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Research Article

Evaluation of bioactive compounds from *Jasminum polyanthum* and its medicinal properties

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ABSTRACT

Jasmine plant species are widely grown in Asia and used for religious offering, is known to have wide range of bioactive compounds and its properties. Most of the jasmine species have been evaluated its bioactive properties and found positive results. This work consists of evaluating bioactive properties of jasmine species, *Jasminum polyanthum* and finding its potential medicinal uses. The plant leaves and flowers were powdered and added water to prepare extract. Both leaves and flower extract was evaluated for phytochemical compounds, anti oxidant, anti diabetic, anti inflammatory, anti microbial, anti cancer and DNA nicking assay. The leaf and flower extract contained most of the phytochemical compounds which leads to presence of various bioactive properties. Leaf possesses good DPPH activity, while flower possess high phenol content and FRAP activity. Leaf possesses good anti diabetic activity, while flower possess good anti inflammatory activity. Flower extract consists of higher antibacterial activity than leaf, while in antifungal it is vice versa.

Keywords: Anti-diabetic, Anti-inflammatory, Antioxidant activity, MTT Assay, DNA Nicking study**Article Info:** Received 31 Jan 2019; Review Completed 07 March 2019; Accepted 09 March 2019; Available online 15 March 2019

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INTRODUCTION

Advances in technology proved to be advantageous to mankind. At the same time, number of diseases also increased. Even though human invented drugs for cure, later it was inferred that those drugs are ineffective in killing pathogens as they developed tolerance¹. Some of those drugs also caused side effects. From olden days, plants were used for treating various disorders. The ancient medicine like Ayurveda and siddha mostly involve plant as curative substances. Medicinal plants have chemical components which produce physiological actions in body like elimination of ROS (anti oxidant), inhibiting enzymes which increase blood glucose level (anti diabetic), inhibiting inflammatory action (anti inflammatory), killing pathogens (anti microbial), inhibiting cell proliferation (anti cancer) etc. So there is a need to explore bioactive compounds in plants as they contribute to medicinal properties and find usage in treating human diseases. These components form class of phytochemicals like alkaloids, steroids, saponin, flavonoids etc². If higher level of any bioactive property was found, then it can be used for applications to treat against human physiological diseases provided it does not cause any side effects.

Jasmine flowers are widely distributed and each species has its own native (mostly Asia). There are about 200 species existing in the world. Its fragrance is well known and so it is popular. These are cultivated in garden and also grown as house plant. It grows on sunny areas and soil must be fertile, well drained. Jasmine species flowers are used for religious purposes like offerings to Hindu gods³. Almost all jasmine species are ingredient for Ayurvedic species having curing qualities. It is used for removing intestinal worms. It is widely used for venereal diseases. Flowers are used to treat ulcers, vesicles, boils, skin diseases and eye disorders. Leaf extract are effective against breast tumours⁴, aphthous, stomatitis, toothache, ulceration in mouth, throat and gums. Traditionally, its leaves were grind into juice and treated for urinary tract infections as sedative, mild anaesthetic and astringent. Jasmine species also finds place in cosmetics and used for making perfumes and scents. Flowers are used for skin toner and conditioner and used in shampoos, soaps, creams etc. Flowers were used as folk remedy for hepatitis, stomatitis, and duodenitis in china⁵, Jasmine flowers are also used for decorative purpose. Oils extracted from flowers are widely used in cosmetic and pharmaceutical industry⁶. In some parts of the world, jasmine tea is consumed for boosting immunity.

Jasminum polyanthum, also known as pink jasmine, is an fast growing evergreen creeper. Its native is china and hence also called Chinese jasmine. It produces pinkish white flower buds in early spring, followed by 5 petalled flowers (white and pink mixed) which are 2 cm in diameter⁷. It develops into dense bush and produces fragrance wherever it is present. Since it grows extremely fast, It also acts as invasive species in some countries. It is propagated through seeds, suckers, and stem cuttings. It grows in moist, fertile, well drained soils and tolerates wide range of pH. It grows well in subtropical climate. . It is widely used in landscape design and used to cover high walls and Fences.

