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RESEARCH ARTICLE

PHARMACOLOGICAL EVALUATION OF CRUDE EXTRACTS OF *ALLAMANDA CATHARTICA* LINN. EXTRACTED IN POLAR AND NON POLAR SOLVENTS

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ABSTRACT

Evaluation of the antibacterial activity of crude extracts from different parts (leaf and flower) of *Allamanda cathartica* (Apocynaceae) Linn. was carried out in the present investigation. Different parts were collected, dried and extracted by using polar (Water and Methanol) and Non-polar (Petroleum ether) solvents. Extracts were screened for antimicrobial activity using 'Disc Diffusion Assay' against 3 Gram negative (*E.coli, K.pneumoniae* and *A.tumifaciens*) and 2 Gram positive (*S.aureus* and *B.subtilis*) bacteria. Minimum inhibitory concentration, Minimum bactericidal concentration & Total activity were studied. Mean and Standard Deviation have also been calculated. *B. subtilis* & *A. tumifaciens* found to be the most susceptible organism. Water extract of flower showed the best activity against *B. subtilis* (IZ= 22 mm, AI= 0.63±0.01, MBC= 0.078 mg ml^{-1}, MIC= 0.039 mg ml^{-1}, TA= 397.43 ml g^{-1}). Pet ether extract of flower (IZ= 21 mm, AI= 0.60±0.01, MBC= 0.156 mg ml^{-1}, MIC= 0.078 mg ml^{-1}, TA= 228.97 ml g^{-1}) and methanolic extract of leaf (IZ= 21.5 mm, AI= 0.67±0.01, MBC= 0.078 mg ml^{-1}, MIC= 0.039 mg ml^{-1}, TA= 5224.36 ml g^{-1}) also showed very good activities against *B. subtilis*. Methanolic extract of flower showed good activity against *E. coli* (IZ= 20 mm, AI= 0.57±0.01, MBC= 0.156 mg ml^{-1}, MIC= 0.078 mg ml^{-1}, TA= 2208.07 ml g^{-1}). The range of MBC & MIC was found to be 0.625-0.039 mg ml^{-1} & 1.25-0.078 mg ml^{-1}, respectively. Results reveal good antimicrobial potential of extracts of *A.cathartica* against tested microorganisms. Hence, may be explored for formation of new antimicrobial drugs.

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INTRODUCTION

A medicinal plant is defined as any plant which contains substances that can be used for therapeutic purposes or are the precursors of chemotherapeutical semi synthesis. Majority of the traditional medicines used in healthcare are obtained from plants (Kala *et al*., 2004). A number of interesting outcomes have been found with the use of a mixture of natural products to treat diseases, most notably the synergistic effects and polypharmacological application of plant extracts (Gibbons, 2003). India with its richness and diversity can be considered as the paradise of medicinal plants. Since prehistoric times, nearly all cultures, both ancient and modern, have used plant as natural resources for medicinal purposes. The study of plants continues principally for the discovery of novel compounds responsible for medicinal activity of plants. Hence in the present study phytochemical screening of some important medicinal plants was carried out. Since ancient times, people have been exploring the particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte, 1998). In the present study *Allamanda cathartica* has been selected for the study. *Allamanda cathartica* (common name Golden trumpet) is a woody climbing evergreen shrub belongs to family Apocynaceae. It is native to Central America and Brazil, cultivated in India for showy flowers, found wild in Karnataka. Various medicinal properties viz. good purgative, Antidote for poisioning, inflammation, constipation, ascites are attributed to this plant. Besides, distilled extract of the plant claims cure of malignancy, fungal and bacterial diseases, for colic and acute abdominal pain. It is used for jaundice and enlarged spleen resulting from malaria. It is active in vivo in mice and in vitro against human carcinoma of nasopharynx. Alcoholic and aqueous extract is hypertensive. It's cathartic (milky sap) posses antibacterial and possibly anticancer activity. The

