INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-8, Issue-1, Jan-Mar-2017 Coden IJABFP-CAS-USA **Copyrights@2017**

Received: 10th Dec 2016 ***Revised: 24th Dec 2016*** ***Accepted: 9th Jan 2017***

DOI: 10.21276/Ijabpt, http://dx.doi.org/10.21276/ijabpt ***Research article***

ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF SOME FOLK MEDICINAL PLANTS AGAINST BACTERIA CAUSING GASTRO-INTESTINAL INFECTIONS

Dr Maya Verma

Department of Botany, University of Rajasthan, Jaipur

ABSTRACT: The present study was carried out to evaluate the antimicrobial activity of methanol, petroleum and dichloromethane extract of *Euphorbi.hirta* L. (Euphorbiaceae), *Emblica officinalis* Gaertn (Euphorbiaceae) and *Butea monosperma* (Lam.) Kuntz. (Fabaceae) against bacteria *Shigella dysenteriae* 3, *Salmonella typhii* 62 and *E.coli* K 88 of gastro-intestinal relevance by agar disc and microbroth dilution methods. The results showed potential antimicrobial activity of tested plant extracts against the screened bacterial strains.It was found that methanolic extract of *E.hirta* (leaves) possessed increased antibacterial activity against *S.typhii*. Lowest minimum inhibitory concentration (0.625 mg/ml) and minimum bactericidal concentration (2.5mg/ml) against *S.typhii* were also observed for methanolic extract of *E.hirta*. The results show that these plant extracts exhibit antimicrobial activity and proved to be most effective as an antibacterial.

Key words: plant extract, antibacterial activity, medicinal plants.

*Corresponding author: Dr Maya Verma, Department of Botany, University of Rajasthan, Jaipur , India maya25verma@yahoo.com

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Antibiotic resistance has become a global concern (Westh et al., 2004).Recent work has revealed the potential of several herbs as sources of drugs (Iwu, 2002).The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of antibiotic prototypes (Afolayan, 2003).Numerous researches have identified compounds within herbal plant s that are effective antibiotics (Basile et al., 2000).Traditional healing systems around the world that utilize herbal remedies are an important source of discovery of new antibiotics (Okpekon et al., 2004).Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria(Kone et al., 2004).The results of this indicate the need for further research in to traditional health systems (Romero et al., 2005).The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Doss et al., 2009).There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increases in the incidence of new and re-imerging infectious diseases (Parivuguna et al., 2008).Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action. Contrary to synthetic drugs, antimicrobial of plant origin not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Geeta singh and Padma Kumar, 2011). .

Infectious diseases is the number one cause of death accounting for approximately one half of all death in tropical countries (Venkataswamy et al., 2010).

Infections of gastrointestinal tract,are the most common intestinal disorder. They have their largest impact in the developing world and are still responsible for the death of up to 3 million pre-school children each year (Farthing and Kelly, 2007).The majority of deaths caused worldwide by diarrheoa occur in children under five years of age (Mohd.et al., 2004).

Diarrhoea is the second leading cause of death and is responsible for killing 1.5 million children every year (WHO, 2009).Among pathogens *Shigella dysenteriae* can cause bloody diarrhoea and high concentration of neutrophils in the stool(Rambaud and Rampol,1993;Pegram et.al,1998).In the case of Salmonella species infections clinical manifestations may proceed to gastroenteritis, bacteraemia or septicaemia, enteric or typhoid fever. *E.coli* being a multiresistant bacteria, present in intestine of human beings causing no harm generally but some enteropathogenic strain of bacteria may cause acute diarrhoea ranging from mild and non bloody diarrhoea to mild watery diarrhoea and abdominal pain (Nataro and Kaper, 1998).*Klebsiella pneumoniae* has been isolated from faecal and clinical specimens. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents. Interest in plants with antimicrobial properties has been revived as a result of current resistance profiles associated with over- and inappropriate use of antibiotics (Chattopadhyay et al., 2009).

MATERIALS AND METHODS

Collection of Plant material

Fresh plants were collected from regional areas of Jaipur and authenticated by taxonomist The leaves were shade dried then coarsely powdered.

Solvent extraction

The dried leaves were powdered with the help of waring blender then powder was filled in thimble and extracted successively with methanol solvent in soxhlet extractor for 48hr.The crude extracts were concentrated using vacuum evaporator.

Antimicrobial screening

All bacterial strains of (*E.col* K 88, *Salmonella typhii* 62, *Shigella dysenteriae* 3), were obtained from S.M.S. Medical college and Microbiology lab, Deptt of botany, university of Rajasthan, Jaipur respectively. The bacteria were maintained on nutrient broth (NB) at 37ºC and fungus were maintained on potato dextrose agar (PDA) at 28º C.

