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In Vitro Assessment of Antisecretory Activity of Root Extracts of *Acacia arabica* Lam.Willd against Biodiversity of *E. coli* Isolated from Bhilai- Durg Region

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Summary

This study has been done to assess antisecretory activity of methanolic and acetone root extracts of *Acacia arabica* Lam. Willd against diarrheagenic clinical, veterinary, environmental and standard isolates of *E. coli*. In addition the present study was also undertaken to evaluate MIC of two different plant extracts against test organism as well as to investigate effect of interaction between plant extracts with antibiotics Ampicillin and Penicillin G against test organisms. Determination of antibacterial activity of methanolic and acetone plant extracts and synergism/ antagonism with various drugs were performed by well-diffusion method. MIC of plant extracts were evaluated by broth microdilution method. Methanolic extract was found to be effective against all isolates of *E. coli* were as acetone extract was found to be effective against four isolates and ineffective against clinical strain of *E. coli.* MIC of methanolic root extract was found to be 0.6 mg/ml for EC (1), EC(3),EC(4) and *E. coli* MTCC 723 and 0.3 mg/ml for EC(2). MIC of acetone root extract for EC(1) was 2.5 mg/ml and 0.6 mg/ml for EC(2),EC(3),EC(4) and *E. coli* MTCC 723. In combination with Ampicillin and methanolic root two cases of synergism were recorded were as antagonism were observed in three different isolates. Synergism was also reported in three different strains of *E.coli* in combination with methanolc root extract and Penicillin G. Additive activity was also observed in case of combined activity of acetone extract with Penicillin G (in two isolates) and Ampicillin (in 3 isolates) of *E. coli.* It is therefore concluded that additive / synergistic effect produced by combination between plant extracts and antibiotics offered an alternative therapy to cure diarrhea caused by MDR- *E.coli* more effectively.

Key Words: Antisecretory, Broth micro- dilution method, Synergistic effect

Introduction

Despite of various infectious diseases associated with microorganisms the most common and frequently occurring disease is diarrhea that persists globally. The etiological agent associated with diarrhea or traveller's disease is *E. coli. E. coli* associated infections is responsible for infantile death annually (Ballal and Ramamurty, 2007). DEC also causes serious health associated problems in adults and also infects dairy animals. Consumption of contaminated water and animal associated dairy and poultry products are the major factors responsible for acquiring pathogenic serotypes of *E.coli*. One of the recent problems faced by various medical and veterinary practitioners to treat the persistent disease associate with *E.coli* is drug resistance with various antibiotics. Multiple –Drug Resistance among various clinical and environmental isolates make treatment complicated. Long term effect of pathogens in developing countries was reported to be more dangerous than short term effect in malnutricious infants (Kahali *et al*., 2004).Wide spread and excessive use of antibiotics and combination of genetic versatility were reported to be the most important cause of drug resistance of previously sensitive microbes (Olila *et al*., 2004). Penicillin, Tetracycline and Erythromycin were reported to be ineffective to various organisms including *E. coli* (Brook *et al*., 2000). Multi Drug Resistant *E. coli* was also isolated from poultry animals and is associated in causing travellers' diarrhea in human by consumption of infected poultry products (Savita *et al.,* 2007). DEC strains were also reported to be resistant to Trimethoprime, Sulfrmethoxazol including Tetracycline and Ampicillin (Hala *et al*., 2007).

Due to increasing prevalence of drug-resistance of *E.coli* among clinical isolates, the search of a new therapeutic remedy is if paramount importance. For this reason with in few years researchers and pharmacuticologists paid much attention to herbal formulations that has an ability to cure drug-resistant strains with out any harmful side effects. For last few years researchers revealed that bioremedies are safe and effective with less or no side effects for various gastrointestinal problems therefore act as suitable health analogues to cure diarrhea (Mukhergee *et al.,* 1998). Abu-Sabin *et al*. (2004) also reported that single and polyherbal preparations were found to be effective against various infectious diseases.

Acacia arabica (Lam.) Willd. Is a medicinal plant belonging to family Fabaceae. Bark extract of *Acacia arabica* was traditionally used for treatment of chronic diarrhea &

blood dysentery (Asolkar *et al*., 1992, Warrier *et al*., 1994). Ethanolic leaf extract of *Acacia arabica* was reported to be effective against EHEC O157 (MIC 35.2 µg/ml) and other pathogenic organisms (Hassan *et al.,* 2009). Roots of *Acacia arabica* was also having various medicinal aspects (Asolkar *et al*., 1992).