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present investigation was undertaken to find out the antibacterial potential of crude extracts of different parts of *A. cathartica* against some Gram positive and Gram negative bacteria. The methanolic extracts of *A. cathartica* showed active inhibition against *Salmonella typhi, salmonella paratyphi, Streptococcus aureus, Klebsiella vulgaris, Shigella dysenteriae, Shigella boydii* and *Escherichia* (Britto *et al*., 2011). Petroleum ether extract of *A.cathartica* showed good inhibition against *Staphylococcus aureus* (20mm), *Escherichia coli* (13mm), *Pseudomonas aeruginosa* (19mm), *Acinetobacter sp* (20mm), *Proteus sp* (18mm) (Rajamanickam and Sudha, 2013). *Allamanda cathartica* (leaf) extract found to exhibit potential antimicrobial properties against human pathogenic bacteria *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* (Md. Chowdhury *et al*., 2013). Petroleum ether and chloroform extracts of *Allamanda cathartica* exhibited promising antifungal activity (Singha *et al*., 2011).

Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant crude extracts for their antimicrobial activity may provide new antimicrobial substances. Review of the current literature reveals that no work has been carried out for extraction and screening of specific compound from selected plant. Hence, in the present work an extraction and screening for antibacterial activity of crude extracts of *A.cathartica* has been undertaken.

MATERIALS AND METHODS

Different parts of *A.cathartica* (leaf and flower) were collected in the month of April to June from the western parts of India (Jaipur, Rajasthan). Plants were identified by senior taxonomist at Department of Botany, University of Rajasthan and voucher specimen no: RUBL 21177 was submitted to the Herbarium, Botany Department, University of Rajasthan.

Preparation of Extracts

Powder of all the two plant parts (Leaf & flower) were taken in different round bottom flasks in different solvents. 20 g powder was taken in each flask and water, methanol and petroleum ether were used as solvent. Dried material and solvents were taken in 1:10 ratio. Those were kept at soxhlet unit for 24 hours. Then extracts were filtered. The filtrates were subjected to evaporation to obtain dried extract. The percentage yield of each dried plant extract was calculated.

Selected test microorganisms

Five pathogenic bacteria were screened, viz., *Escherichia coli* (MTCC no.46), *Bacillus subtilis* (MTCC no. 121), *Staphylococcus aureus* (MTCC no. 3160), *Klebsiella pneumoniae* (MTCC no.4030) and *Agrobacterium tumifaciens* (MTCC no. 431). The pathogens were procured from IMTECH (Chandigarh, Punjab, India). Bacterial strains were grown and maintained on Muller-Hinton Agar medium. *E.coli* is one of the most frequent causes of many common bacterial infections including bacteremia, urinary tract infection (UTI), traveler's diarrhea, neonatal meningitis (Venier *et al*., 2007) and pneumonia. Some virulent strains cause serious illness or death in the elderly, the very young or the immunocompromised (Hudault *et al*., 2001; Nataro and Kaper, 1998). Intestinal mucosa associated *E. coli* is observed in increased number in the inflammatory bowel diseases, Crohn's diseases and ulcerative colitis (Rolhin and Darfeuille-Michaud, 2007; Toder, 2007). *S.aureus* is the most common hospital acquired pathogen and cause staph infections which is responsible for various diseases including: mild skin infections e.g. folliculitis, invasive diseases e.g. wound infections and bacteremia etc., and toxin mediated diseases e.g. food poisoning, toxic shock syndrome (TSS) and scaled skin syndrome etc. In infants its infection can cause a severe disease Staphylococcal scalded skin syndrome (SSSS) (Curran and Al-Salihi, 1980). Recently, the serious emergence of antibiotic resistance staph occurred with a specific strain is Methicillin-Resistant *Staphylococcus aureus* (MRSA) and research being done to investigate hospital acquired MRSA. *B.subtilis* bacteria are nonpathogenic. They can contaminate food; however, they seldom result in food poisoning. *K.pneumoniae* cause destructive changes to human lungs inflammation and hemorrhage with cell death (necrosis). The range of clinical disease includes pneumonia, thrombophlebitis, UTI, cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis and bacteremia and septicemia. *Agrobacterium tumifaciens* is a tumor producing pathogenic bacteria and do not benefit the plant. Economically, this pathogen is a serious pathogen of walnuts, grape vines, and stone fruit.