In this paper, extract of *Jasminum polyanthum* leaves and flowers were prepared and evaluated its composition of bioactive compounds, anti oxidant, anti diabetic, anti inflammatory, antimicrobial against urinary tract infection, anti cancer and DNA nicking assay. These activities were compared between leaf and flower extracts and inferred which one is best among those medicinal properties.

MATERIALS AND METHODS

Preparation of plant extract

Fresh flowers and leaves of *Jasminum polyanthum* were collected from nearby regions of Coimbatore, Tamilnadu, India. Leaves and flowers were dried under shade of sunlight for 48 hours. The dried leaves and flowers were grinded and powder were stored in air tight container and used for the further studies.

2gm of the powdered sample was dissolved in water and methanol, incubated at 40°C, 70-80 rpm for 24 hours. Then the solution was filtered through filter paper and these extracts were further analysed.

Qualitative Analysis of Bioactive Compounds⁸

The four extracts (leaf water, leaf methanol, flower water and flower methanol) were evaluated for qualitative bioactive compounds of alkaloids (mayer's test), terpenoids (concentrated sulphuric acid), phenol (FeCl₃ test), sugar (Fehling's test), saponin (Foam test), flavonoids (concentrated HCl), quinines (NaOH test), protein and steroid (chloroform and Sulphuric acid).

Anti oxidant activity

DPPH Assay

Leaf and flower powder of different concentrations (10-30 mg) were taken in different test tube and 0.1ml of 0.1M DPPH solution was added and mixed well. After 5 minutes of incubation 0.4ml of 50 mM Tris HCl was added and make up to 2ml with distilled water and incubated in dark room for 30 minutes. OD reading was taken at 517 nm using spectrophotometer. Ascorbic acid was used as a standard to calculate the mg/g of DPPH.

Flavonoids

1ml of the leaf and flower extract was taken separately and added 0.1ml of 10% AlCl₃. 0.1ml of 1M potassium acetate, 2ml of distilled water also added and incubated at room temperature for 30 minutes. OD measurement were taken at 415 nm using Spectrophotometer. Quercetin was used as a standard to calculate the mg/g of flavonoids.

Total phenol

0.5ml of the extract was mixed with 0.5ml of folin's phenol reagent (10%), 2ml of 20% sodium carbonate solution, after adding all the reagent the tubes were incubated at 45°C for 45 minutes. OD were measured at 765 nm using

Spectrophotometer. Gallic acid was used as a standard and its standard curve plotted.

FRAP assay

Radical scavenging activity was measured by dissolving 1ml of the sample with equal amount of Phosphate buffer solution and 0.1% potassium ferric cyanide solution. After adding the tubes were incubated at 50°C for 20 minutes. 1 ml of the 10% TCA solution, 1ml of the distilled water and 0.1% FeCl₃ solution was also added to the sample and OD reading was taken at 700 nm using Spectrophotometer. Ascorbic acid was taken as standard to calculate the mg/g of the FRAP content.

Anti Diabetic Activity

α- Amylase activity⁹

1ml of the sample was mixed with 0.1% starch solution in 16mM of sodium acetate buffer and 0.2 ml of the alpha – amylase enzyme which is prepared by mixing of 27.5gm in 100ml distilled water. The colorimetric reagent was prepared by mixing sodium potassium tartarate and 3,5 di nitro salicylic acid solution in the concentration of 96mM. After adding, the tubes were incubated in alkaline condition at 25°C for 3-5minutes. The generation of maltose was quantified by the reduction of 3,5di nitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This was detected at 540nm using spectrophotometer. The % of alpha amylase inhibitory activity was calculated by using the following formula;

$$\text{Percentage of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

α - Glucosidase activity¹⁰

The inhibitory activity was determined by mixing of 1ml of 2% starch solution and 1ml of the sample with 1ml of the 0.2M tris buffer (pH-8), incubated at 37°C for 5minutes. The reaction was initiated by adding 1ml of the alpha-glucosidase enzyme (1U/ml) incubated for 40minutes at 35°C. The reaction was terminated by adding 2ml of 6N HCl and the reading was measured by 540nm using spectrophotometer. The % of alpha glucosidase inhibitory activity was calculated by using the following formula;

$$\text{Percentage of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Anti Inflammatory Activity