Materials and Method

Collection of Plant material

Roots of *Acacia arabica* was obtained from natural habitat from road side area in Bhilai, shade dried and finely powdered using domestic mixer. Plant was identified from Department of Botany, St.Thomas College, Bhilai.

Preperation of Media/inoculum

The media used for the drug sensitivity purpose were Muller- Hinton's agar (Hi-media) and Muller- Hinton's broth (Hi-media). Cultures were adjusted according to Mc Farland turbidity 0.5 standard in MH- Broth,

Preparation of Plant Extracts

Finely homogenized and powdered plant part was soaked in measured amount of solvents (methanol & acetone) for 24-48 hours and shaken occasionally at 120 r.p.m (Nair *et al.,* 2005, Dhanabalan *et al*., 2008, Sharma & Patel, 2009). The process was repeated to recover large quantity of extract and was stored under refrigeration at -4^{0}C for further use to test antibacterial sensitivity. The yield was recovered as % of quantity of initial root powder used (7.5 g) in 100 ml of solvents taken.

Yield (%) = yield × 100/7.5

Bacterial Strains

E. coli strains were obtained from diarrheagenic calve stool veterinary isolates, infantile stool, environmental water samples and a reference standard from IMTECH Chandigarh, India. Enterotoxigenic *E. coli* MTCC 723. Clinical, veterinary and environmental isolates were isolated, identified and characterized by conventional biochemical tests.

Evaluation of Antibacterial activity by well-diffusion method

Antibacterial activities of methanol and acetone root extracts were tested against different DEC by well-diffusion method (Ncube *et al*., 2008). About 100 µl of Muller-Hinton's broth containing test organisms were aseptically spread over Muller-Hinton's agar plate and spread uniformly with swab and allowed to dry for half an hour. With a sterile well cutter of 6 mm diameter wells were cut in all petriplates. Five different concentrations of plant extracts 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml of both extracts were taken and 100 µl of each dilution was placed into wells and kept for 1 hour for complete diffusion inside laminar hood. All plates were incubated for 37^{0}C for 24 hours. All experiments were performed in triplicates and average of inhibition zone diameter (IZD) was recorded.

Determination of Minimum Inhibitory Concentration

Minimum Inhibitory Concentration of two different root extracts was estimated by broth microdilution technique in 96-multiwell micro-titer plates (Kashikar & George,2006), About 10- fold dilutions of plant extracts from 10 mg/ml to 0.01 mg/ml was prepared). About 10 µl of double strength of Muller-Hinton's broth was placed into each well, then same amount of test organisms of different isolates were placed into each well. Exactly same amount of plant extracts of decreasing concentration (two fold step wise serial dilution) starting from highest to lowest were inserted into each well and incubated for 37^{0}C for 24 hrs. On next day 10 µl of 0.2 mg/ml of 2,3,4-triphenyl tetrazolium chloride (INT) (Kashikar & George, 2006) was introduced into each well and kept for 6 hrs for determination of exact inhibition well. Lowest concentration of extract that shows no growth was taken as MIC. Colorless tetrazolium act as electron acceptor and react with organism and turn pink to red in color to detect the presence of growth (Al-Bayati, 2009).The well that turns pink after a certain stage of dilution indicates that activity of biological active compound stops and growth persists.

Estimation of Synergistic /Antagonistic Activity

Synergistic and antagonistic activity between plant extracts and antibiotics Ampicillin and Penicillin G between acetone and methanolic extracts were performed by well-diffusion method (Odunbaku *et al.*, 2008).Concentration of plant extracts was taken having moderate zone diameters. About 100 µl of two different antibiotics of 0.2 mg/ml were added to each well individually as well as in combination with plant extracts to estimate additive activity of combination to inhibit test organism. Estimation of synergistic /antagonistic activity was performed by measuring zone diameter of plant extract, antibiotics and combination of both (extract +antibiotics) on next day after incubation under standard condition for 24 hrs at 37^{0}C.

Results

Yield of plant extracts

Methanolic extract shows limited yield as compared to acetone extract revealed that acetone shows better solubility as compared to methanol.

Determination of antidiarrheal activity on the basis of diameter of zone of inhibition

The present study revealed that methanolic and acetone extracts showed effective inhibitory response against all diarrheagenic isolates of *E. coli.* Five different *E. coli* strains from different sources showed effective zone diameter in both extracts. Both extracts were also found to be highly effective against diarreagenic standard MTCC strain of *E. coli* mentioned in table 1.

Minimum Inhibitory Concentration of Extracts

Minimum inhibitory concentration of both methanolic and acetone root extracts was performed by microdilution method using micro- titer multi well plates. Minimum inhibitory concentrations of both extracts were mentioned in table 2. MIC of methanolic and acetone extract in most cases were found to be 0.6 mg/ml.