Antimicrobial assay

'Disc Diffusion Assay' was performed for screening (Andrews, 2001). MH agar base plates were seeded with the bacterial inoculum (inoculum size 1×10^8 CFU ml^{-1}). Sterile filter paper discs of Whatmann no.1 (6mm in diameter) were impregnated with 100µl of each of the extract of concentration 10mg ml^{-1} to give a final concentration of 1 mg per disc. Discs were left to dry in vaccuo so as to remove residual solvent, which might interfere with the determination. Discs with extract were then placed on the corresponding seeded agar plates. Each extract was tested in triplicate along with streptomycin (1mg per disc) as standard drug for bacteria. The plates were kept at 4°C for diffusion of extract, thereafter were incubated at 37°C for 24h. Activity index for each extract was calculated (Table I) by the standard formula viz.

Activity index = IZ produced by the extract/ IZ produced by standard

Where, IZ = inhibition zone.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC)/ Fungicidal (MFC) Concentration

Minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity against the test pathogens. 'Broth micro dilution' method was followed for determination of MIC values (Basri and Fan, 2005). Plant extracts were resuspended in acetone (which has no activity against test microorganisms) to make 10 mg ml^{-1} final concentration. Two fold serially diluted extracts were added to

broth media of 96-wells of micro titer plates. Thereafter 100µl inoculum (1×10^8 CFU ml^{-1}) was added to each well. Bacterial suspensions were used as negative control, while broth containing standard drug was used as positive control. Micro titer plates were then incubated at 37°C for 24 h. Each extract was assayed in duplicate and each time two sets of micro plates were prepared, one was kept for incubation while another was kept at 4°C for comparing the turbidity in the wells of micro plate. The MIC values were taken as the lowest concentration of the extracts in the well of the micro titer plate that showed no turbidity after incubation. The turbidity of the wells in the micro titer plate was interpreted as visible growth of microorganisms. The minimum bactericidal concentration (MBC) was determined by sub culturing 50 µl from each well showing no apparent growth (Table II). Least concentration of extract showing no visible growth on sub culturing was taken as MBC.

Total activity (TA) determination

Total activity is the volume up to which test extract can be diluted without losing the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1g plant material by the MIC of the same extract or compound isolated and is expressed in ml g^{-1} (7) (Table III) Total Activity= Amount of extract from 1gm dry plant material/MIC value of the extract

RESULTS

Antimicrobial efficiency of crude extracts was assessed by using IZ, AI (Table I), MIC & MBC (Table II). Quantity of extract per gram of plant material was also calculated (Table III). In the present investigation 6 extracts were tested against five pathogenic bacteria, including three Gram-ve (*E.coli, K.pneumoniae* and *A.tumifaciens*) and two Gram+ve (*S.aureus* and *B.subtilis*) bacteria. Among all the tested extracts, water extract of flower found to be the least active as showed activity against only two of the five pathogens. *E. coli* found to be the most resistant organism amongst all the five pathogens as 3 out of 6 tested extracts showed activity against it. The most susceptible organism in the study was *B. subtilis* & *A. tumifaciens*, against which all the 6 tested extracts showed activity. Best activity was observed in water extract of flower against *B. subtilis* (IZ= 22 mm, AI= 0.63±0.01, MIC= 0.078 mg ml^{-1}, MBC= 0.039 mg ml^{-1}, TA= 397.43 ml g^{-1}) followed by *A. tumifaciens* (IZ= 14 mm, AI= 0.50±0.02, MIC= 0.312 mg ml^{-1}, MBC= 0.156 mg ml^{-1}, TA= 99.36 ml g^{-1}), while methanolic extract of flower showed very good activities against *E. coli* (IZ= 20 mm, AI= 0.57±0.01, MIC= 0.156 mg ml^{-1}, MBC= 0.078 mg ml^{-1}, TA= 2208.07 ml g^{-1}) followed by *B. subtilis* (IZ= 18 mm, AI= 0.51±0.02, MIC= 0.156 mg ml^{-1}, MBC= 0.078 mg ml^{-1}, TA= 2208.07 ml g^{-1}).