Antibacterial activity

Agar disc diffusion assay

The antibacterial activity of the extracts was determined by the disc diffusion method (Rios et al.,1988).Briefly overnight bacterial cultures were diluted in the Mueller-Hinton broth (O.D. 600 = 0.08) to obtain a bacterial suspension of 10^8 CFU/ml. Petri plates containing 20 ml of Mueller hinton agar were inoculated with 200 µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. Filter paper discs of whatman no.1 (6mm diameter) were impregnated with 50µl,100µl and 150µl of the extract which is equivalent of 5,10 and 15 mg/ml, were placed on the inoculated agar surface and allowed to dry completely. Standard antibiotic Gentamycin (20 µg) placed as controls. Plates were incubated at 37º C for 24 hrs. The same procedure was followed for the fungus also. The antibacterial activity was assessed by measuring the inhibition zone. All the tests were performed in triplicates.

Determination of minimum inhibitory concentration (MICs)

A minimum inhibitory concentration (MICs) is the lowest concentration of an antimicrobial that inhibits growth of a microorganism after 18-24 hrs. The extracts that showed antibacterial activity were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration. Briefly, the stock solutions of the extracts were subjected to two-fold serial dilution of Mueller-Hinton broth to obtain a concentrations from 100mg/ml to 0.19 mg/ml. Streptomycin was placed as control-A 10 µl of 10^7 (CFU) bacterial cultures were added to the tubes and were incubated at 37º C for 18 hrs. MICs was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration.

Preparation of the inoculums

Stock cultures were maintained at 4º C on nutrient broth. Active cultures for experiment were prepared by transferring a loopful of cells from the stock cultures to the test tubes of Mueller-Hinton agar (MHA) for bacteria that were incubated without for agitation for 24 hrs at 37º C and 25º C respectively. The cultures were diluted with fresh Mueller-Hinton to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/ml) for bacteria strains.

Statistical analysis

The data of all the parameters were statistically analyzed (statistical software used Minitab 14-state college, PA, USA) and zone of inhibition diameter values are expressed as Mean Diameter ± SEM (n= 3)

RESULTS AND DISCUSSION

Results obtained in the present study revealed that the tested medicinal plant extracts possess potential antimicrobial activity against tested micro-organisms (Table-1).In vitro antimicrobial screening using gentamycin as a positive control clearly indicated that extracts of *E.hirta* show promising antimicrobial activity against all the three microorganisms. Highest antimicrobial activity was observed with methanolic extract of *E.hirta* against *S.typhii*(16.8mm) respectively while minimum activity was observed with petroleum ether extract of *E.hirta* against *S.dysenteriae* (6.2 mm).Results obtained in the current investigation revealed that studied herbal extract possess potential antimicrobial activityagainst entire tested organisms, methanolic extract was found to have shown the strongest and broadest spectrum.

E.hirta extract showed moderate inhibition activity with the zone range of 6.2-16.8mm.Maximum inhibition was observed against *S.typhii* (16.8mm) and minimum inhibition against *S.dysenteriae* (6.2mm).The crude extract of *E.officinalis* were active against all bacterial strains showing maximum zone of inhibition (15.6 mm) against *E.coli* from methanol extract and minimum inhibition against *S.typhii* (6.5mm).The antimicrobial activity of extract of *E.officinalis* may be attributed to the presence of active ingredients (quercitin, phyllemblin, gallic acid alkaloids, phyllantine, phyllantidine and tannins. *B.monosperma* extract showed inhibitory activity with the zone range of (6.9-16.0mm).

Table 1 - Antimicrobial screening of plants against *Shigella dysenteriae 3, Salmonella typhii* 62 and *E.coli* k88 using disc diffusion method.

Zone.of inhibition (mm)									
	S.dysenteriae			*S.typhii*			*E.coli*		
	ME	PE	DCM	ME	PE	DCM	ME	PE	DCM
Euphorbia hirta (leaves)	15.6±0.06	6.2±0.16	12.2±0.12	16.8±0.13	7.4±0.08	9.8±0.03	15.4±0.08	NA	11.2±0.6
Emblica officinalis (fruit)	14.7±0.15	NA	13.9±0.05	13.9±0.06	6.5±0.01	8.6±0.08	15.6±0.06	9.0±0.06	12.7±0.3
Butea monosperma (stem bark)	13.8±0.15	7.4±0.12	10.2±0.08	12.5±0.09	6.9±0.06	7.6±0.07	16.0±0.5	9.06±0.03	NA
Gentamycin		16.42±0.02			17.56±0.02			18.1±0.08	

PE= Petroleum ether extract; DCM = Dichloromethane extract;ME = Methanoloc extract;NA = No antibacterial activity.Values are means ± SEM (mm) of three measurements ; *P<0.05.

Table-2: Minimum inhibitory concentrations (MIC) and minimium bactericidal concentrations (MBC) of active methanolic extracts of Medicinal plants and gentamycin

Methanol exracts of medicinal plants	*S.dysenteriae*		*S.typhii*		*E.coli*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
E.hirta	2.5	2.5	0.625	2.5	2.5	2.5
E.officinalis	na	na	1.25	>5	1.25	5
B.monosperma	1.25	>5	2.5	5	1.25	5
Gentamycin	0.413	0.62	0.064	0.143	0.054	0.246
na - No antibacerial activity. Results are average of three measurements.						