Anti proteinase action¹¹

0.06mg of trypsin was added to 1ml of the sample with 1ml of 20 mM Tris HCl solution. The mixture was incubated for 10 minutes at 37°C. 1ml of the 0.8% casein solution was added and incubated for 20 minutes. Followed by added 2ml of 70% perchloric acid to arrest the reaction. Cloudy suspension was centrifuged at 5000 rpm for 5 minutes and supernatant was collected, OD reading were taken at 210 nm using Spectrophotometer and tris HCl was used as a blank. Inflammatory inhibitory activity was found using following formula;

$$\text{Percentage of inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Antimicrobial Activity¹²

Agar well diffusion method

Well diffusion method was used to evaluate anti-microbial activity. Mueller Hinton agar media was used for antibacterial study. 39gm of media was dissolved in 1000ml of distilled water and sterilised for 121°C at 15lbs. The

media was poured into sterile petri plates and 70µl of bacteria were swabbed. The samples were evaluated for antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Gentamicin was used as a positive control and sterile distilled water was taken as negative control. Wells were loaded with extracts and incubated at 37°C for 24 hours. After incubation zone of inhibition was measured.

Antifungal activity

Malt extract media was used for anti-fungal study. 38gm of malt extract agar media was dissolved in 1000ml of distilled water and sterilised. The media was poured into sterile Petri plates. 70µl of *Aspergillus flavus* and *Aspergillus niger* were swabbed on to the plate and wells were loaded with leaf and flower extracts. Petri plates were sealed tightly and kept aside at 30°C for 3 -5 days. After incubation zone of inhibition was measured.

Anticancer Activity¹³

Preparation of cell lines media

DMEM media (19.75 g of DMEM, 3.7g of sodium carbonate, 4.5g of glucose) was prepared in T- flask and the addition of HeLa cell lines the flask was incubated in CO₂ incubator with 5% CO₂, 37°C temperature, at 70-80% of humidity for 24-72hrs.

In vitro cytotoxicity Assay

Leaf and flower extracts of different concentration (10µl, 20µl, 30µl) were taken and to that 100µl of cell lines were added. DMSO was taken as blank and cell lines were used as a control. The plate was incubated for 24 hours in CO₂ incubator. DMSO and trypsin was used to lysis the cells and also for the washing. After washing 20µl MTT dye was added to all the wells and incubated for 24 hours. OD values were measured using ELISA reader (Robonic read well touch ELISA plate reader). Percentage of cell death was calculated using the following formula;

$$\text{Percentage of cell death} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

DNA Nicking Assay¹⁴

DNA nicking assay was done to find the presence of DNA damaging property of extracts using Fenton's method (30 mM Hydrogen peroxide, 80 mM FeCl₃ solution, 50mM Ascorbic acid). 3µl of pBR322 plasmid DNA was mixed with 10µl of the Fenton's reagent, 5µl of the sample and make up to 20 µl with distilled water to evaluate DNA damage. The tubes were incubated 37°C for 2-3 hours and runned at 1% agarose gel. While preparing the agarose 2µl of ethidium bromide was added to visualise the DNA under UV-Transilluminator. Samples were loaded with the dye of bromophenol blue and run at 50 millivolts along with pBR322 as a control DNA.

RESULTS

Bioactive Compound Analysis

The analyses of bioactive compounds in four extracts – leaf water, leaf methanol, flower water, flower methanol are given in table 1. Leaf and flower extracts containing water has most of bioactive compounds and hence these two extracts are taken for further study.