Table I: Antimicrobial activity of crude extracts of *Allamanda cathartica* Linn. against some pathogenic bacteria

Plant part	Extract	Microorganisms									
		E.coli		*B.subtilis*		*S.aureus*		*K.pneumoniae*		*A.tumifaciens*	
		IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
Leaf	P1	12	0.40±0.01	14.5	0.45±0.01	9.5	0.64±0.01	11	0.44±0.05	14.5	0.41±0.01
	M1	-	-	21.5	0.67±0.01	9	0.60±0.09	10.5	0.42±0.01	9.5	0.27±0.01
	W1	-	-	14.5	0.45±0.01	-	-	7	0.28±0.01	14.5	0.41±0.01
Flower	P2	10	0.28±0.02	21	0.60±0.01	7	0.58±0.02	-	-	11	0.39±0.03
	M2	20	0.57±0.01	18	0.51±0.02	8	0.67±0.04	8	0.36±0.04	11.5	0.41±0.01
	W2	-	-	22	0.63±0.01	-	-	-	-	14	0.50±0.02

P1, P2 = Pet ether extract of respective plant parts,
M1, M2= Methanolic extract of respective plant parts,
W1, W2= Water extract of respective plant parts,
IZ=Inhibition zone in mm (value: including 6mm diameter of disc),
AI= Activity index (IZ developed by extract/IZ developed by standard),
(-)= no activity, ±=SEM.

Table II: MIC and MBC of active crude extracts of *Allamanda cathartica* Linn. against different pathogens

Plant parts		Leaf			Flower		
Microorganisms	**MIC & MBC (mg ml^{-1})**	**P1**	**M1**	**W1**	**P2**	**M2**	**W2**
E.coli	MBC	0.312	-	-	0.625	0.156	-
	MIC	0.156	-	-	0.312	0.078	-
B.subtilis	MBC	0.312	0.078	0.312	0.156	0.156	0.078
	MIC	0.156	0.039	0.156	0.078	0.078	0.039
S.aureus	MBC	0.625	0.625	-	1.25	1.25	-
	MIC	0.312	0.312	-	0.625	0.625	-
K.pneumoniae	MBC	0.312	0.625	1.25	-	1.25	-
	MIC	0.156	0.312	0.625	-	0.625	-
A.tumifaciens	MBC	0.312	0.625	0.312	0.312	0.312	0.312
	MIC	0.156	0.312	0.156	0.156	0.156	0.156

P1, P2 = Pet ether extract of respective plant parts,
M1, M2= Methanolic extract of respective plant parts,
W1, W2= Water extract of respective plant parts,
MIC= Minimum inhibitory concentration (in mg ml^{-1}),
MBC=Minimum bactericidal concentration (in mg ml^{-1}),
(-)= no activity.

Table III: Quantity & Total activity of crude extracts of *Allamanda cathartica* Linn.

Plant part	Extract	Quantity of extract (mg g.d.wt.$^{-1}$)	Total Activity(ml g^{-1})				
			E.coli	***B.subtilis***	***S.aureus***	***K.pneumoniae***	***A.tumifaciens***
Leaf	**P1**	35	224.36	224.36	112.18	224.36	224.36
	M1	203.75	-	5224.36	653.04	5224.36	5224.36
	W1	112.5	-	721.15	-	180	721.15
Flower	**P2**	17.86	57.24	228.97	28.57	-	114.48
	M2	172.23	2208.07	2208.07	275.57	275.57	1104.04
	W2	15.5	-	397.43	-	-	99.36

P1, P2 = Pet ether extract of respective plant parts,
M1, M2= Methanolic extract of respective plant parts,
W1, W2= Water extract of respective plant parts,
TA= total activity (extract per gm dried plant part/MIC of extract) in ml g^{-1},
(-)= no activity.

