the number of bacteria in Suspensions used for calculating the Opsonic index and for vaccines, J.Am.Med.Assoc.,1907;49:1176.
16. Mittal R.K. Cultural, metabolic, toxicogenic, biochemical analysers and drug sensitivity of mastitis S. aureus. M.V.Sc. thesis submitted to Rajasthan Agricultural University, Bikaner.
17. Rajesh and Sharma G.L. Studies on Antimycotic properties of Datura metel. Journal of ethnopharmacology,2002;80:193-197.
18. Thomas K. A blue peroxide slide catalase test Mon bull Minist health lab serv,1964;22:124
19. Tian B and Wang G.L. Relationship between antibacterial activity of Aloe and its anthraquinone compounds. 2003;28: 1034-1037.
20. Tsuchia H, et al,. Comparative study on the antibacterial activity of phytochemical flavonones against methicillin resistant Staphyllo coccus aureus. Journal of Ethanopharmacology 1996;50: 27-34.
21. Uzun E, et al.,. Traditional medicine in turkey and antimicrobial activities of selected species. Journal of Ethanopharmacology,2004;49:287-296.
22. Zhang Y and Lewis k. Fabatins: New antimicrobial plant peptides FEMS microbial Litt. 1997;149:59-64.