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Research Article

Evaluation of antimicrobial efficacy of alkaloids, flavonoids and steroids of *Allamanda cathartica* Linn. against some pathogenic bacteria

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ABSTRACT

Plants have been investigated for their antimicrobial properties and used as precursor for synthesizing medicine. Present work deals with assessing antimicrobial activity of *A.cathartica* against some multidrug resistant pathogenic bacteria. Different parts (leaf and flower) of *A.cathartica* were collected, dried and then extracted using standard methods for alkaloids, flavonoids and steroids. Extracts were then screened for antimicrobial activity using 'Disc Diffusion Assay'. Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) & Total activity were studied. Mean and Standard Deviation have also been calculated. *B. subtilis* found to be the most susceptible organism followed by *A. tumifaciens* and *S. aureus*. Free flavonoid extract of leaf showed the best activity against *K. pneumoniae* (IZ= 18mm, AI= 1±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 783.34 ml/g) followed by *B. subtilis* (IZ= 16mm, AI= 0.61±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 783.34 ml/g) and *A. tumifaciens* (IZ= 16mm, AI= 0.57±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 783.34 ml/g). Free flavonoids extract of flower also showed very good activities against *A. tumifaciens* (IZ= 17mm, AI= 0.71±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 399.67 ml/g) followed by *B. subtilis* (IZ= 16mm, AI= 0.67±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 399.67 ml/g). Bound flavonoid and alkaloid extract of leaf also showed good activities. The range of MBC and MIC was found to be 1.25-0.156mg/ml & 0.625-0.078mg/ml respectively. Results reveal that extracts of *A.cathartica* have good antimicrobial potential against tested microorganisms and may be exploited for future antimicrobial drugs.

Keywords: Disc Diffusion Assay, MIC, MBC and Total activity.

INTRODUCTION

Medicinal plants are gifts of nature to cure limitless number of diseases of human beings[1]. The abundance of plants on the earth's surface has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants, as potential source of new antimicrobial agents[2]. Increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to screening of several medicinal plants for their potential antimicrobial activity[3]. Hence, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drug against microbial infections[4].

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents[5]. The natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action[6,7]. They are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[8]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine, and to reveal the

active principle by isolation and characterization of their constituents. Systematic screening of plant extracts may result in the discovery of novel active compounds[9]. 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 poisoning, 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. Its cathartic (milky sap) posses antibacterial and possibly anticancer activity. The present investigation was undertaken to find out the antibacterial potential of flavonoids, alkaloids and steroids of different parts of *A. cathartica* against some Gram positive and Gram negative bacteria.

Alkaloids are known to have pharmacological effects and are used in medications, as recreational drugs or in entheogenic rituals. Presence of Alkaloids, Sterols and Flavonoids in leaf of *A.cathartica* and their antifungal activity against *candida albicans* were reported[10].

Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity [11]. Different phyto-constituents such as alkaloids, phenolic compounds, flavonoids, saponins, glycosides, terpenoids, steroids, coumarins, quinones, phytosterols, proteins and carbohydrates were identified in the aqueous, acetone, petroleum ether, chloroform and ethanol extracts of the flowers *Allamanda cathartica Linn*.[12].

Steroids and their metabolites are frequently used as signalling molecules, represents highly concentrated energy stores, along with phospholipids function as components of cell membranes. Literature related to antimicrobial activity of steroids of *Allamanda cathartica* was not yet found.

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 the flavonoids, alkaloids and steroids of *A.cathartica* has been undertaken.

MATERIAL 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:

Alkaloids Extraction:

Alkaloids were extracted from different parts of the selected plant by well established method [13]. Finely powered sample (100g) of plant parts were extracted in 20ml methanol after shaking of 15 min. After filtration, filtrate kept for drying then residual mass were treated with 1% H_2SO_4 (5ml. 2 times).Extraction was then done in 10ml. Chloroform ($CHCl_3$) by using separating funnel. Organic layer of chloroform was rejected and aqueous layer was basified with 30% NH_4OH (P^H=9-10). Now again, extraction was done in 10ml. chloroform & organic layer of chloroform (lower layer) was collected in a flask and repetition of step was done with fresh chloroform. Extracts was then dried in vaccuo for further use.