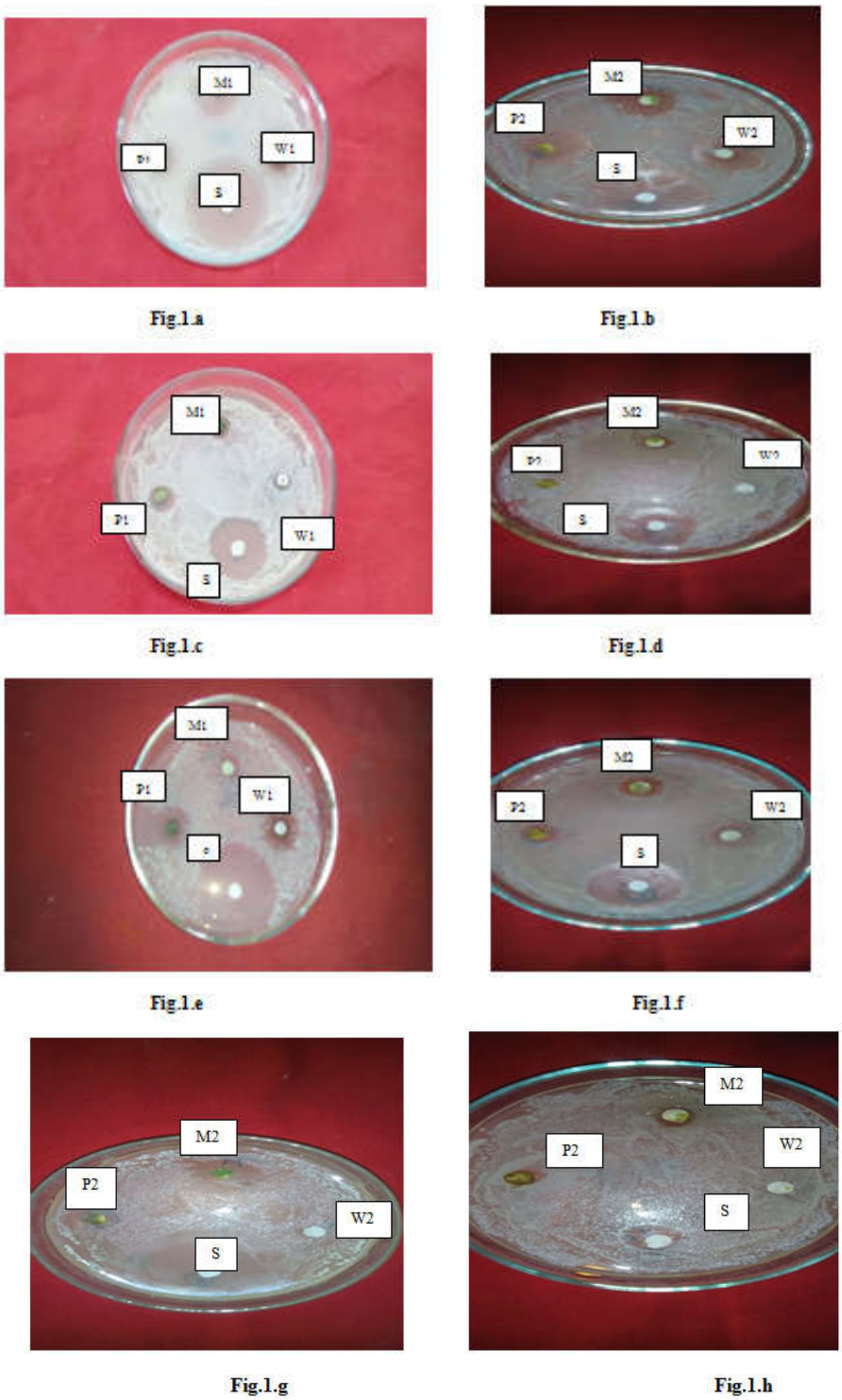

Fig.1.a Fig.1.b

Fig.1.c Fig.1.d

Fig.1.e Fig.1.f

Fig.1.g Fig.1.h

Figure 1. Antimicrobial activity of extracts of leaves & flowers of *Allamanda cathartica* Linn.

