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Research Article

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Evaluation of Phytochemical Screening Antioxidant Using By DPPH, H_2O_2 Methods and Antibacterial activity of the Crude flower extracts of *Allamanda cathartica* L. (Apocynaceae)

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Abstract

Objevtives*:** To investigate Phytochemical analysis and in-vitro antioxidant and antimicrobial activity of flower 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. ***Methods: The presence of phytochemicals and antioxidant was also assessed using standard methods.Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics compound and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins- Ciocalteau reagent method and aluminum chloride method respectively. The In-vitro antioxidant activity of methanolic extract and aqueous extract of the flowers was assessed against DPPH assay method using standard protocols. The agar well diffusion method was used to determine the susceptibility of bacterial strains to crude extracts of the plant. The minimum inhibitory concentration was determined by the microdilution test. It is used to detect the antibacterial activity by comparing the standard drugs Ciproflaxin. ***Result:*** Phytochemical assay revealed the presence and equivalent quantity of alkaloids, tannins, flavonoids, saponins and phenolic acid in the extracts. The water and ethanol extracts were also shown as the best solvents of extraction for the phytochemicals. The DPPH radical scavenging activity of *Allamanda cathartica* was evaluated and compared with ascorbic acid. The presence inhibition of flowers extract was calculated at various concentration (50-250 µg/ml) as well as standard ascorbic acid. The highest scavenging activity of ethanolic and aqueous extract were 175.1±0.41and 124.1±0.37%at concentration of 250 µg/ml. Hydrogen peroxide Scavenging antioxidant activity of ethanol and aqueous extract flower was found to be 46.71µg/ml and 48.52µg/ml, respectively. The highest scavenging activity of ethanolic and aqueous extract were 276.1±0.86 % and 217.9±0.79 % at concentration of 250 µg/ml. The antibacterial activity of the microorganism: Bacillus subtilis, Staphylococcus aureus, Escheria coli, Pseudomonas aeruginosa among the various solvents tested . The maximum activity was observed against all organisms in both extract. The highest zone of inhibition was observed against Escheria coli (8.7±0.29) and (7.3±0.74) at 100µg/ml of ethanol and aqueous extract. ***Conclusion:*** The ethanol and aqueous extracts of *Allamanda cathartica* exhibited a significant antibacterial and antioxidant activities, suggesting the presence of either good bioactive potency or the high concentration of the active principle in the extracts which may serve as a guide for selecting bio- medicinal substances of plant origin in pharmacological drugs. This study helps to predict the formula and structure of biomolecules which can be used as drugs and further investigation may lead to the development of drug formulation.

Keywords: *Allamanda cathartica*, Antibacterial activity, Phytochemicals, antioxidant activity, zone of inhibition, DPPH, H_2O_2 Scavenging

1. Introduction

Medicinal plants are very useful in folk medicine due to present different phyto-components whose properties show medicinal importance. All over world for thousands of years, these medicinal plants are used as a natural medicine [1]. Today, according to the World Health Organization (WHO) primary health-care is associated with traditional medicine which is used by 80% of world's people [2]. The world of medicine has been fascinated by the group of compounds called coumarins because of the huge number of biological activities they possess and their structural adaptability [3]. The medicinal properties of plants are explored due to presence of their potent pharmacological activities [4]. Various type of free radical scavenging molecules is found in plants which act as a natural product, rich in antioxidant properties [5]. That's why plants extract is displayed various biological activities such as antioxidant properties [6]. Antioxidant molecules can be suppressed the oxidative reaction which is instigated by the free radicals [7].

Allamanda cathartica belongs to the family Apocynaceae. It is used as herbal medicine due to its wide range of phytoconstituents and pharmacological properties[8]. *Allamanda cathartica* Linn. (Apocynaceae) is one of the most studied species of the Allamanda genus. *Allamanda cathartica,* also known as trumpetvine, golden trumpet or yellow allamanda

is an ornamental flowering plant. 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 [9]. The beauty of the plant is the fact that the plant extracts have medicinal properties whereas, at the same time, the extracts can be toxic if they are not properly prepared [10]. A recent report suggests that *Allamanda cathartica* extracts have been found useful not only for treating malaria, jaundice, cough, wounds and constipation, but also shows activity against leukemia and human carcinomamia [10].The need to develop newer drugs of plant origin forms the basis of this study. Thus, this study evaluates the in vitro antimicrobial activity of the root extracts of Allamandcathartica against Bacillus subtilis, Staphylococcus aureus, Escheria coli, Pseudomonas aeruginosa using ethanol and water as the solvent.The aim of the present study to find out the maximum antioxidant activity in comparison of standard chemical compounds and correlation with the Total phenolic and total flavonoid compounds of the specific Flowerpart.