Flavonoid extraction:

Selected plant parts were separately washed with sterilized water; shade dried, and finely powdered using a blender. Each sample was subjected to extraction, following the method of Subramanian and Nagarjan [14]. One hundred grams of each finely powdered sample was soxhlet extracted with 80% hot methanol (500ml) on a water bath for 24 h and filtered. Filtrate was re- extracted successively with petroleum ether (fraction I), diethyl ether (fraction II), and ethyl acetate (fraction III) using separating funnel. Petroleum ether fractions were discarded as being rich in fatty substances, where as diethyl ether and ethyl acetate fractions were analyzed for free and bound flavonoids respectively. The ethyl acetate fraction of each of the samples was hydrolyzed by refluxing with 7% H_2SO_4 for 2 h (for removal of bound sugars) and the filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract obtained was washed with distilled water to neutrality. Diethyl ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried in vaccuo and weighed. The extracts were stored at 4^oc and were re-suspended in their respective solvents to get 10mg/ ml concentration for antimicrobial assay.

Steroid Extraction:

Steroids were extracted from different parts of the selected plant by well established method [15] after preliminary detection of steroids. Finely powdered sample (100g) of plant parts were extracted in petroleum ether for 2-4hr.After filtration, residual mass was treated with 15% ethanolic HCl for 4hr. Extraction was then done in ethyl acetate followed by washing in dis. water to neutralize the extract. Neutral extract was then passed over sodium sulphate to remove moisture contents and was dried in vaccuo. Chloroform was used for reconstitution of extract, filtered and dried for further use.

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 [16] and pneumonia. Some virulent strains cause serious illness or death in the elderly, the very young or the immunocompromised[17, 18]. Intestinal mucosa associated *E. coli* is observed in increased number in the inflammatory bowel diseases, Crohn's disease and ulcerative colitis [19, 20]. *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) [21]. 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.pneumonae* 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. *A. 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 [22]. MH agar base plates were seeded with the bacterial inoculum (inoculum size 1×10^8 CFU/ml). Sterile filter paper discs of Whatmann no.1 (6mm in diameter) were impregnated with 100µl of each of the extract of concentration 10mg/ml to give a final concentration of 1 mg/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/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 1] 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 [23]. Plant extracts were resuspended in acetone (which has no activity against test microorganisms) to make 10 mg/ml 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) 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 [Table2]. 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 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g [24] [Table 3].

RESULTS

Alkaloids, flavonoids & steroids were for their antimicrobial potency by using IZ, AI (Table1), MIC & MBC (Table 2). Quantity of extract per gram of plant material was also calculated (Table 3). In present study, 8 extracts were tested against five pathogenic bacteria, including both gram+ve and gram-ve strains. Among all the tested extracts, B.F. and alkaloid of flower found to be active against only one pathogen where as all other extracts were found to be more active. *E.coli* and *K.pneumoniae* found to be resistant as very few of the tested extracts showed activity against them. The most susceptible organism in the study was *B.subtilis*, against which 7 out of 8 extract showed IZ which were persistent as compared with the standard. Best activity was observed in the F.F. extract of leaf against *K. pneumoniae* (IZ= 18mm, AI= 1±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 783.34 ml/g) followed by against *B. subtilis* (IZ= 16mm, AI= 0.61±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 783.34 ml/g) and *A. tumifaciens* (IZ= 16mm, AI= 0.57±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 783.34 ml/g). F.F. extract of flower were also showed very good activities against *A. tumifaciens* (IZ= 17mm, AI= 0.71±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 2243.58 ml/g) followed by *B. subtilis* (IZ= 16mm, AI= 0.67±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 2243.58 ml/g), *K. pneumoniae* (IZ= 14mm, AI= 0.78±0.01, MBC= 0.312 mg/ml, MIC= 0.156 mg/ml, TA= 1121.79 ml/g) and *S. aureus* (IZ= 13mm, AI= 0.54±0.01, MBC= 0.312 mg/ml, MIC= 0.156 mg/ml, TA= 1121.79 ml/g). B.F. extract of leaf showed good activities against *E.coli* (IZ= 13mm, AI= 0.54±0.01, MBC= 0.312 mg/ml, MIC= 0.156 mg/ml, TA= 799.36 ml/g), *K. pneumoniae* (IZ= 13mm, AI= 0.72±0.01, MBC= 0.312 mg/ml, MIC= 0.156 mg/ml, TA= 799.36 ml/g), *S. aureus* (IZ= 12mm, AI= 0.48±0.02, MBC= 0.312 mg/ml, MIC= 0.156 mg/ml, TA= 799.36 ml/g). Alkaloid extract of leaf showed very good activity against *B. subtilis* (IZ= 15.5mm, AI= 0.59±0.01, MBC= 0.156 mg/ml, MIC= 0.078 mg/ml, TA= 557.69 ml/g). Steroidal extract of leaf, B.F. & steroidal extract of flower were showed satisfactory activities against *S. aureus* (IZ= 10.5 mm, AI= 0.42 ±0.04, MBC= 0.625 mg/ml, MIC= 0.312 mg/ml, TA= 2825.86 ml/g), *B. subtilis* (IZ= 10.5 mm, AI= 0.44 ±0.02, MBC= 0.625 mg/ml, MIC= 0.312 mg/ml, TA= 1116.47 ml/g) & *A. tumifaciens* (IZ= 10.5 mm, AI= 0.44 ±0.01, MBC= 0.625 mg/ml, MIC= 0.312 mg/ml, TA= 1442.31 ml/g), respectively. Among all the tested extracts, flavonoids found to be most bioactive substance in *A. cathartica* plant as showed activities against all tested pathogens. MIC and MBC values (Table 2) were evaluated for plant extracts which had shown activity in diffusion assay. The range of MIC and MBC of extracts recorded was 0.625-0.078 mg/ml & 1.25-0.156 mg/ml respectively. In present study, lowest MIC value 0.078 mg/ml was recorded against *B. subtilis, K. pneumoniae* and *A. tumifaciens.* Such low values of MIC showed excellent antibacterial efficacy of tested extracts. Quantification and total activity calculation was done and recorded (Table 3). Total activity (TA) is the volume after dilution, at which extract retain their ability to kill microorganisms. High values of TA were observed against *S. aureus* (2825.86 ml/g) followed by *B. subtilis* (2243.58 ml/g) as well as *A. tumifaciens* (2243.58 ml/g).

