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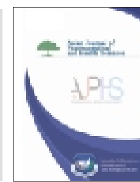

Phytochemical analysis and In-Vitro antimicrobial activity of leaf extract of *Allamanda Cathartica* Linn

Ramya Madhiri*, Sonali Jadav, Venkata Abhinay Musunuri, Charugundla Harini, Yathakula Vinay Sree

Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Secunderabad, Telangana, India.

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***Corresponding author:**

Email : madhiriramya2709@gmail.com
Phone : +91-9581552204

ABSTRACT

To investigate Phytochemical analysis and in- vitro antimicrobial activity of leaf extract of *Allamanda cathartica* linn. Plants have been the major source of drugs in medicine and other ancient systems in the world. In traditional systems of medicine, Indian medicinal plants have been used in the successful management of various disease conditions. *Allamanda cathartica* (AC)Linn. (Apocynaceae) is one of the most studied species of the Allamanda genus. This method has been by using aqueous methanol (80%solvent) and was evaluated for phytoconstituents present in them so the preliminary phytochemical screening method was done with the help of standard literature. The Aqueous Methanolic extract of AC consists of alkaloids, carbohydrates, glycosides, tannins, phytosterols, flavonoids, and phenols. So the present study provides evidence that it is having a moderate antimicrobial effect on organisms *E coli, Strephylococcus aureus*, and *candid albicans. Allamanda cathartica* contains medicinally important bioactive compounds and it is having a moderate antimicrobial effect on organisms *E coli, Strephylococcus aureus*, *Candida albicans*. We suggest for extensive research studies on the plant and formulations should be encouraged with proper evaluation.

INTRODUCTION

Medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety being economical, effective, and their easy availability[1] In traditional systems of medicine, Indian medicinal plants have been used in the successful management of various disease conditions like bronchial asthma, chronic fever, cold, cough, malaria, dysentery, convulsions, diabetes, diarrhea, arthritis, emetic syndrome, skin diseases, insect bite and in treatment of gastric, hepatic, cardiovascular and immunological disorders[2]. The family Apocynaceae consists of several important medicinal plants with wide range of biological activities and interesting phytochemical constituents [3] Allamanda cathartica Linn. (Apocynaceae) is one of the most studied species of the Allamanda genus. Lavender-red, trumpet-shaped flowers explode into bloom during the warm months and cover the vine in vibrant color. The spiny, yellow-green fruit follows and can be seen on the plant simultaneously with the spectacular blooms. The dark green, glossy leaves are produced on slender, green, twining stems which become woody with age[4] The Allamanda cathartica Linn. is a native of South America (French Guyana, Guyana, Brazil, surenam, Venezuela) where it

Figure 1 : Allamanda cathartica flower

lies preferably in sunny locations, at the borders of forests, and on the banks of the rivers. The genus was honored by Linnaeus to the Swiss naturalist Fredricks louis allamanda (1736-1803) scholar of the Brazilian flora; the name of the species comes from the Greek "kathartikos"= which purifies concerning the presumed cathartic activity[23] . Based on the literature review as the plant is dwelled with greater bioactive principles we would like to investigate the antimicrobial activity of the aqueous methanolic extract of leaves of *Allamanda cathartica.*

2. MATERIALS AND METHODS

Based on the literature review Allamanda cathartica proved to have the following activities

It have been proved that Se nanoparticles were stabilized with *Allamanda cathartica* L. flower extract showed property of inhibiting phytopathogens and promoted mustard growth under salt stress and Encapsulation of nodal segments of *Allamanda cathartica* was used for short-term storage and germplasm exchange[5]. Studies showed that Chemical variability of *Allamanda cathartica* L. flowers was assessed by multivariate data analysis *Allamanda cathartica* provn to have antioxidant potential with leaf extract. Studies have been conducted on the Establishment of adventitious root cultures of *Allamanda cathartica* L. for the production of iridoid glycosides and its identification using Industrial Crops and Products. Allamanda cathartica flower's aqueous extract was proven to mediate green synthesis of silver nanoparticles with excellent antioxidant and antibacterial potential for biomedical application. Establishment of an HPLC-PDA method for analysis of derivative products from the flowers of *Allamanda cathartica* was performed and reported . Antibacterial and Antifungal activity of was also reported by *Allamanda Cathartica* Linn. Studies reveal that Plumieride which was obtained from *Allamanda cathartica* is an inhibitory compound to plant pathogenic fungi.