Fig 1: Flowering plant of *Allamanda cathartica* Linn.

2. Materials and Methods

2.1 Collection of Plant Material

The flower of plants *Allamanda cathartica* was authentified by Prof. Rijwan Ulla, Department of Botany, Rajeev Gandhi Govt. Autonomous Post Graduate College Ambikapur, Surguja, Chhattisgarh, India. They were collected from different areas of Mainpat forest districts of Surguja, India. Collected materials was washed in running tap water, rinsed properly in distilled water and then subjected to drying at room temperature for about 5 days in open air. This air-dried material was grind into powdered and stored under refrigeration until their further utilization

2.2 Preparation of Plant Extracts

Dry powder of plant parts was percolated in a soxhlet apparatus with solvents such as ethanol and aqueous. The filtrates were evaporated to get concentrated residue. This residue treated as experimental drug for the present study. The extract was stored at 4^{o}C until assay was completed.

2.3 Phytochemical analysis

The qualitative preliminary phytochemical analysis of plant extract was performed according to the protocol reported[11-13]

(1) Alkaloids

Five milliliters of plant extract in a test tube was concentrated to yield a residue. Thus, the obtained residue was dissolved by adding 1.5 mL of 2% (v/v) HCl followed by adding three drops of Meyer's reagent (0.679 g of HgCl2 and 2.5 g of KI in 50 mL distilled water). The formation of white precipitates indicates the presence of alkaloids.

(2) Phenolic compounds

The plant extract was mixed with 2 mL of 2% FeCl3 solution and observed for the formation of blue, green, or black coloration.

(3) Flavonoids

The plant extract was mixed with a few pieces of magnesium followed by a dropwise addition of conc.HCl. The formation of a pink scarlet color after a few minutes indicates the presence of flavonoids.

(4) Tannins

Two milliliters of 5% FeCl3 was added to 2 mL of plant extract and observed for the formation of yellow or brown precipitates.

(5) Reducing sugars

A total of 2.5 mL of Benedict's solution (17 g of trisodium citrate dihydrate, 10 g of Na2CO3, and 1.74 gof copper sulfate pentahydrate in 100 mL of distilled water) was taken in a test tube followed by adding0.5 g of plant extract and then warmed in a hot water bath for about 5 min. The formation of green/red or yellow coloration indicates the presence of reducing sugar.

(6) Saponins

The plant extract was mixed with 5 mL of distilled water and then shaken vigorously. The formation of stable foam indicates the presence of saponins.

(7) Terpenoids

A small amount of plant extract was dissolved in chloroform and an equal volume of conc. $H_2SO_4$was added. Reddish-brown coloration at the junction of two liquids indicates the presence of terpenoids.

(8) Steroids

Two milliliters of plant extract was mixed with chloroform. Overall, 1–2 mL of acetic anhydride was added followed by adding one or two drops of conc. $H_2SO_4$from the side of the test tube. An array ofred, blue, and green colors indicates the presence of sterols.

(9) Glycosides

Two milliliters of glacial acetic acid, one drop each of 5% $FeCl_3$ and conc. H_2SO_4 was added to 5 mL of plant extract. The appearance of a brown ring indicates the presence of glycosides.

(10) Proteins

Plant extract is mixed with 2 mL of Millon's reagent and observed for a white precipitate that turns redon gentle heating.

2.4 Quantitative Phytochemical Screening

The quantitative phytochemical screening was performed by determining total phenolic content (TPC) and total flavonoid content (TFC) of the extracts [14]. The TPC and TFC of the extracts were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of extracts and milligrams quercetin equivalent per gram (mg QE/g) of extracts, respectively [15].

(i) Determination of Total Phenolic Content (TPC)

Folin–Ciocalteu reagent was used for the determination of total polyphenolic content. 0.5 ml of each extract (5 mg/ml), Folin–Ciocalteu reagent (5 ml, 1:10 v/v diluted with distilled water) and aqueous sodium carbonate (4 ml, 1 M) solution were mixed together. The mixture was allowed to stand in the dark for 15 min at room temperature, and the absorbance at 765 nm was measured with the help of ultraviolet (UV-visible) spectrophotometer. Then, the total polyphenolic content was determined in terms of mg GAE/g of dry weight of the extract with the help of a calibration curve prepared with a series of gallic acid standards [16]

(ii) Determination of TotalFlavonoid Content (TFC)

0.5 ml of each extract (50 mg/ml) was separately mixed with 1.5 ml methanol and 0.1 ml aluminium trichloride (10%). Then, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water was added into each test tube. Then, absorbance at 415 nm was measured after it was allowed to stand in the dark for 30 min using a UV-visible spectrophotometer. Finally, a calibration curve was prepared with a series of quercetin standards and the total flavonoid compound concentration was determined in terms of mg QE/g of the extract [17].