DISCUSSION

Multi drug resistance has become a global concern. The development of bacterial super resistant strains is resulting in failing of currently used antibiotic agents to end many bacterial infections. Hence, a continuous and urgent need to discover new antimicrobials, with less or no side effects, cost effective and have ability to affect a wide range of pathogens. Literature indicates that work has been carried out in this direction. The antibacterial activity of the compound quercitrin isolated from *Allamanda cathartica* Linn. was tested with *Staphylococcus aureus* and *Escherichia coli* micro organisms[25]. *A.cathartica* petroleum ether extract showed good antimicrobial activity against *S.aureus* (20mm), *E.coli* (13mm), *P. aeruginosa* (19mm), *Acinetobacter sp* (20mm) and *Proteus sp* (18mm) [26]. *A. cathartica* found to be bioactive against *Staphylococcus aureus* and *Escherichia coli* [27]. Present study is also an effort towards this direction to strengthen the discovery of new antimicrobial drugs. In the present investigation *A. cathartica* has shown antimicrobial potential against all the five tested pathogens which are the major causative agents of various human diseases. Evaluation of IZ, AI, MIC, MBC & TA have been done for each extract. Extracts recorded for low MIC values, indicating strong bioefficacy of the plant. The

findings of the present study indicate the presence of broad spectrum antibiotic compounds. The results were in agreement with the findings of previous studies. Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and provides biochemical tools for the study of infectious diseases.

CONCLUSION

Present study concluded that among all the tested extracts of different plant parts, free flavonoid extract was found to be the most active substances while alkaloid extract was found to be the least active. *B. subtilis* was recorded as the most susceptible organisms while *E. coli* & *K. pneumoniae* were found to be the most resistant throughout the study. Lowest MIC value 0.078 mg/ml was recorded against *B. subtilis*, indicating significant antimicrobial efficacy of the tested extracts. High values of TA (2825.86 ml/g) were observed against *S. aureus*, indicating strong antimicrobial potential, even in the diluted forms of the extracts. Therefore, study may be beneficial for further studies related to the production of novel antimicrobial drugs.