Figure 1: *Jasminum polyanthum* plant

Table 1: Composition of bioactive compounds in different extracts

Compounds	Flower water	Leaf water	Flower methanol	Leaf methanol
Alkaloids	Present	Present	Present	Absent
Terpenoids	Absent	Present	Present	Absent
Phenol	Present	Present	Present	Present
Sugar	Absent	Present	Absent	Present
Saponin	Present	Present	Absent	Present
Flavonoids	Present	Absent	Present	Absent
Quinines	Present	Present	Present	Present
Protein	Present	Present	Present	Absent
Steroid	Absent	Absent	Present	Absent

Figure 3: Results of bioactive compound analysis of 4 extracts – flower water, leaf water, flower methanol, leaf methanol. Analysis were done for following type of compounds (from the left) alkaloid, terpenoid, phenol, sugar, saponin, flavonoid, quinine, protein, steroids.

Antioxidant Activity

DPPH assay

DPPH radical scavenging activity study is extensively using to screen the antioxidant properties of the sample. In this study the antioxidant concentration from plant powder were determined from ascorbic acid standard curve given in table 2. From the curve, leaf powder has higher antioxidant. Antioxidant concentration in leaf and flower is in range of 6 to 19mg/g and 11 to 36 mg/g respectively.

Flavonoids

Flavonoids are well famous for their antioxidant properties and it is a universal plant pigment. Flavonoids content were calculated by using quercetin as standard shown in table 3. Flower extract has higher flavonoid content than leaf. Flavonoid concentration in leaf and flower are 30 and 19 mg/g respectively.

Total phenol

Phenolic constituents are important in plants because of scavenging activity due to the hydroxyl group. Total phenol concentrations were determined from standard curve using gallic acid as standard shown in table 4. Flower extract has slightly high phenol content than leaf. Total phenol content in leaf and flower are 5 and 7mg/g respectively.

FRAP assay

Reducing power of an antioxidant reacting with the ferric cyanide was calculated with the standard ascorbic acid. From the study flower extract has higher antioxidant concentration than leaf. Antioxidant concentration in leaf and flower are 33 and 59mg/g respectively which are shown in the table 5.

Antidiabetic Activity **α - Amylase activity**

Amylase activity was calculated in terms of percentage and leaf extract showed 29.78% while flower extract showed 34.07% shown in table 6. Flower Extract has lower amylase inhibitory activity than leaf.

 α - Glucosidase activity

Glucosidase inhibitory properties are starch blockers, which inhibit was calculated in terms of percentage and leaf extract showed 31.92% while flower extract showed 37.76% shown in table 7. Leaf extract has lower glucosidase inhibitory activity than flower. Therefore, flower has higher anti diabetic activity than flower.

Anti Inflammatory Activity**Anti-proteinase action**

Anti-proteinase activity was evaluated for leaf and flower extracts and inflammation inhibitory activity of leaf and flower were 94.46% and 88.93% respectively shown in table 8. Further, both these extracts have higher amount of inflammatory inhibitory activity. Leaf extract has higher inflammatory inhibitory activity than flower extract.

Antimicrobial Activity

The zone of inhibition of bacterial organisms along with extracts is shown in table 9. Leaf and flower extract shown

significant inhibition effect on growth of bacteria. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are inhibited more effectively by the plant extract than *Escherichia coli* and *Klebsiella pneumoniae* due to bigger zone of inhibition. Comparing the extracts, flower extract shown more antibacterial activity than leaf extract.

The zones of inhibition of fungal organisms along with extracts are shown in table 10. The extracts have antifungal activity against *A. flavus* but extracts had no effect on *A. niger*. Thus, we can say that both extracts have low inhibitory activity against the used organisms and its range on anti-fungal activity is limited.