2.5 Antioxidant activity

Antioxidant activity of ethanolic and aqueous extract were evaluated by the free radical scavenging methods like as the DPPH scavenging method and H_2O_2 scavenging assay.

(i) 2, 2-Diphenyl- picryl- hydrazyl (DPPH) Free Radical Scavenging Activity:

This assay was used in many studies for testing antioxidant activity. 2,2-Diphenyl-1-picryl hydrazyl stable radical (DPPH) evidently offers a convenient and accurate method for titrating the oxidizable groups of natural and synthetic antioxidants This assay was based on the relation of a methanolic solution of the colored free radical (DPPH) by free radical scavenger (20). The degradation of DPPH was evaluation by composition with a control sample without hydrogen donating compounds. The decrease in absorbance of DPPH at its absorbance maximum of 517nm was proportional to be conformed of free radical Scavenger added to DPPH reagent solution. Lower absorbance of reaction mixture indicated higher antioxidant activity. In this experiment methanolic solution of DPPH (100mm.2.5ml). 0.05ml of each extract dissolved in methanol was added different concentrations (50-250µg/ml) reaction mixture was shaken and after 30minut at room temperature, the absorbance value were measured 517nm and converted into percentage of antioxidant activity (%AA) Ascorbic acid was used as standard[18-19]. The degree of discoloration indicator the scavenging efficacy of the extract was calculated by the following equation –

Radical scavenging activity = $(A_0 - A_T / A_0) \times 100$

Where, A_0 is the absorbance of pure DPPH solution (0.01mM), and A_T is the absorbance of (DPPH) and compounds/ samples.

(ii) Hydrogen peroxide (H_2O_2) scavenging activity

Solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 560nm using UV spectrophotometer. 0.1mg/ml of the extract was added to hydrogen peroxide solution and absorbance measured at 560nm using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard [20]. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using the given formula:

Percentage scavenged [H_2O_2] =

1- Abs (standard)/Abs (control) x100

Where, Abs control was the absorbance of the control (without extract) at 560nm; Abs sample was the absorbance in the presence of the extract at 560nm.

2.6 Antibacterial assay

(i) Test organisms

Reference bacterial strains were obtained from the Columbia Institute of Pharmacy, Raipur (C.G.) which included *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*. The strains were kept at 4 °C on agar slant and sub cultured at 37 °C for 24 h on nutrient agar before any susceptibility test. The antibacterial assays were carried out using Nutrient Agar (Biolab) and broth.

(ii) Antimicrobial Susceptibility Testing

Agar well Diffusion Assay

Mueller Hinton agar medium was prepared and distributed into suitable containers. It was sterilized in an autoclave at 121 ^{0}C for 15 minutes. The sterile medium was cooled at 45 ^{0}C. 0.1ml of standardized inoculums (0.5m McFarland standard) was introduced into each 20ml of the prepared and sterilized Mueller Hinton Agar [MHA]. It was then poured aseptically into sterile Petri dishes. The plates were then allowed to stand in a horizontal position until the agar solidified. Discs of agar were removed from the agar layer with the aid of a cork borer of 6 mm diameter in order to produce wells on the agar plates. The various concentration of the plant extract (50µg/ml, 100µg /ml) was then introduced into the wells using a micro pipette. This was carried out in triplicates for each concentration. The plates were then incubated at 37^{0}C for 24hrs after which they were observed for zones of inhibition. The inhibition zones obtained were is compound with a positive control were set using standard antibiotics ciprofloxacin [21-22].

3. Results and Discussion

3.1 Phytochemical Constituents

The study of the chemical constituents and the active principles of the medicinal plants have acquired a lot of importance all over the world. The present study includes the phytochemical screening of the plants *Allomanda catharita*. The qualitative chemical tests for the ethanolic extracts were performed. The investigation showed that *Allomanda Catharita* contains, flavonoids, alkaloids, saponins, tannins, Steroidal glycosides, Steroids, Carbohydrate, Phenolic compound, Protein present in ethanol and flavonoids, saponins, Steroids, Carbohydrate, Phenolic compound, Protein amino acid were present in aqueous extract.