ACKNOWLEDGEMENT

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Table 1
Antimicrobial activity of extracts of *Allamanda cathartica* against some pathogenic bacteria

Plant part	Extract	Microorganisms									
		E.coli		*B.subtilis*		*S.aureus*		*K.pneumoniae*		*A.tumifaciens*	
		IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
Leaf	**E1**	-	-	16	0.61±0.01	14	0.56±0.01	18	1±0.01	16	0.57±0.04
	E2	13	0.54±0.01	10	0.38±0.02	12	0.48±0.02	13	0.72±0.01	10	0.36±0.07
	A1	-	-	15.5	0.59±0.01	-	-	-	-	10	0.36±0.02
	S1	7	0.29±0.01	7	0.27±0.01	10.5	0.42±0.01	-	-	8	0.28±0.01
Flower	**E1**	-	-	16	0.67±0.01	13	0.54±0.01	14	0.78±0.01	17	0.71±0.01
	E2	-	-	10.5	0.44±0.02	-	-	-	-	-	-
	A2	-	-	-	-	9	0.37±0.01	-	-	-	-
	S2	7	0.29±0.01	7	0.29±0.01	-	-	-	-	10.5	0.44±0.01

IZ=Inhibition zone in mm (value: including 6mm diameter of disc),
AI= Activity index (IZ developed by extract/IZ developed by standard),
E1 = Free flavonoids, E2= Bound flavonoids,
A1, A2, A3= Alkaloids of respective plant parts,
S1, S2, S3= Steroids of respective plant parts,
(-)= no activity, ±=SEM.

Table 2
MIC and MBC of active extracts of *Allamanda cathartica* against different pathogens

Plant parts		Leaf				Flower			
Microorganisms		**E1**	**E2**	**A1**	**S1**	**E1**	**E2**	**A2**	**S2**
E.coli	MBC	-	0.312	-	1.25	-	-	-	1.25
	MIC	-	0.156	-	0.625	-	-	-	0.625
B.subtilis	MBC	0.156	0.625	0.156	1.25	0.156	0.625	-	1.25
	MIC	0.078	0.312	0.078	0.625	0.078	0.312	-	0.625
S.aureus	MBC	0.312	0.312	-	0.625	0.312	-	0.625	-
	MIC	0.156	0.156	-	0.312	0.156	-	0.312	-
K.pneumoniae	MBC	0.156	0.312	-	-	0.312	-	-	-
	MIC	0.078	0.156	-	-	0.156	-	-	-
A.tumifaciens	MBC	0.156	0.625	0.625	1.25	0.156	-	-	0.625
	MIC	0.078	0.312	0.312	0.625	0.078	-	-	0.312

E1 = Free flavonoids, E2= Bound flavonoids,
A1, A2, A3= Alkaloids of respective plant parts,
S1, S2, S3= Steroids of respective plant parts,
MIC= Minimum inhibitory concentration,
MBC=Minimum bactericidal concentration,
(-)= no activity.

Table 3
Quantity & Total activity of extracts of *Allamanda cathartica* Linn.

Plant part	Extract	Quantity of extract mg/g dwt	Total Activity(ml/g)				
			E.coli	***B.subtilis***	***S.aureus***	***K.pneumoniae***	***A.tumifaciens***
Leaf	**E1**	61.1	-	783.34	391.67	783.34	783.34
	E2	124.70	799.36	399.67	799.36	799.36	399.67
	A1	43.5	-	557.69	-	-	139.42
	S1	881.67	1410.67	1410.67	2825.86	-	1410.67
Flower	**E1**	175	-	2243.58	1121.79	1121.79	2243.58
	E2	348.34	-	1116.47	-	-	-
	A2	674.12	-	-	2160.64	-	-
	S2	450	720	720	-	-	1442.31

E1 = Free flavonoids, E2= Bound flavonoids,
A1, A2, A3= Alkaloids of respective plant parts,
S1, S2, S3= Steroids of respective plant parts,
TA= total activity (extract per gm dried plant part/MIC of extract).