Synergistic/ Antagonistic activity between plant extracts and different drugs:

Combined administration of methanolic root extract and Ampicillin showed synergism in two strains and antagonism in three strains of *E. coli* whoever synergism was reported with combination with Penicillin G in three strains of *E. coli* where as two strains showed antagonism. Combined activity of acetone root

extract and Ampicillin showed synergism in three *E. coli* strains. Synergism was also reported with combined administration of Penicillin G and acetone root extract mentioned in table 3.

Table:1 Mean Diameter of Zone of Inhibition of Methanolic & Acetone Root Root extract ± Standard Error

	Mean Diameter of Zone of Inhibition of Methanolic Root extract ± Standard Error					Mean Diameter of Zone of Inhibition of Acetone Root extract ± Standard Error				
Organisms used	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	31.25 mg/ml	500mg/ml	250mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml
*EC(Clinical diarrheagenic infant stool isolate 1)	9.6±4.5	8.6±0.5	4.0±0	0±0	0±0	3.0±0	0±0	0±0	0±0	0±0
*EC(vet calve stool isolate2)	7.3±0.5	5.3±0.4	3.3±0.4	3.0±0	0±0	10±0	8.3±1.1	7.0±0.3	5.3±0.4	4.3±0.8
*EC(Environmental isolate 3)	8.0±0	2.6±0.2	2.0±0	0±0	0±0	21.3±0.5	19.6±0.9	15.3±0.5	10.6±0.5	9.3±0.1
*EC(Environmental isolate4)	19.3±0.1	17.0±0.3	15.3±0.1	9.6±0.1	5.6±0.1	15.6±0.9	11.3±0.5	10.0±0	9.0±0.3	7.6±0.1
E. coli MTCC723	14.0±0.9	12.6±1.4	11.3±0.5	10.0±0.5	8.0±0.5	19.3±0.4	14.6±0	14.0±0.3	13.3±0.5	12.6±0.5

*EC- *E. Coli*

Table: 2 Minimum Inhibitory Concentration of Methanolic & Acetone Root Extract (in mg/ml)

Organism used	MIC of Methanolic extract	MIC of Acetone extract
EC(Clinical diarrheagenic infant stool isolate 1)	0.6	2.5
EC(vet calve stool isolate2)	0.3	0.6
EC(Environmental isolate 3)	0.6	0.6
EC(Environmental isolate 4)	0.6	0.6
E.coli MTCC723	0.6	0.6

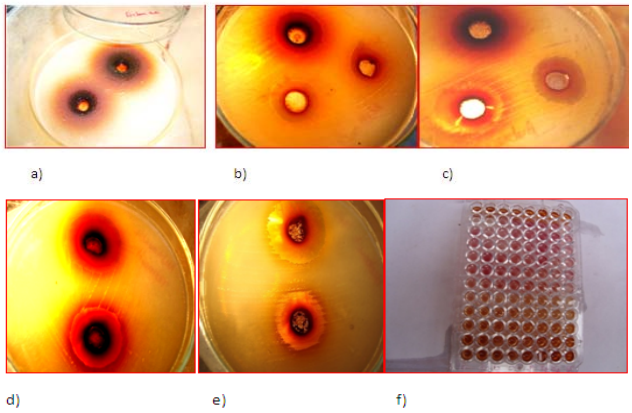
*EC- *E. Coli*

Table 3: Synergistic/ Antagonistic Activity of Methanolic/ Acetone root extract with Ampicillin & Penicillin G. Mean zone diameter ±SE

Organisms used	Ampicillin	Penicillin G	Methanolic root extract	Ampicillin + Methanolic root extract	Result	Penicillin G+ Methanolic root extract	Result	Acetone root extract	Acetone root extract + Ampicillin	Result	Penicillin G + Acetone root extract	Result
EC(1)	0±0	0±0	8.6±0.5	6±0	A	8.5±0	n.d	3.0±0	6.6±0.6	S	0±0	n.d
EC(2)	0±0	0±0	3.3±0.4	6±0.2	S	10.0±0	S	8.3±1.1	8±0	A	0±0	A
EC(3)	0±0	0±0	2.6±0.2	10.6±0.2	S	8±0	S	15.3±0.5	0±0	A	20±0	S
EC(4)	0±0	0±0	11.3±0.5	6±0.1	A	8±0	A	10.0±0	14±0	S	32±0	S
E.coli MTCC 723	0±0	0.6±0.6	12.6±1.4	2.3±0	A	16±0	S	13.3±0.5	15±0.2	S	8.6±0.6	A

*EC: *Escherichia coli*, A: Antagonism, S: Synergism, n.d: not determined

Photographs of results obtrained



a) Antibacterial activity of acetone root extract on *E. coli* MTCC 723, b) Antibacterial activity of methanolic root extract on EC(3) c) Effect of methanolic root extract on EC(4), d) Synergism between methanolic root extract with Ampicillin and Penicillin G on EC(3), e) synergism between acetone root extract with Ampicillin and Penicillin G on EC(4), f) MIC of methanolic and acetone extract in micro-titer plates: pink colour indicates growth.