2.1 SELECTION OF PLANT

Allamanda is an evergreen, vigorous, perennial, woody compact, or creeping shrub belonging to the family Apocynaceae. The plant *Allamanda Cathartica* has many therapeutic properties such as Anti-inflammatory , Antioxidant, Antidermatophytic, Hepatoprotective, wound healing, thrombolytic, Antibacterial, and Antifungal [10]. It was considered to be very important and necessary to identify the properties which may be useful in the future for therapeutic use. In *Allamanda cathartica*, the above activities have been confirmed with reference to the literature review.

2.2 COLLECTION OF LEAVES, IDENTIFICATION, AND AUTHENTICATION OF PLANT.

Leaves of this plant and a small twig (specimen) were collected from our college garden at MRIPS maisammaguda, secunderabad.Our plant specimen with vide voucher no. 380 has been identified and authenticated as *Allamanda cathartica* L.(Apocynaceae) By B. Kamalakar , professor Department of Botany University college of science Osmania University, Hyderabad. The identification was done based on macroscopic studies of the sample (small twig) followed by scrutiny of literature.200 gms of Leaves of *Allamanda Cathartica* were collected cleaned, and dried under shade for around 1 week. Dried leaves were grounded into coarse powder by using the grinder. 100gms of the leaves were subjected to Extraction.

2.3 EXTRACTION

Soxhlet extraction was a modern extraction technique in which we circulate the same solvent through the extractor several times. It was a type of continuous extraction technique but we can call it a series of short maceration. Soxhlet extractor was used to extract the desirable compound according to the polarity of the solvent and according to the boiling point of the solvent .we can perform the extraction process by using the desired amount of solvent for multiple times as many times to get the maximum amount of the yield of the desired compound [11].Soxhlet extractor makes the extraction process much more efficient than that of the traditional method.The Leaves were air-dried and powdered to form a coarse powder. The powder was Subjected to extraction by Soxhlet apparatus using aqueous methanol as a solvent for about 3 hours .Chemicals were procured from Sigma Aldrich Aqueous Methanol (80%) , Ethanol , Dragendroffs reagent , Wagner's reagent ,Mayer's reagent , Molisch reagent ,Barford's reagent , Seliwanhoffs reagent, Lead acetate, GAA Gelatin solution ,conc. Sulphuric acid , Acetic anhydride. The extract obtained was dark green it was semisolid .

2.4 PRELIMINARY PHYTOCHEMICAL ANALYSIS [12]

DETECTION OF ALKALOIDS : DRAGENDROFF'S TEST 1mL of extract was taken and placed into a test tube. Then 1mL of potassium bismuth iodide solution (Dragendorff's reagent) was added and shaken, An orange-red precipitate formed indicates the presence of alkaloids. WAGNER'S TEST :1mL of extract was taken and placed into a test tube. Then 1mL of potassium iodide (Wagner's reagent) was added and shaken. Appearance of reddish-brown precipitate signifies the existence of alkaloids. MAYER'S TEST :1mL of extract was taken and placed into a test tube. Then 1mL of potassium mercuric iodide solution (Mayer's reagent) was added and shaken. The emergence of whitish or cream precipitate implies the presence of alkaloids.