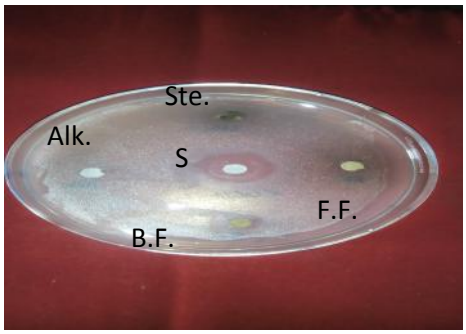

Fig. 1(a) IZ (*A.c.*/L/*E.coli)*

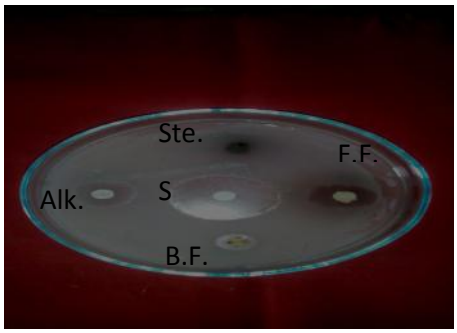

Fig. 1(b) IZ (*A.c.*/L/*B.subtilis)*

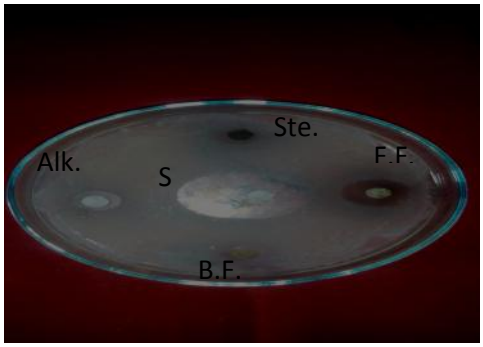

Fig. 1(c) IZ (*A.c.*/L/ *A.tumifaciens)*

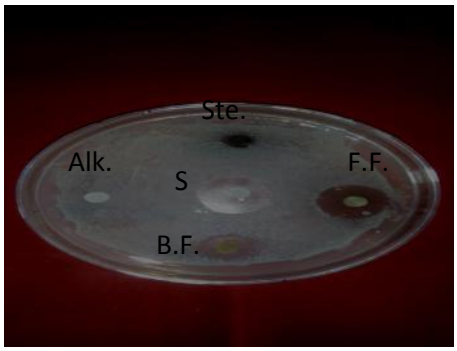

Fig. 1(d) IZ (*A.c.*/L/*K.pneumoniae)*

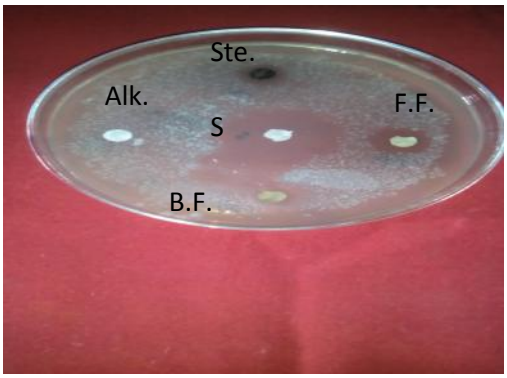

Fig. 1(e) IZ (*A.c.*/L/ *S.aureus)*

Fig. 1
Inhibition zone of extracts of *Allamanda cathartica* leaves against pathogenic bacteria-
Fig. 1(a) *Allamanda cathartica* /Leaf/ *Escherichia coli*,
Fig. 1(b) *Allamanda cathartica* /Leaf/ *Bacillus subtilis*,
Fig. 1 (c) *Allamanda cathartica* /Leaf/ *Agrobacterium tumifaciens*,
Fig. 1(d) *Allamanda cathartica* /Leaf/ *Klebsiella pneumoniae*,
Fig. 1 (e) *Allamanda cathartica* /Leaf/ *Staphylococcus aureus*
[Alk. = Alkaloid, Ste.= Steroid, F.F. = Free flavonoid, B.F. = Bound flavonoid,
S= Standard]

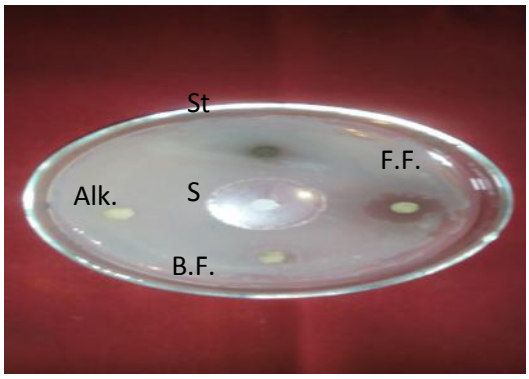

Fig. 2(a) IZ (A.c./F/ *B.subtilis*)