Discussion

The yield of methanolic extract was found to be less (11.6%) as compared to acetone extract (22.8 %) due to dissolving ability of plant material. It was therefore concluded that solvent extraction and yield were highly effected antimicrobial activity (Ekwene & Elegalam, 2005).

Acacia arabica (Lam.) Willd. Root extracts exhibited effective *in vitro* anti-diarrheal response against diarrheagenic clinical and environmental isolates of *E. coli.* Antibacterial activity of various plant extracts used in this work were dependent upon concentration of plant extracts used by well-diffusion method. Table 1 represents that mean zone diameter was found to be largest in EC4 (Environmental isolate) and Standard MTCC 723 reference strain of *E. coli* in methanolic extract whereas moderate IZD (Inhibition Zone Diameter) were observed in three different isolates. Acetone root extract revealed that strongest inhibitory activity was observed in EC(2), EC(4) and MTCC 723 reference strain of *E. coli* where as EC(1) clinical isolate showed resistance to acetone extract. Both extracts were found to have wider spectrum of anti-diarrheal activity against *E. coli* isolated from various sources. However, well diffusion method faced large amount of disadvantages since its diffusion rate was quite unreliable (Silva *et al*., 2005, Ncube *et al*., 2008).

Minimum inhibitory concentration of methanolic root extract Table: 2 was found to be same in case of four different isolates of *E. coli* including EC(1), EC(3), EC(4) and MTCC 723 of 0.6 mg/ml where as MIC of EC(2) was found to be 0.3 mg/ml. MIC (Minimum inhibitory concentration) of Acetone root extract Table: 2 was found to be 2.5 mg/ml in EC (1) and 0.6 mg/ml for other isolates including standard strain of *E. coli*.The best method used for detection of inhibitory activity used in this work was broth micro dilution method since it provides accurate results of inhibition at very limited concentration of drugs used (Ncube *et al*., 2008). However, in view of the results obtained both well-diffusion method and broth- micro dilution technique were found to be effective against test organism but well diffusion method requires much amount of extract to show visible results of zone inhibition but micro dilution method was found to be highly sensitive in very small amount of extract to inhibit test organisms.

Synergistic/ Antagonistic Activity: Synergistic/ Antagonistic activity between extracts and antibiotics were studied with Ampicillin and Penicillin G. The two cell wall synthesis inhibitor were found to be completely ineffective against any strains of *E. coli* and no IZD was observed against any organism used for investigation. However, combination of Ampicillin and methanolic root extract showed additive response against EC (2) & EC (3). Combined synergistic or additive inhibitory response was also observed with methanolic root extract and Penicillin G against EC (2), EC (3) and *E. coli* MTCC 723. Antagonism was observed in EC (1), EC (4) and MTCC 723 strain of *E. coli* on combined administration with methanolic root extract and ampicillin as well as also observed in EC (4) in combination with methanolic extract and Penicillin G. Combined activity between acetone root extract and Ampicillin showed additive response against EC(1), EC(5) and MTCC 723 standard strains of *E. coli.* EC (3) shows synergism in combination with acetone extract and penicillin G however EC (4) shows strongest synergistic response of 32 mm zone diameter with combination with acetone extract and penicillin G.

Reference

Abu-sabin,B,; Adwan,G. and Abu-Safia, O. (2004). Antibacterial Activity of Some Plant extracts utilized in popular medicines in Palestine.*Turkish Journal of Biology*, 28: 99-102.

Al-Bayati, F.A. (2009). Isolation and identification of antimicrobial compound from *Mentha longifolia* L. leaves grown wild in Iraq. *Ann Clin Microbiol Antimicrob*.; 8: 20.

Asolkar, L.V.; Kakkar, K.K.and Chakve, O.L. (1992). *Second Supplement to Glossary of Indian Medicinal Plants with Active Principles.* Part 1, 1 st Edition, Publication & Information Directorial (CSIR), New Delhi, India, pp. 10-11.