DETECTION OF CARBOHYDRATES MOLISCH TEST, BARFOEDS TEST, SELIWANOFF'S TEST were performed DETECTION OF GLYCOSIDES : BONTRAGER'S TEST One gram of the crude extract was first weighed, placed into a test tube, and dissolved in 5mL of dilute hydrochloric acid. Then 5mL of ferric chloride (5%) solution was added. The mixture was shaken and placed over the water bath. Then the mixture was allowed to boil for 10min, cooled, and filtered. Afterward, the mixture was then extracted again with benzene. Finally, an equal volume of ammonia solution was added to benzene layer. The appearance of pink color indicates the presence of anthraquinone glycosides. KELLER KILLIANI'S TEST : 2mL of the extract was taken and diluted with equal volume of water. Then 0.5mL of lead acetate was added, shaken, and filtered. Again, the mixture was extracted with an equal volume of chloroform, evaporated, and dissolved the residue in glacial acetic acid. Then few drops of ferric chloride were added. Again, the whole mixture was placed into a test tube containing 2 mL of sulfuric acid. The emergence of reddish brown layer that turns bluish green indicates the presence of digitoxose.

DETECTION OF FLAVONOIDS :ALKALINE REAGENT TEST 1mL of extract was taken and placed into a test tube. Then few drops of sodium hydroxide solution were added and shaken. The emergence of intense yellow color that turns to colorless after adding dilute acid implies the existence of flavonoids LEAD ACETATE TEST: To detect the presence of flavonoids, 1mL of extract was taken and placed into a test tube.

Then few drops of lead acetate are added and shaken. The formation of a yellow precipitate signifies the presence of flavonoids.

DETECTION OF PHENOLS FERRIC CHLORIDE TEST **:**1mL solution of an extract was taken and placed into a test tube. Then 1% gelatin solution containing sodium chloride was added and shaken. The formation of bluish-black color indicates the presence of phenols. GELATIN TEST A solution of plant extract was placed into a test tube followed by 2mL of 1% gelatin solution and shaken. The appearance of a white precipitate indicates the presence of phenols.

DETECTION OF TANNINS : PHENAZONE TEST[20] To 5 ml of aqueous solution of tannin-containing drug, add 0.5 g of sodium acid phosphate. Warm cool, and filter. Add 2% phenazone solution to the filtrate. All tannins are precipitated as a bulky, colored precipitate. 10% NAOH TEST :0.4 ml of plant extract, add 4 ml of 10% NaoH, shake well, formation of emulsions indicates the presence of hydrolyzable tannins

DETECTION OF PHYTOSTEROLS ; SALKOWSKI'S TEST :Aqueous extract of filtrate of plant sample was taken, add a few drops of conc. Sulphuric acid, shake well, and allow it to stand, red color was formed in a lower layer. LIBERMAN BURCHARD TEST:test for steroids To 0.2 g of each portion, 2 ml of acetic acid was added, and the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Color development from violet to blue or bluish-green indicate the presence of a steroidal ring i.e. aglycone the portion of cardiac glycoside

DETECTION OF FIXED OILS AND FAT : SPOT TEST / STAIN TEST Little quantity of plant extract is pressed in between filter papers, oil stain on the filter was observed .SAPONIFICATION TEST :1 ml of extract was taken and a few drops of 0.5 alc. KOH and a drop of phenolphthalein which is heated for 2 min heated for 2 hrs. soap formation or partial neutralization of alkali was observed.

2.5 PHYSICO-CHEMICAL PARAMETRES[13]

Determination of foreign matter: 10 gms of the sample was weighed and spread on a white tile uniformly without overlapping, then the sample was inspected utilizing 5x lens and foreign organic matter were separated, and after complete separation the sample was weighed and the percentage w/w was determined.

Determination of Ash Value : a. Determination of total ash: Total ash was determined by weighing 2 gm of the air-a dried crude drug in the tared platinum or silica dish and incinerated at a temperature not exceeding 450°C until free from carbon and then it has been cooled and weighed. % Total ash value = (Wt. of total ash /Wt. of crude drug taken) * 100.