Active methanolic extract wre further evaluated for their MICs and MBCs. The lowest MICs against *S.dysenteriae* (1.56 mg/ml),*S.typhii* (1.56mg/ml) and *E.coli* (3.125mg/ml) were recorded. Similarly lowest MBCs against *S.dysenteriae* (6.25mg/ml), *S.typhii* (6.25mg/ml) and *E.coli* (6.25mg/ml) were also recorded.

Against all the tested bacterial strain, we observe methanol extract of all the samples showing much better antibacterial activities in contrast to other extracts, which may be because of organic nature of methanol and also for the reason of its high capacity to dissolve more organic and active antimicrobial compounds (Cowan, 1999).
The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments but several studies have also reported various type of contamination of herbal medicines which include microorganisms and toxins produced by microorganisms, pesticides and toxic heavy metals (Talaly and Talaly, 2001).
These results suggest that these plants can serve as potential source of bioactive healthy compounds in diet. Further research is needed towards isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

REFERENCES

A.Basile, S.Sorbo, S.Giordano, L.Ricciardi ,S.Ferrara and D.Montesano (2000). Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves.Fitorapia :vol.71,110-116

A.Doss, H.Mohammed Mubarak and R.Dhanabalan (2009). Indian Journal of science and Technology: vol.2,2, 41-43

A. J. Afolayan (2003). Extracts from the shoots of *Arctotis artotoides* inhibit the growth of bacteria and fungi.Pharm Biol :vol.41,22-25

A.K.Mohd, A.K.Naeem, A.Q.Iqbal, A.Ghofran, Z. Shadad (2004). Protec.effect of Arque-Ajeeb on acute experiment Diarrhoea in rats.BMC complementary & alt.medicine :vol.4,8

C.D.Romero, S.F.Chopin, G.Buck, E.Martinez, M.Garcia and M, Bixby (2005). Antibacterial properties of common herbal remedies of the southwest. J.Ethnopharmacol: vol.99,253-257

G.C.Pegram, N.Rollins and Q.Espay (1998). Estimating the cost of diarrhoeal & epidemic dysentery in Kwa-2ulu-Natal & S.Africa.Water S A: vol. 24,11-20

Geeta Singh and Padma Kumar (2011). Journal of pharmacy research: vol. 4,4,1228-1230

H.Westh,C. S. Zinn , V. T.Rosdahl (2004). Sarisa Study Group. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries .Microbial Drug Resistance: vol.10,169-176

J.C.Rambaud and P.Rampol (1993). Diarrhees aigues infectieuses. Progres en hepatp-gastroienterologie – collection publiee sovs la direction du Pr.R.Zittoun doin,Ed.Paris

J.L.Rios, M C Recio and A.Villar (1988). Screening of methods for natural products with antimicrobial activity: A review of the literature.J.Ethnopharmacol: vol.23, 127-149

J.P.Nataro and J.B.Kaper(1998). Diarrhea genic *Escherichia coli*.Clinical Microbiology Reviews: vol.11,142-201

M. J. G. Farthing and P Kelly (2007). Infection Diarrhoea. Medicine Gastroenterology: vol.3,251-256

M.M.Cowan (1999). Plant products as antimicrobial agents.Clin.Microbiol.Rev.vol.12, 564-582

M.M.Iwu (2002). In: Therapeutic agents from Ethnomedicine and Drug Discovery.Iwu MM.Wotton JC(Eds) Elsevier Science , Amsterdam

Talaly P and P.Talaly (2001). The importance of using scientific principles in the development of medicinal aspects of plants.Acad.Med.vol.76, 238-247

R.R.Chattopadhyay, S K Bhattacharya, C Medda , S Chanda , A Bag (2009). A comparative evaluation of antibacterial potential of some plants used in india traditional medicine for the treatment of microbial injections.Braz Arch Biol Technol: vol. 52 ,1123-1128

R.Venkataswamy,A.Doss,H.Muhamed Mubarak and M.Sukumar (2010). Phytochemical HPTLC finger printing and antibacterial activity of *Acacia nilotica* (L.) Delile.Hygeia. J.D.Med :vol.2,2,38 – 42

T.Okpekon ,S.Yolou ,C.Gleye, F.Roblot ,P.Loiseau , CET Bories (2004). Antiparasitic activities of medicinal plants use in ivory coast.J Ethnopharmacol :vol.90,91-97

V.Parivuguna, R. Gnanaprabhal, R.Dhanabalan and A.Doss (2008). Antimicrobial properties and phytochemical constituents of *Rhoeo discolor* Hance.Ethnological leaflets:vol.12,841- 845

W.N.Kone ,K.K.Atindehou,C. Terreaux, K.Hostettmann , D. Traore and M. Dosso (2004).Traditional medicine in North Cote-d' Ivoire screening of 50 medicinal plants for antibacterial activity.J Ethnopharmacol: vol. 93, 43-49

WHO (2009). Diarrhoeal disease.WHO media centre.fact sheet no 330 August 2009. availaible at< http://www.who.int/mediacentre/factsheets/fs330/en/index.html>.consulted on 16 feb 2012

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com