Table1.1: Phytochemical investigation of *Allomanda catharita* Flower on ethanol and aqueous extract

S.N	: Phytochemical Test	Ethanol Extract	Aqueous Extract
1	Carbohydrate	+	+
2	Protein	+	+
3	Alkaloid	+	-
4	Flavonoid	+	+
5	Steroid	-	+
6	Amino Acid	-	+
7	Tannin	+	-
8	Steroidal glycosides	+	-
9	Phenolic compound	+	+
10	Saponins	+	+

(+) :Presence, (-): Absent

3.2 Quantitative analysis

The results given in table 2 show that the total phenol content and total flavonoid of *Allomanda catharita* flowers are ethanolic 310± 0.28, 295.22± 0.18and aqueous extract 143.20± 0.46113.75± 0.24 respectively.

Table 1.2 :Quantitative analysis of *Allomanda catharita* flower

S.N.	Extract	Test Parameter	Results(mg/g) (±SEM)
1.	Ethanol	Total phenolic Total Flavonoids	310± 0.28 295.22± 0.18
2.	Aqueous	Total phenolic Total Flavonoids	143.20± 0.46 113.75± 0.24

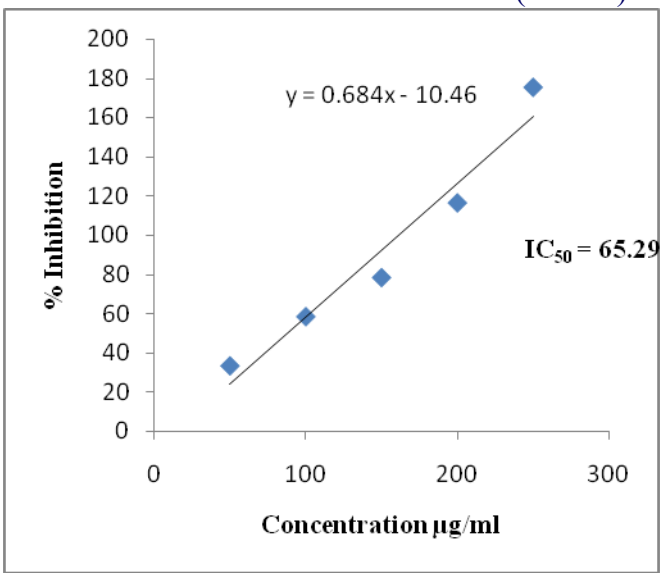
3.3 Antioxidant activity

a) DPPH scavenging activity

DPPH scavenging activity of *Allomanda Catharita* flower extracts against DPPH radical were determined and the results are shown in table (4,5,6). DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the in vitro antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517nm. IC_{50} for standard ascorbic acid was found to be 51.36 µg/ml and for ethanol and aqueous extract flower was found to be 65.29µg/ml and 95.21µg/ml, respectively. In order to study the effects of these compounds on biological system more studies are needed as these compounds might be responsible for use of this plant in different diseases [23-25]. The DPPH radical scavenging activity of Allomanda Catharita was evaluated and compared with ascorbic acid. The presence inhibition of flowers extract was calculated at various concentration (50-250µg/ml)as well as standard ascorbic acid. The highest scavenging activity of ethanolic and aqueous extract were 175.1±0.41and 124.1±0.37% at concentration of 250 µg/ml.

Table 1.3: Free radical scavenging capacity of ethanol extract of AllomandaCatharita (Flower)

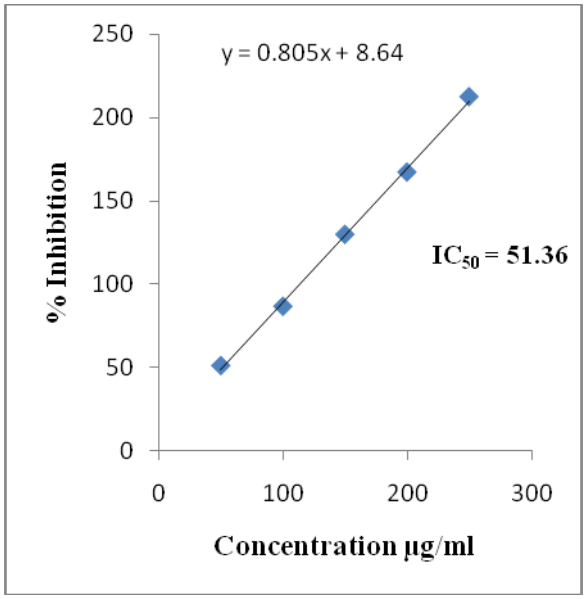
Concentration (µg/ml)	DPPH Scavenging %
	Ethanol Extract
50	33.1±0.32
100	58.2±0.78
150	78.1±0.65
200	116.2±0.38
250	175.1±0.41
IC_{50}	65.29