Determination of acid insoluble ash: The ash obtained from the previous process was boiled with 25ml of 2M Hcl for 5 min The insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a desiccator and weighed. Percentage of acid-insoluble ash was calculated concerning the air-dried drug. % Acid insoluble ash value = (Wt. of acid insoluble ash/ Wt. of crude drug taken) *100**.**

Determination of water-soluble ash: The ash was boiled with 25ml of water for 5 min. The insoluble matter was collected on ash-less filter paper and was washed with hot water, and ignited for 15min, at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water-soluble ash. The percentage of water-soluble ash was calculated concerning the air dried drug % Water soluble ash value =(Wt. of Water soluble ash/Wt. of crude drug taken) *100.

Determination of solvent extractive value:

Determination of water-soluble extractive value: Five gm of the powdered drug was macerated with 100ml of water closed flask for 24 hr and was occasionally shaken within 6hr time period and was allowed to stand for 18hr After filtration the 25ml of the filtrate evaporated to dryness in a tarred flat bottomed shallow dish. Dried at 105°C and weighed. The percentage of water-soluble extractive value was calculated concerning the air dried drug.

Determination of alcohol soluble extractive value: Five gm of the powdered drug was macerated with 100ml of ethanol closed flask for 24 hr and was occasionally shaken for 6hr time period and was allowed to stand for 18hr After filtration the 25ml of the filtrate evaporated to dryness in a tared flat-bottomed shallow dish. Dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated concerning the air-dried drug.

Fluorescence analysis:A fluorescence study of leaf powder was performed. A small quantity of the leaf powder was placed on a grease-free clean microscopic slide and 1-2 drops of the freshly prepared reagent solution were added, mixed by gently tilting the slide and waited for a few minutes. Then the slide was placed inside the UV chamber and observed in visible light short (254 nm) and (365 nm) ultraviolet radiations. The color observed by application of different reagents in different radiations was recorded.

Determination of swelling index: Transfer 1 gram of dried leaf powder to a 25 ml to stoppered measuring cylinder upto 20 ml mark. Agitate gently occasionally for 2 hours and allow to stand. Measure the volume occupied by swollen the given powder of leaves.

Determination of foaming Index: Weigh accurately about 1 gram of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml boiling water maintained at moderate boiling at 80 -90 C for about 30 minutes. Then make it cool, filter it into a volumetric flask and add a sufficient amount of water through the filter to make the volume upto 100 ml, clean the stoppered test tubes, 10 ml are taken in each test tube. After doing test tubes with the stoppers, shake them for 15 seconds and allow them to stand for 15 seconds, allow for 15 minutes then measure the height.If the height of the foam in each test tube is less than 1 cm the foaming index is less than 100, if it is more than 1cm height after dilution of plant material in the 6th test tube then the corresponding no. of the test tube is the index(V1)If the height of the foam is more than 1000 in this case 10 ml of the first test tube of plant material needs to be measured and transferred to a volumetric flask of 100 ml capacity V2 and volume is used to be maintained. The foaming index can be calculated by using the following index,

Foaming index=1000/a in case of V1

Foaming index=1000*10/a in case of V2

Determination of moisture content: Weigh rapidly 0.5 gm of finely powdered crude drug in a flat-bottomed dish, dry it in an oven at 100 -105 C for 10 minutes, continue the procedure until