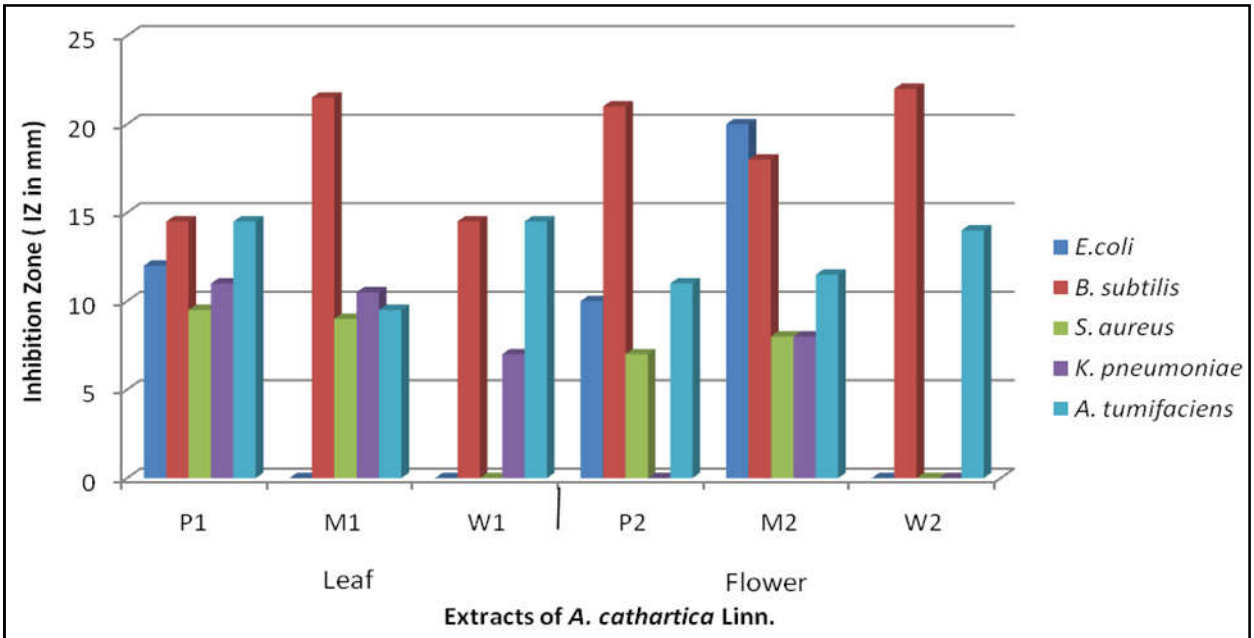

Figure 2: Antimicrobial activity of crude extracts of *A. cathartica* Linn. against selected microorganisms