Ballal, M. and Ramamurthy,T. (2007). Virulence characteristics and Molecular Epidermology of Diarrheagenic *Escherichia coli* (DEC) Associated with Sporadic cases in a Tertiary

Care Hospital in Manipal-Southern India. *World Journal of Medical Sciences*, 2(1): 63-64.

Brook, L.; Gouch, W.M.; Jenkine, S.G.;Pichicharo, M.E.; and Reines,S.R.(2000). Medical Management of Acute Bacterial sinusitis recommendations of Clinical Advisory committees on Pediatrics and Acute sinusitis*. Ann. Otol. Rhind. Laryngot*.109: 1-19.

Dhanabalan, R.; Doss, A.; Jagadeeshwari, M; Karthic, R.; Palaniswamy, M. and Angayarkanni, J. (2008). Preliminary Phytochemical Screening and Antimalarial Studies of *Spathodea campanulatum* P. Beauv Leaf Extracts. *Ethnobotanical Leaflets,* 12: 811-19

Ekwenye, U.N. and Elegalam, N.N. (2005). Antibacterial Activity of Ginger (*Zingiber officinale*) and Garlic (*Allium sativum* L.) Extracts on *Escherichia coli* & *Salmonella typhi. International Journal of Molecular Medicines & Advanced Science*, 1: 411- 416.

Hala, A.K.; Metaully, E.I, Ibrahim, H.A, El- Alhamana, M.N. and Amen, M.A. (2007). Multipex PCR for Detection of Diarrheagenic *Escherichia coli* in Egyptian Children. *Journal of Medicinal Sciences*, 7:255-262.

Hassan, A.; Rahaman, S.; Deeba, F. and Mahmud, S. (2009). Antimicrobial Activity of Some Medicinal Plant Extracts Having Hepatoprotective Effect. *Journal of Medicinal Plant Research*, 3: 20-23.

Kahali,S; Sarkar, B.; Rajendran, K.; Khanan, S.; Yamasala, S.; Nandy, R.K.; Bhattacharya, S.K and Ramamurthy,T. (2004). Virulence Characteristics and Molecular Epidermology of Enteroaggregative *Escherichia coli* Isolates from Hospitaized Diarrheal Patients in Kolkata, India. *Journal of Clinical Microbiology*, 42(9); 4111-4120.

Kashikar, N.D. and George, I. (2006). Antibacterial activity of *Cissus quadrangularis* Linn. *Indian Journal of Pharmaceutical Sciences,* 68: 245-247.

Mukhergree, P.K.; Saha, K.; Murughesan, T. and Mandal, S.C. (1998). Screening of Anti- diarrheal Profile of Some Plant Extracts of a Specific Region in West Bengal. *Journal of Ethanopharmacology*, 60: 85-89.

Nair, R.; Kalariya, T. and Sumitra, C. (2005). Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turkish Journal of Biology,* 29: 41-47.

Ncube, N.S.; Afolayan, A.J. and Okoh, A.I. (2008). Assessment Techniques of Antimicrobial Properties of Natural Compounds of Plant Origin: Current Methods and Future Trends. *African Journal of Biotechnology,* 7: 1797-1806.

Olila,D.; Olwa- Odyek and Opuda- Asibo, N. (2001). Antibacterial and Antifungal Activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandenisis*, Uganda Medicinal Plants. *African Health Sciences*, 1(2);66-72.

Odunbaku, O.A.; Ilusanya, O.A.and Akasora, K.S. (2008). Antibacterial Activity of Ethanolic Leaf Extract of *Ficus exasperata* on *Escherichia coli* & *Staphylococcus aureus*. *Scientific Research & Assay*, 3: 562-564.

Warrier, P.K.; Namber, V.P.K. and Ramankutty, C. (1994). *Indian Medicinal Plants*. 1st Edition, Vol 1, Orient Longman Ltd, Madras, India, pp .21-29.

Savita; Kusumakar, R.K. and Malik, Y.P.S. (2007). *In vitro* Characterization of multiple- drug resistant diarrheagenic avian *Escherichia coli* isolates. *Indian Journal of Poultry Science*, 42(3), 1153-1158.

Sharma, A.; Patel, V.K. and Ramteke, P. (2009). Identification of Vibriocidal Compounds from Medicinal Plants using Chromatographic Fingerprinting. *World Journal of Microbiology & Biotechnology*, 25: 19-25.

Silva, M.T.G,;Simas, S.M.; Batista T.G.F.M.; Cardarelli ,P. and Tomassini, T.C.B. (2005). Studies on antimicrobial activity of *Physalis angulata* L. (Solanaceae) fraction and physalin B bringing out the importance of assay determination. *Memorias do Instituto Oswaldo Cruz*,100(7): 779-782.