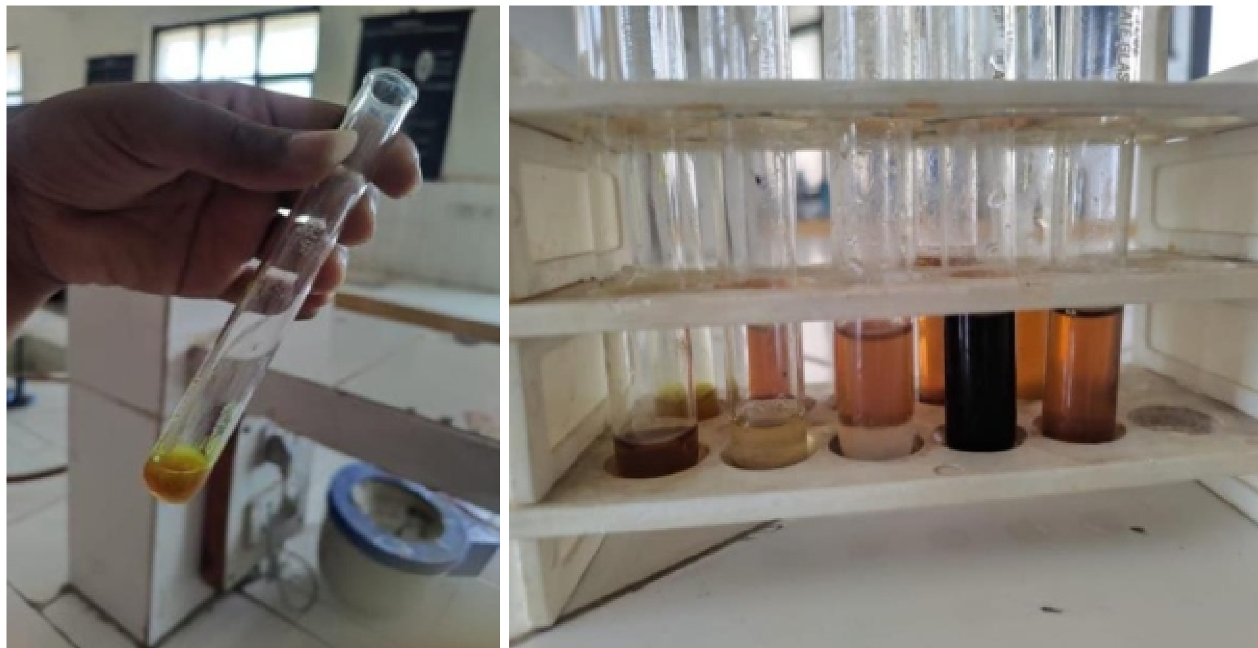

Figure 3.1 and 3.2 : Preliminary phytochemical screening

the powder weighs constant %moisture content by% moisture content =initial weight the weight of the crude drug after drying / initial weight

2.6ANTIMICROBIAL ACTIVITY BY AGAR WELL DIFFUSION METHOD OF ASSAY[14] :PREPARATION AND STERILIZATION OF AGAR MEDIA COMPOSITION OF NUTRIENT AGAR MEDIA

Meat extract - 3gm,Peptone - 5gm

Sodium chloride - 5gm ,Agar - 20gmPurified water - 100mlpH - 7.4+_0.2

* The required quantities of ingredients are weighed and dissolved in specific quantities of water. If the broth is not clear filter through filter paper [18] pH is adjusted to 7.4±0.1 After pH adjustment add the agar and heat the contents on the water bath, until the agar Dissolves.

Melted agar medium is distributed into the test tube and flask which are then plugged with non-absorbent cotton. The test tubes and the flasks are sterilized in an autoclave at 121ºC,151 lbs pressure for 15-20 min

Agar well diffusion Method of the assay:

Agar well diffusion assay[19] has been employed to determine the antimicrobial potential of the leaf extract of A.cathartica by measuring the zones of inhibition obtained with different concentrations of the test samples. 100 µl of microbial suspension (106 cfu/ml) was swabbed uniformly on the surface of Mueller-Hinton agar (HI Media) and placed in Petri plates. Wells of 6mm diameter were made with cork borer and different concentrations of 1ml of leaf extract was added to each well. Different concentrations of test samples were prepared by dissolving the dried plant extracts separately in 1 ml of water to reach a final concentration of 400, 800, 1000, 1600, and 2800 ug/ 1ml The inoculated and test sample loaded plates were incubated at 37°C for 16 hours. Along with this standard antibiotic. Ciprofloxacin (100, 200 µl/ml) and control solvent water (5% 50µl) were used as positive and negative controls. After incubation, the inhibition zones formed around the wells in test and control plates were measured with the HI Media zona scale. The results reported were the mean values of the triplicates run in each concentration Results were given in table and figure along with minimum inhibitory concentrations.