Values are mean ± SEM of three determinations

Table 1.4: Free radical scavenging capacity of Ascorbic acid

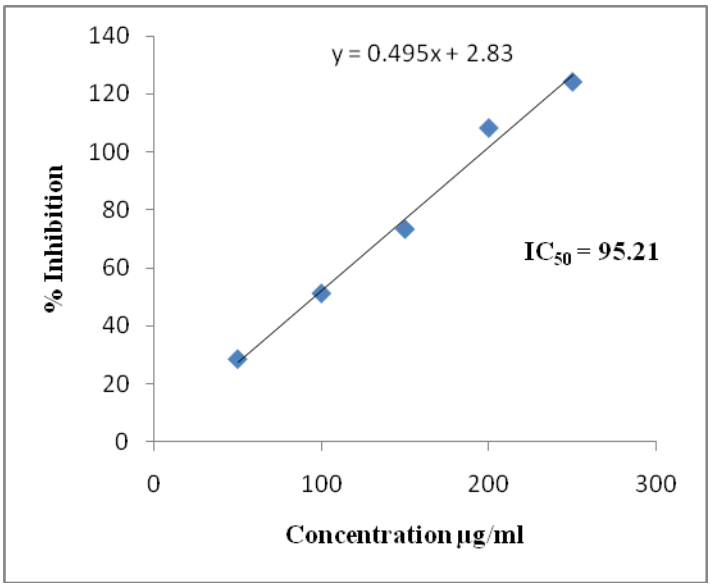
Concentration (µg/ml)	DPPH Scavenging % Ethanol Extract
50	51.2±0.36
100	86.7±0.27
150	129.8±0.98
200	167.1±0.24
250	212.3±0.76
IC_{50}	51.36



Values are mean ± SEM of three determinations

Table 1.5: Free radical scavenging capacity of aqueous extract of Allomanda Catharita (Flower

Concentration (µg/ml)	DPPH Scavenging % Aqueous Extract
50	28.7±0.76
100	51.3±0.56
150	73.4±0.83
200	108.2±0.92
250	124.1±0.37
IC_{50}	95.21



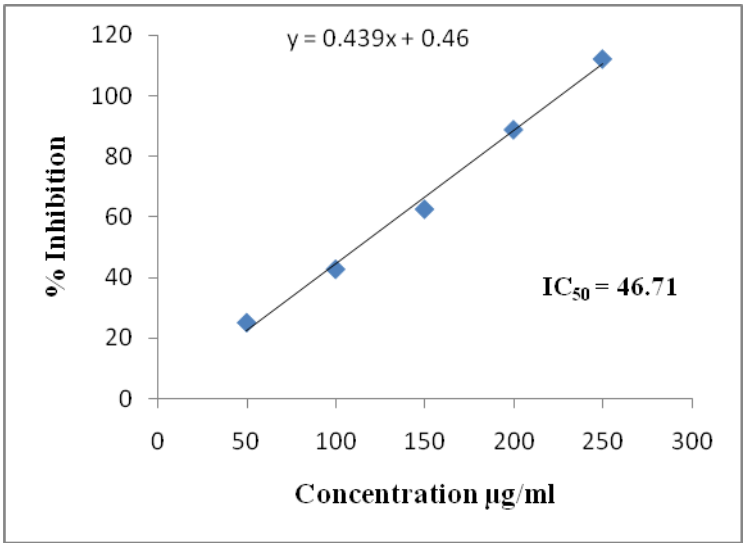
Values are mean ± SEM of three determinations

b) Hydrogen peroxide(H_2O_2) Scavenging antioxidant activity

Hydrogen peroxide Scavenging antioxidant activity of ethanolic and aqueous flower extract as shown in table no. 7, and 8.IC_{50} for standard ascorbic acid was found to be 51.36 µg/ml and for ethanol and aqueous extract flower was found to be 46.71µg/ml and 48.52µg/ml, respectively. The highest scavenging activity of ethanolic and aqueous extract were 276.1±0.86 % and 217.9±0.79 % at concentration of 250 µg/ml.