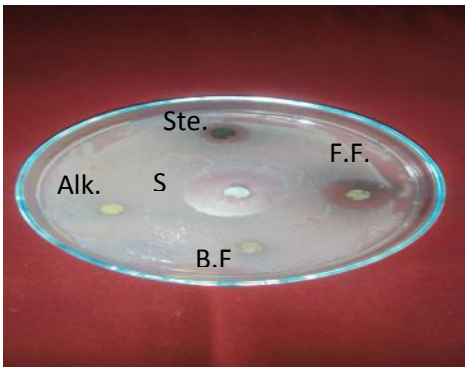

Fig. 2(b) IZ (A.c./F/*A.tumifaciens*)

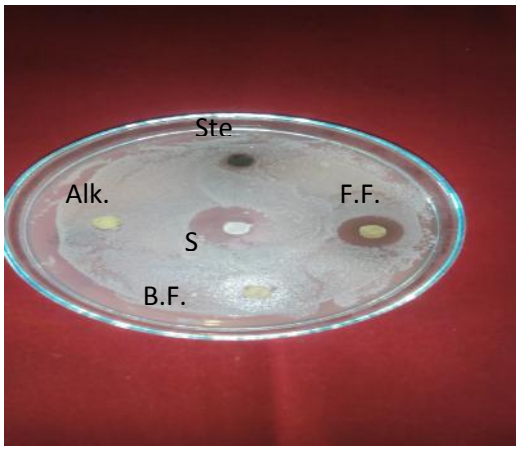

Fig. 2(c) IZ (A.c./F/ *K.pneumoniae*)

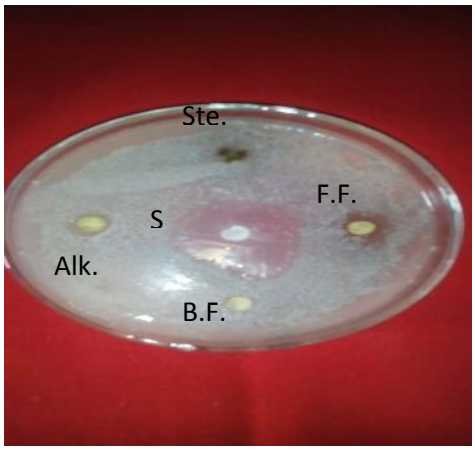

Fig. 2(d) IZ (A.c./F/*S.aureus*)

Fig. 2
Inhibition zone of extracts of *Allamanda cathartica* flowers against pathogenic bacteria-
Fig. 2(a) *Allamanda cathartica* /Flower/ *Bacillus subtilis*,
Fig. 2(b) *Allamanda cathartica* / Flower / *Agrobacterium tumifaciens*,
Fig. 2(c) *Allamanda cathartica* / Flower / *Klebsiella pneumoniae*,
Fig. 2(d) *Allamanda cathartica* / Flower / *Staphylococcus aureus*
[Alk. = Alkaloid, Ste.= Steroid, F.F. = Free flavonoid, B.F. = Bound flavonoid,
S= Standard]