RESULTS

Qualitative Preliminary Phytochemical tests were performed for Aqueous methanolic extract of AC and it indicates the presence of phytoconstituents like Alkaloids, Flavanoids, carbohydrates, Glycosides, Phenols, Tannins, Phytosterols, saponins .Physico-chemical parameters like determination of foreign matter, ash value was , solvent extractive value, fluorescence analysis, determination of swelling index, foaming index and moisture content were performed and the results obtained for Ash value is 9.5% , Acid insoluble Ash was 4.93% ,Water soluble ash was 3.5% extractive value in which water

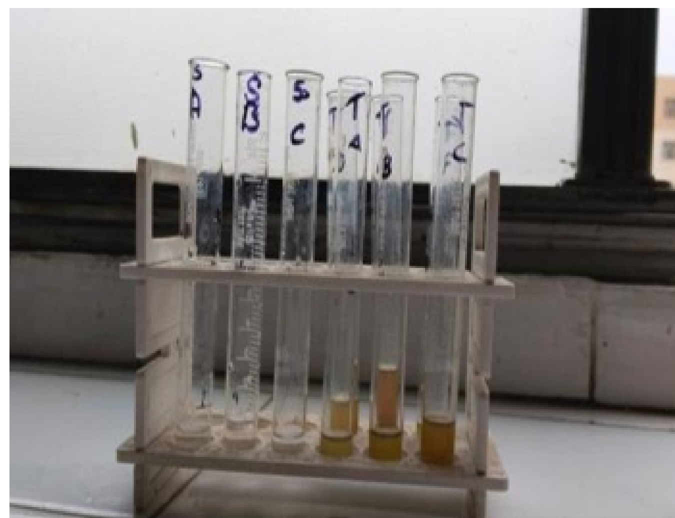

Figure 3.3 : Preparation of concentrations of standard and test

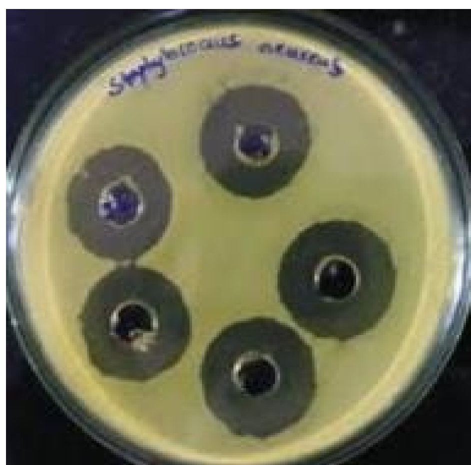

Figure 7.1 : Zone of Inhibition for *S Aureus*

Figure 7.2 : Zone of Inhibition for *E.coli*

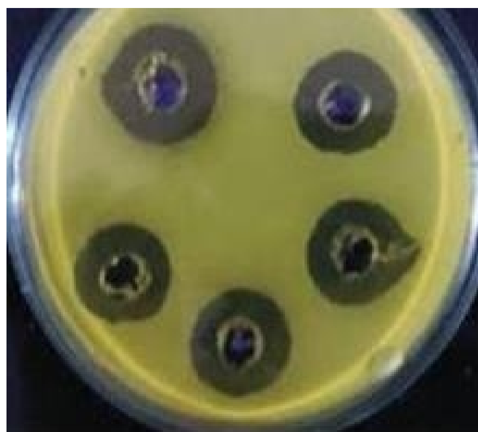

Figure 4 : Zone of inhibition for *Candida albicans*

soluble extractive value was 20.80% and Alcohol soluble extractive value was 12%, Moisture content was 15.89%, Foaming index was 100 . Antimicrobial activity of Aqueous methanolic leaf extract of *Allamanda cathartica* against micro-organisms *E.coli, S. Aureus, C. Albicans* was performed and Zone of inhibition for Aqueous Methanolic Extract with different concentrations like 400µg/ml 400µg/ml, 800µg/ml 1000µg/ml, 2800µg/ml were compared to standard antibiotic ciprofloxacin by 100µg/ml, 200µg/ml concentration and it was having Moderate antimicrobial activity against *E.coli, S. Aureus, C. Albicans.*