Anticancer Activity

The percentages of cell death of extracts in different concentrations are given in the table 11. Both the extracts have significant anticancer activity. Comparing extracts, leaf extracts have higher anticancer activity than flower extract.

DNA Nicking Assay

Sample was run on agarose gel electrophoresis and bands were observed on UV- illuminator. The first well contains control which contains only DNA and second well contains DNA marker. 3rd well contains DNA with leaf extract and 4th well DNA with flower extract. As DNA remains single band when extracts were added, so we conclude that DNA was not cleaved and hence extracts do not have DNA damaging property.

Table 2: antioxidant concentration of extracts in different amount from DPPH assay

Sample	Amount (mg)	Antioxidant conc (mg/g of powder)
Flower	10	6
	20	8
	30	19
Leaf	10	11
	20	31
	30	36

Table 3: Flavonoid concentration of extracts

Sample	Flavonoid content (mg/g)
Flower	19
Leaf	30

Table 4: total phenol content of extracts

Sample	Total phenol content (mg/g)
Flower	7
Leaf	5

Table 5: Antioxidant concentration of extracts from FRAP assay

Sample	Antioxidant concentration (mg/g)
Flower	59
Leaf	33

Table 6: Amylase inhibitory activity of extracts

Sample	α - Amylase inhibitory activity (%)
Leaf	29.87
Flower	34.07

Table 7: glucosidase inhibitory activity of extracts

Sample	α - Glucosidase inhibitory activity (%)
Leaf	31.92
Flower	37.76

Table 8: Inflammation inhibitory activity of extracts

Sample	Inflammation inhibitory activity (%)
Leaf	64.46
Flower	58.93

Figure 4: Antibacterial activity against *P. aeruginosa*, *S. aureus*, *E. coli* and *K. pneumoniae* Figure 5: Antifungal activity against *A. flavus* and *A. niger*

Table 9: zone of inhibition of extracts against different bacteria

Microorganism used	Leaf extract (mm)	Flower extract (mm)	Gentamicin disc (mm)	Sterile distilled water
<i>E coli</i>	7	8	10	NIL
<i>K pneumoniae</i>	8	9	10	NIL
<i>S aureus</i>	11	13	10	NIL
<i>P aeruginosa</i>	12	13	10	NIL

Table 10: zone of inhibition of extracts against different fungi

Microorganism used	Leaf extract	Flower extract	Distilled water
<i>A. flavus</i>	10 mm	8 mm	NIL
<i>A.niger</i>	NIL	NIL	NIL

Table 11: percentage of cell death of extracts of different volumes

Sample	Volume (µl)	Percentage of cell death (%)
Leaf	10	41.55
	20	45.6
	30	49.47
Flower	10	34.5
	20	44.89
	30	57.22

Figure 6: Agarose gel visualised in UV- transilluminator after electrophoresis. 3rd and 4th well from left contains DNA with leaf and flower extracts, respectively. Note that they form single band and was not damaged due to reaction with extracts

DISCUSSION

In the study of Ali esmail⁴ on phytochemical studies in common jasmine (*Jasminum officinale*), aqueous extracts of *Jasminum officinale* leaves indicated presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, leuco anthocyanins, steroids, anthocyanins, phlobatinins, essential oil and saponins. In the study of mittalet al¹⁶ on phytochemical studies in *Jasminum auriculatum*, solvent extracts of plant leaves revealed presence of alkaloids, carbohydrates, flavonoids, steroids, terpenoids, saponins, tannins and phenolic compounds. In the study of Bhagat et al¹⁵ on phytochemical studies in *Jasminum arborescens*, solvent extracts revealed presence of terpenoids, flavonoids, steroids, glycosides, tannins and saponins. In the present study, leaves and flower extracts indicated presence of alkaloids, terpenoids, sugars, saponins, flavonoids, quinines, proteins and phenol.