Table 1.6: H_2O_2 scavenging capacity of ethanol extract of Allomanda Catharita (Flower)

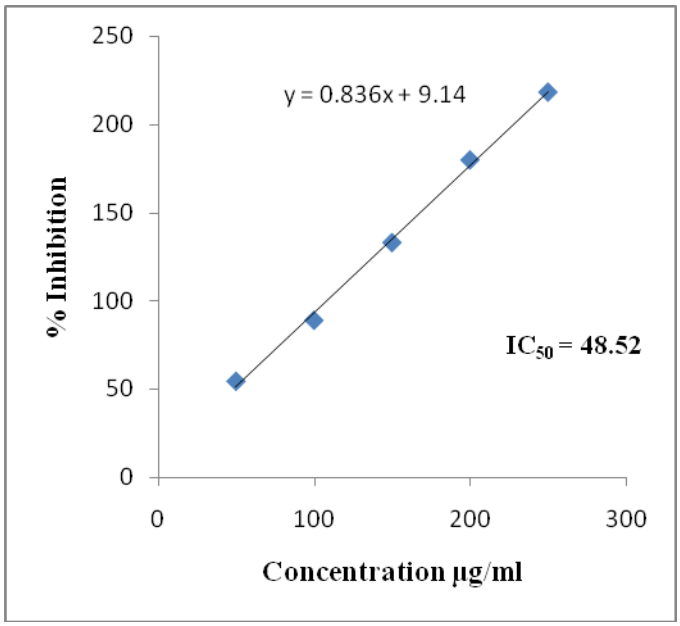
Concentration (µg/ml)	H_2O_2 Scavenging %
	Ethanol Extract
50	61.7±0.41
100	98.1±0.20
150	162.7±0.31
200	214.7±0.45
250	276.1±0.86
IC_{50}	46.71



Values are mean ± SEM of three determinations

Table 1.7: H_2O_2 scavenging capacity of aqueous extract of Allomanda Catharita (Flower)

Concentration (µg/ml)	H_2O_2 Scavenging %
	Aqueous Extract
50	54.2±0.65
100	88.7±0.35
150	132.7±0.29
200	179.5±0.17
250	217.9±0.79
IC_{50}	48.52



Values are mean ± SEM of three determinations

3.4 Antibacterial activity:

Study on the antibacterial activity of ethanol and aqueous extract of dry flowers of Allamanda cathartica was coducted using agar disc diffusion method was shown in table [1.8 and 1.9]. The antibacterial activity of the microorganism: Bacillus subtilis, Staphylococcus aureus, Escheria coli, Pseudomonas aeruginosa among the various solvents tested . The maximum activity was observed against all organisms in both extract. The highest zone of inhibition was observed against Escheria coli (8.7±0.29) and (7.3±0.74) at 100µg/ml of ethanol and aqueous extract.

It is used to detect the antibacterial activity by comparing the standard drugs Ciproflaxin (10µg/ml). The *Allomanda Catharita* flowers are known to contain various active principle of therapeutic value and possess biological activity against various diseases[26-27]. The antibacterial properties of *Allomanda Catharita* extracts may be due to the presence of phenolicconstituents.

Table1.8: Antibacterial activity of ethanol extract of *Allomanda Catharita (Flower).*

Concentration of extract	**Zone of inhibition (mm)**			
	S. aureus	***E. coli***	***B. subtilis***	***P. aeruginosa***
50 µg/ml	3.4±0.86	5.2±0.73	2.9±0.38	4.7±0.67
100 µg/ml	6.1±0.06	8.7±0.29	5.3±0.15	8.3±0.25
Ciprofloxacin (10 µg/ml)	17.3±0.51	19.8±0.27	17.6±0.46	22.1±0.34

Values are mean ± SEM of three determinations

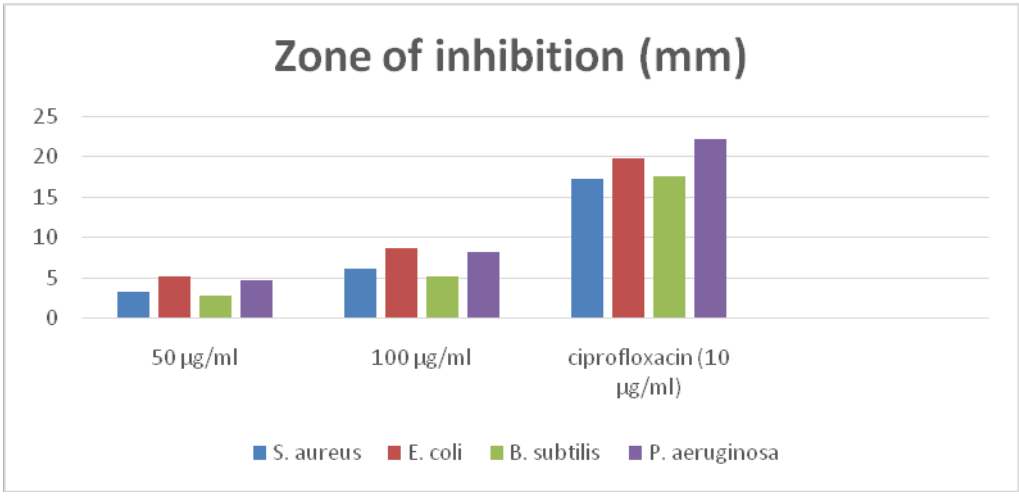


Fig 2: Antibacterial activity of ethanol extract

Table1.9:Antibacterial activity of aqueous extract of *Allomanda Catharita (Flower)*