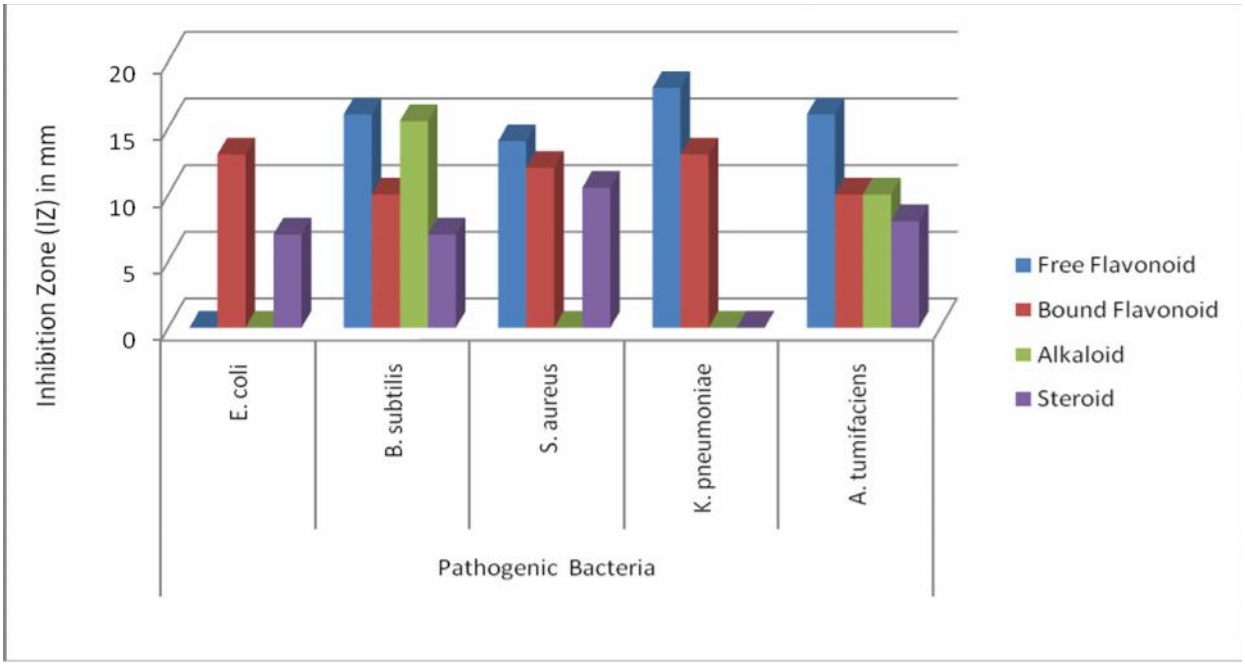

Fig. 3
Antimicrobial activity of extracts of *Allamanda cathartica* leaves against pathogenic bacteria

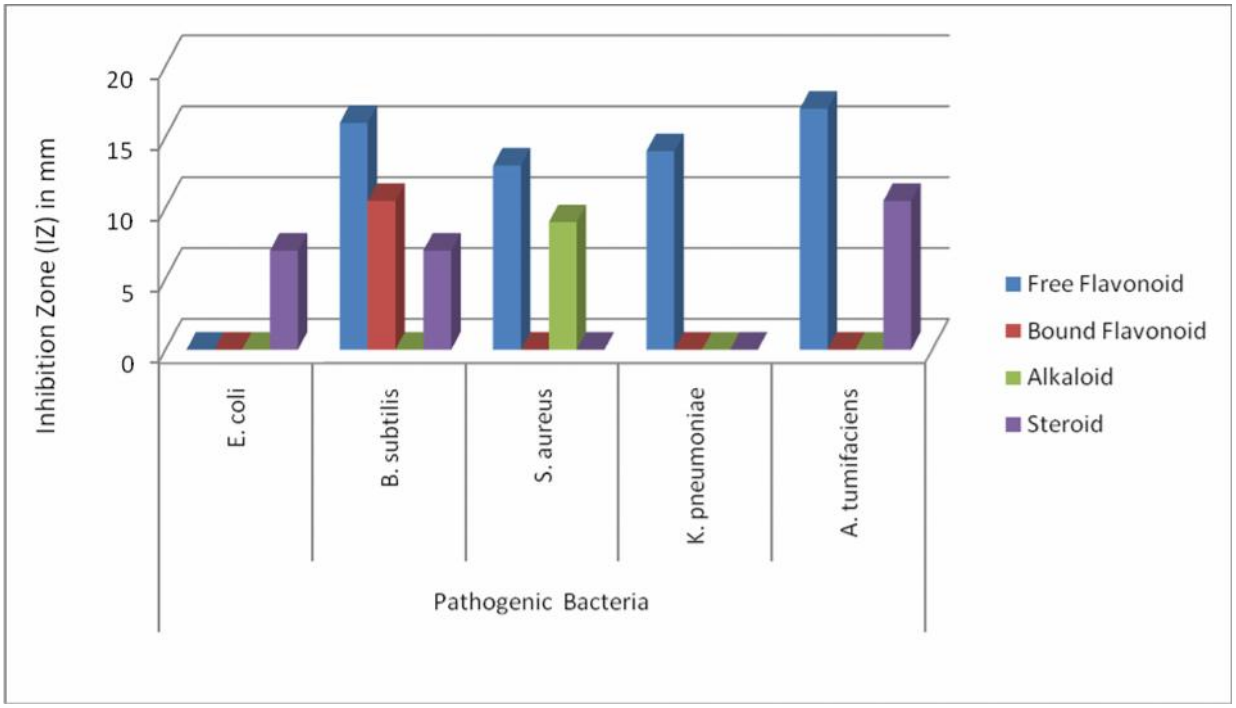

Fig. 4
Antimicrobial activity of extracts of *Allamanda cathartica* flowers against pathogenic bacteria

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