Pet ether extract of flower showed very good activity against *B. subtilis* (IZ= 21 mm, AI= 0.60±0.01, MIC= 0.156 mg ml^{-1}, MBC= 0.078 mg ml^{-1}, TA= 228.97 ml g^{-1}). Water extract of leaf showed good activities against *B. subtilis* (IZ= 14.5 mm, AI= 0.45±0.01, MIC= 0.312 mg ml^{-1}, MBC= 0.156 mg ml^{-1}, TA= 721.15 ml g^{-1}) as well as against *A. tumifaciens* (IZ= 14.5 mm, AI= 0.41±0.01, MIC= 0.312 mg ml^{-1}, MBC= 0.156 mg ml^{-1}, TA= 721.15 ml g^{-1}) , while methanolic extract of leaf showed very good activity against *B. subtilis* (IZ= 21.5 mm, AI= 0.67±0.01, MIC= 0.078 mg ml^{-1}, MBC= 0.039 mg ml^{-1}, TA= 5224.36 ml g^{-1}). Pet ether extract of leaf also showed good activities against *B. subtilis* (IZ= 14.5 mm, AI= 0.45±0.01, MIC= 0.312 mg ml^{-1}, MBC= 0.156 mg ml^{-1}, TA= 224.36ml g^{-1}) as well as *A. tumifaciens* (IZ= 14.5 mm, AI= 0.41±0.01, MIC= 0.312 mg ml^{-1}, MBC= 0.156 mg ml^{-1}, TA=224.36 ml g^{-1}). Pet ether extract of leaf showed satisfactory activities against *E. coli* (IZ= 12 mm, AI= 0.40±0.01, MIC= 0.312 mg ml^{-1}, MBC= 0.156 mg ml^{-1}, TA= 224.36 ml g^{-1}) & *K. pneumoniae* (IZ= 11 mm, AI= 0.44±0.05, MIC= 0.312 mg ml^{-1}, MBC= 0.156 mg ml^{-1}, TA= 224.36ml g^{-1}), while pet ether extract of flower (IZ= 11 mm, AI= 0.39±0.03, MIC= 0.312 mg ml^{-1}, MBC= 0.156 mg ml^{-1}, TA= 114.48ml g^{-1}) & methanolic extract of flower (IZ= 11.5 mm, AI= 0.41±0.01, MIC= 0.312 mg ml^{-1}, MBC= 0.156 mg ml^{-1}, TA= 1104.04 ml g^{-1}) showed satisfactory activities against *A. tumifaciens*. Among all the tested extracts pet ether extract of leaf and methanolic extract of flower found to be the most active substances as they showed activities against all the tested pathogens. Plant extracts, which had shown activity in diffusion assay, were evaluated for their MIC & MBC values (Table II). The range of MIC & MBC of extracts recorded was 0.625- 0.039 mg ml^{-1} & 1.25- 0.078 mg ml^{-1}, respectively. In the present investigation, lowest MIC value 0.039 mg ml^{-1} was recorded against *B. subtilis*, indicating significant antimicrobial efficacy of test extracts. Quantity of extract obtained per gram from plant parts & TA was calculated and recorded (Table III). TA indicates the volume at which extracted can be diluted without losing ability to kill microorganisms. High values of TA were observed against *B. subtilis* (5224.36 ml g^{-1}) as well as against *K.pneumoniae* (5224.36 ml g^{-1}) & *A.tumifaciens* (5224.36 ml g^{-1}).

DISCUSSION

Due to indiscriminate use of antimicrobial drugs, the microorganisms have developed resistance to many antibiotics. This has created immense clinical problems in the treatment of infectious diseases (Davis, 1994). Hence, a continuous research for getting new antimicrobial agents is the need of the present scenario, either by designing and synthesizing new agents, chemically or through the search of new natural sources for antimicrobial agents (Bhavnani and Ballow, 2000). Present investigation is an effort towards this direction. In present study, *A. cathartica* has shown antimicrobial potential against all the five tested bacteria which are the major causative agents of various human diseases. Although the plant has been studied previously for its antimicrobial activity but only restricted to the determination of IZ and that too without AI, MIC, MBC & TA evaluation. Hence could not explore the preparation of antibiotics. Such studies could only indicate their antimicrobial potential but can't replace the existing antibiotics. In present investigation, IZ, AI, MIC, MBC & TA have been evaluated for each extract to determine their antimicrobial activity. The activity of plant extracts against both Gram+ve and Gram-ve bacteria may be an indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. From this study it can be concluded that crude extracts of different parts of plant exhibited potential bactericidal properties. Present investigation together with previous studies; provide support to

the antimicrobial properties of *A. cathartica*. Therefore it can be used as antimicrobial supplements in the developing countries towards the development of new therapeutic agents. Further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of these plant extracts in treating various infectious diseases. The demonstration of broad spectrum of antimicrobial activity by *A. cathartica* may help to discover new chemical classes of antibiotic substances that could serve as alternatives or second line treatment for infectious diseases and their control.

Conclusion

Present study concluded that among all the tested extracts of different plant parts, pet ether extract of leaf and methanolic extract of flower found to be the most active substances while water extract of flower found to be the least active. *B. subtilis* & *A. tumifaciens* were found to be the most susceptible organisms while *E. coli* found to be the most resistant throughout the study. Lowest MIC value 0.039 mg ml^{-1} was recorded against *B. subtilis*, indicating significant antimicrobial efficacy of the tested extracts. High values of TA (5224.36 ml g^{-1}) were observed against *B. subtilis, K.pneumoniae* & *A.tumifaciens*, indicating strong antimicrobial potential, even in the diluted forms of the extracts.

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