Concentration of extract	**Zone of inhibition (mm)**			
	S. aureus	***E. coli***	***B. subtilis***	***P. aeruginosa***
50 µg/ml	2.7±0.52	4.6±0.54	2.3±0.33	3.9±0.65
100 µg/ml	4.1±0.14	7.3±0.74	3.8±0.48	6.8±0.73
Ciprofloxacin (10 µg/ml)	16.5±0.59	18.4±0.66	17.2±0.48	21.5±0.58

Values are mean ± SEM of three determinations

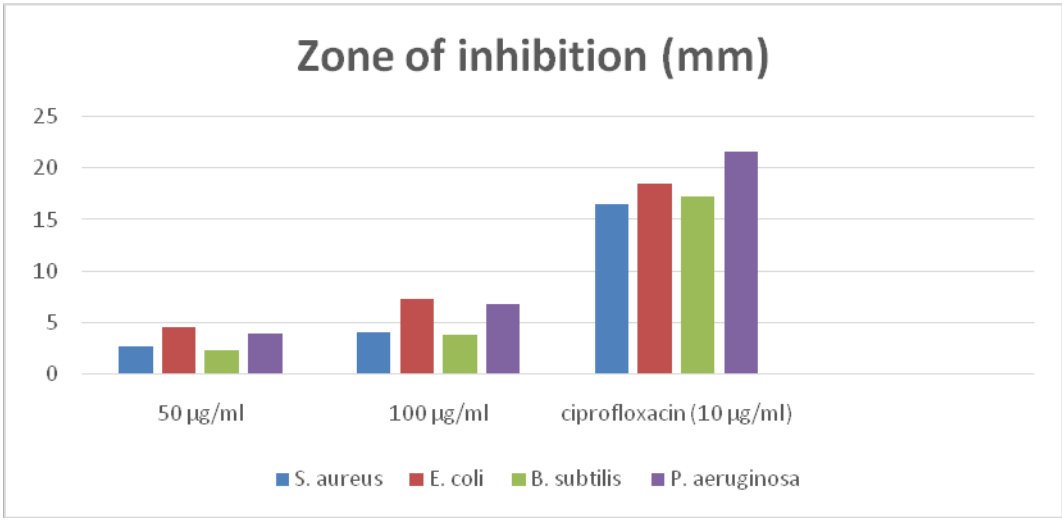


Fig 3: Antibacterial activity of aqueous extract

3.5 Statistical analysis:

All experiments were performed in triplicate and data were reported as mean±SD. The half-maximal inhibitory concentration (IC_{50}) value was calculated using the linear regression analysis. Data are expressed as mean ± standard errors of the mean. All statistical tests were computed by MS excel- 2013.

4. Conclusion

Antioxidant properties play an important role in plant physiology. In medicinal plant, different type of bioactive compound can influence the anti-oxidant activity. These bioactive compounds play a vital role in blocking of free radical generation. Mainly phenolic compound are involved in antioxidant property. Not only phenolic compound, other phytocompounds are also influence antioxidant activity. Like as glycoside have a therapeutic value in treatment of inherited deficiencies in man.Ethanol and aqueous extracts of AllomandaCatharitaare rich in phenolic and flavonoid content. They display significant antioxidant and antibacterial activities. Plant extracts inhibited the growth of both gram-positive and gram-negative bacteria, but significant activity was not observed against fungal species.Antibacterial activity was higher in ethanol extracts than in their aqueous counterparts. The present study supports the use of Allomanda Catharita flower in traditional medicine. It also validates the high antioxidant and antibacterial activities of the plant extract that needs to be explored. Further work should be carried out on the isolation, purification, characterization, and standardization of bioactive compounds.

5. Conflict of interest

The authors declare that there is no conflict of interest for this publication.

6. Acknowledgments

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7. References

(1) Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F. et al., Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases, J. of ethnopharm, 2003; 84(2 3):131-138.