Table 1 : PRELIMINARY PHYTOCHEMICAL SCREENING

S.NO	CHEMICAL CONSTITUENTS	RESULTS
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4.	Flavanoides	+
5.	Proteins and amino acids	-
6.	Tannins	+
7.	Phytosterols	+
8.	Coumarins	-
9	Phenolic compounds0-0	+

Table 2 : PHYSICO - CHEMICAL PARAMETERS

S.NO	Physicochemical analysis	
1	Total ash	9.5%
2	Acid insoluble ash	4.93%
3	Water soluble ash	3.53%
4	Swelling index	-
5	Moisture content	15.89%
6	Foaming index	100
1	Water soluble extractive value	20.80%
2	Alcohol soluble extractive value	12%

Table 3 : FLOURESCENCE ANALYSIS

REAGENT	LONG UV	SHORT UV	FLOURECENT LIGHT
Dry powder (P)	Light green	Light green	Brown
P+1N NaOH (alc)	Brownish	Green	Light brown
P+1N NaOH (Aq)	Light green	Yellow	Yellow
P+1N Hcl	Green	Green	Brown
P+50%Sulphuric acid	Light green	Light green	Brown
P+50%Nitric Acid	Green	Green	Brown
P+Picric Acid	Green	Light green	Brown
P+Acetic acid	Light green	Green	Brown
P+Ferric chloride	Green	Green	Yellow

Table 4 : ZONE OF INHIBITION FOR SELECTED MICROORGANISMS

S. No.	Micro organisms	ZONE OF INHIBITION FOR AQUEOUS METHANOLIC EXTRACT				ZONE OF INHIBITION FOR STANDARD ANTIBIOTIC (CIPROFLOXACIN)	
		400µg/ml	800 µg/ml	1000 µg/ml	2800 µg/ml	100 µg/ml	200 µg/ml
1.	E. Coli	7 mm	9mm	12mm	12 mm	22 mm	24 mm
2.	Staphylococcus aureus	8 mm	7mm	10mm	8 mm	20 mm	25 mm
3.	Candida albicans	8 mm	11mm	12 mm	12 mm	22 mm	25 mm

Pls. share the original table

DISCUSSION

Antimicrobial activity of medicinal plants is being widely reported. Studies have indicated that the extract of different parts of Allamanda catharticawas reported to have potential antibacterial, antifungal and invitro hepatoprotective properties ,used as a treatment for gonorrhea, dysentery and hepatitis.In the present study the aqueous methanolic leaf extract of AC showed mild antimicrobial activity against selected strains .The results were compared with standard antibiotic drugs They confirm that the extract was active against the microorganisms because of the presence of phytoconstituents like Alkaloids, Flavanoids, carbohydrates, Glycosides, Phenols, Tannins, Phytosterols, saponins. The presence of phenols ,tannins and flavonoids shows the effect on microorganisms.

CONCLUSION

Allamanda cathartica belongs to the family Apocynaceae. It is used as herbal medicine due to its wide range of phytoconstituents and pharmacological properties. As there are only a few reports on the phytochemical analysis of leaf extract with aqueous methanol (80%solvent) of Allamanda cathartica. the selection was done based on a literature review. Investigation and collection of the leaves of *Allamanda cathartica* from college garden of the MRIPS campus were performed The extraction method has been performed by using aqueous methanol (80%solvent) and was evaluated for phytoconstituents present in them so the preliminary phytochemical screening method was done with the help of standard literature. The extract consists of alkaloids, carbohydrates, glycosides, tannins, phytosterols, flavonoids, and phenols. So the present study provides evidence that aqueous methanolic extract(80%) of Allamanda cathartica contains medicinally important bioactive compounds and it is having a moderate antimicrobial effect on organisms E coli, Strephylococcus aureus, Candid albicans. We suggest for extensive research studies on the plant and formulations would be encouraged with proper evaluation.

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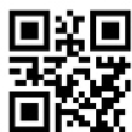

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