Aliesmail⁴ also investigated antioxidant activity in *Jasminum officinale*. The total phenolic contents of the aqueous extract of *Jasminum officinale* leaves was 104.02 mg/g gallic acid equivalent, the total flavonoids content was 10.76 mg/g quercetin equivalent, the total antioxidant capacity was 155.40 µg/ml and reducing power was 44.28 µg/ml. In study of Bhagat et al¹⁶ on anti oxidant activity in *Jasminum arborescens*, DPPH scavenging activity inhibition was in the range of 40 to 80 %. FRAP assay revealed 50 to 85 % inhibition. In the study of Jyothsana et al¹⁷ on antioxidant activity in *Jasminum auriculatum*, DPPH scavenging activity was in range of 22 to 70 %. Reducing power assay was in

range of 40 to 80 %. In this study, DPPH antioxidant concentration was in the range of 6 to 36 mg/g. Total flavonoid of flower and leaf was 19 mg/g equivalent quercetine and 30 mg/g equivalent quercetine respectively. Total phenol content of flower and leaf was 7 mg/g equivalent gallic acid and 5 mg/g equivalent gallic acid respectively. FRAP antioxidant concentration in flower and leaf were 59 mg/g equivalent ascorbic acid and 33 mg/g equivalent ascorbic acid respectively.

In the study of Shivakumarswamy et al¹⁸, using plant extract of *Jasminum grandiflorum*, glucose reduction level ranges from 0.4 to 70% which is indicator of anti diabetic activity. In this study, using extracts of *Jasminum polyanthum*, antidiabetic activity of extracts ranges from 29 to 38%.

Nurulislam et al¹⁹, investigated anti-inflammatory activity using plant extract of *Jasminum sambac* and was found to be in the range of 6 to 51%. In the study of Sangita kumara et al²⁰, using extract of *Tabernaemontana divaricata* (crepe jasmine), anti inflammatory activity was in the range of 22 to 58%. In this study, using the plant extract of *Jasminum polyanthum*, anti inflammatory activity of leaf and flower extract was found to be 64.46% and 58.93% respectively.

Srinivas ampati et al²¹ done antibacterial activity in *Jasminum officinale*, solvent extracts showed zone of inhibition ranging from 14 to 24 mm. In study of Aliesmail⁴ on antibacterial activity of *Jasminum officinale*, solvent extracts revealed zone of inhibition ranging from 10 to 20 mm. In the study of Usman et al²² in antibacterial activity of *Jasminum officinale*, aqueous extracts of flower showed zone of inhibition in the

range of 9-18 mm. In our study, aqueous extracts of flowers and leaves shows zone of inhibition ranging from 7 to 13 mm.

Research work of Manokaran et al²³, using plant extracts of *Jasminum sambac*, anti cancer activity on HeLa cells revealed percentage of cell death ranges from 17 to 98%. In this study, using plant extracts of *Jasminum polyanthum*, percentage of cell death ranges from 35 to 57% for different concentrations of leaf and flower extracts.

Report of Srinivas ampati²¹ on antifungal activity in *Jasminum officinale*, solvent extracts showed zone of inhibition ranging from 12 to 20 mm and Aliemail⁴ on antifungal activity in *Jasminum officinale*, solvent extracts shown zone of inhibition from 14 to 20 mm. The present

study, antifungal activity is lower and it shows zone of inhibition maximum of 10 mm

CONCLUSION

In the present study the Jasmine leaf and flower extracts were used to find out the various bioactive compounds. The qualitative screening showed the presence of various bioactive compounds and the antioxidant properties were also studied. The DPPH, FRAP, total phenol and Flavonoids content were estimated. The antibacterial activities were also studied against *E.coli*, *K.pneumoniae*, *S.aureus*, *P.aeruginosa* bacteria and *A.niger* and *A.flavus* for fungi. The antidiabetic activity was also studied. The anticancer activity against HeLa cells showed 35 to 55% of cell death. The DNA fragment study was carried out to find out DNA damaging property and was found to have no DNA damaging property.

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