(2) Choi C W, Kim S C, Hwang S S, Choi B K, Ahn H J, Lee M Y. et al., Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay guided comparison, Plant sci, 2002; 163(6):1161-1168.
(3) Al-Majedy Y.K., Kadhum A.A.H., Al-Amiery A.A., Mohamad A.B., Coumarins: The Antimicrobial agents, Systematic Reviews in Pharmacy, 2017, 8:24.
(4) Jaberian H, Piri K, Nazari J, Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants, Food chem, 2013; 136(1):237-244.
(5) Biapa P C, Agbor G A, Oben J E, Ngogang J Y, Phytochemical studies and antioxidant properties of four medicinal plants used in Cameroon, Afr J Tradit Complement Altern Med, 2007; 4(4):495-500.
(6) Borris RP. Natural products research: Perspective from a major Pharmaceutical company. J. Ethnopharmacol. 1996; 51:29-38
(7) Agbor G A, Kuate D, Oben J E, Medicinal plants can be good source of antioxidants: case study in Cameroon, Pak. J. Biol. Sci, 2007; 10(4):537-544.
(8) Kalita P, Barman KT, Pal KT, Kalita R. Estimation of total flavonoids content (tfc) and antioxidant activities of methanolic whole plant extract of biophytumsensitivum linn. J Drug Delivery Ther 2013;3:33-7.
(9) Sethi A, Prakash R, Bhatia A, Bhatia G, Khanna KA, Srivastava PS. Hypolipidemic, hypoglycemic and antioxidant activities of flower extracts of *Allamanda violacea* A. DC. (Apocynaceae). Trop J Pharm Res 2012;11:225-34.
(10) Uduak AE, Udo SE. Comparative phytochemical screening and nutritional potentials of the stems, leaves and flowers of *Allamanda Cathartica* (Apocynaceae). Int J Sci Technol 2015;4:248-53.
(11) Harborne, J. B. In Phytochemical Methods. Chapman Hall: London, 1998.
(12) Trease GE, Evans WC. Treas and Evans Pharmacognosy. (14th ed) India. W.C. Saunders company limited, 1989.
(13) Sofowora A. Medicinal and Traditional medicin in Africa. Ibadan Nigeria spectrum books Limited. 1998, 130.
(14) Geedhu D, Krishnakumari S. Quantitative analysis of primary and secondary metabolites in aqueous hot extract of *eugenia uniflora* (l.) leaves. Asian J Pharm Clin Res 2015;8:334-8.
(15) Sreedevi P, Vijayalakshmi K, Venkateswari R. Phytochemical evaluation of *punica granatum* l. Leaf extract. Int J Curr Pharm Res 2017;9:14-8.
(16) Hema K, Krishnaveni R. Antibacterial and antifungal Activities of Allamanda cathartica Linn. international journal of pharma and Bio sciences. 2014; (1):588-598.
(17) Sofowora A. Medicinal and Traditional medicin in Africa. Ibadan Nigeria spectrum books Limited, 2008, 150-153.
(18) Yadav RNS, Agarwala M, Phytochemical analysis of some medicinal plants, J. Phytol, 2011; 3(12).
(19) Bargah R.K. (2017). Preliminary phytochemical screening analysis and therapeutic potential of Tecoma stans (L.), International Journal of Applied Chemistry, Vol.13(1): 129-134.
(20) Ainsworth, E. A.; Gillespie, K. M. Estimation of Total Phenolic Content and Other Oxidation Substrates in PlantTissues Using Folin–Ciocalteu Reagent. Nat. Protoc. 2007, 2(4), 875–877.
(21) Okore VC. Principles of Pharmaceutical Microbiology: Evaluation of chemical antimicrobial agents (2nd ed). Enugu Nigeria. El` Demak Press, 2005, 86-87.
(22) Hema K, Krishnaveni R. Antibacterial and antifungal Activities of Allamanda cathartica Linn. international journal of pharma and Bio sciences. 2014; (1):588-598
(23) Okore VC. Principles of Pharmaceutical Microbiology: Evaluation of chemical antimicrobial agents (2nd ed). Enugu Nigeria. El` Demak Press, 2005, 86-87.
(24) Nandagoapalan V, Doss A, Marimuthu C, Phytochemical analysis of some traditional medicinal plants, Biosci. Discov, 2016; 7(1):17-20.

(25) Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N, Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal, Plants, 2019; 8(4):96-103.
(26) Bargah R.K., Kushwaha A.,Tirkey, A., Hariwanshi. B., (2020). In Vitro Antioxidant and Antibacterial Screening of flowers Extract from Cassia auriculata Linn." Research Journal of Pharmacy and Technology Vol.13(6), pp.2624-2628.
(27) K. Arun, A. N. Kalia, H. Singh. (2020). In-vitro antioxidant potential of polyherbal formulation of three different Herbal drugs. Indo Global Journal of Pharmaceutical sciences, 10(4):70-76.

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