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Kumaresan M
Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Kannan
Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

A Sankari
Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

CN Chandrasekhar
Department of Crop Physiology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

D Vasanthi
Department of Soil Science and Agricultural Chemistry, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
M Kannan
Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Phytochemical screening and antioxidant activity of *Jasminum multiflorum* (pink Kakada) leaves and flowers

Kumaresan M, M Kannan, A Sankari, CN Chandrasekhar and D Vasanthi

Abstract
Jasminum multiflorum is an evergreen shrub belongs to the family Oleaceae which is used as a traditional medicine from ancient times. The leaves and flowers have a rich source of phytochemicals like alkaloids, phenols, flavonoids, tannins, etc., which are capable of curing various diseases. In this regard, an experiment was conducted to screen the phytochemical components and antioxidant activity in leaves and flowers of *Jasminum multiflorum.* The extraction was carried out by using different solvents *viz.*, methanol, ethanol, ethyl acetate, chloroform, petroleum ether and aqueous extracts for phytochemical screening and methanol, ethanol, ethyl acetate and aqueous extracts for antioxidant analysis. Antioxidant assay was done by determining DPPH, ABTS and Chelating potential of leaves and flower extracts. Phytochemical screening of leaves and flower extracts revealed the presence of alkaloids**,** tannins**,** carbohydrates**,** sterols and terpenoids, flavonoids**,** cardiac glycosides, proteins and amino acids. The quantitative determination of total phenolic, total flavonoids and various antioxidant activities (DPPH, ABTS and chelating potential) was carried out using colorimetric method and the results revealed that the total phenolic and total flavonoids were maximum in ethanol extract of leaves. The antioxidant activity was expressed in terms of EC_{50} value and maximum reduction percentage was observed in ethanol extract of leaves.

Keywords: Jasmine, phytochemicals, antioxidant and solvents

Introduction
Jasminum multiflorum is a species of jasmine commonly known as Indian jasmine, Winter jasmine and Downy jasmine. It is an ornamental flowering shrub native to India and Southeast Asia. In ancient age, people used local flora and fauna as a traditional medicine for their survival. Mostly leaves, roots, flowers and fruits are used as traditional medicines to maintain health and also treat fever, cough, indolent ulcer, abdominal distention, diarrhoea, lowering the blood glucose level, regulating menstrual flow, to clean kidney waste, inflamed and blood shot eyes (Stary and Hans, 1998) [1]. One such example is tea (black & green tea) which is frequently used as beverage all over the world and is a rich source of polyphenolic compounds (Borneo *et al.,* 2009) [2]. In modern world, the synthetic drugs are readily available and more effective in curing numerous diseases; there are people who still prefer using traditional folk medicines, because of their less harmful effects due to non-toxic in nature and easy availability at reasonable price. Plants are producing good source of secondary metabolites such as phenolic compounds, nitrogen containing compounds, vitamins and minerals. These compounds have antidiabetic (Upaganlawar, 2003) [3], antitumor (Radu and Kqueen, 2002) [4], antimicrobial (Hussaini and Mahasneh, 2009) [5], antioxidant (Latif *et al*., 2010) [6] anti-acne activity (Harisaranraj *et al.,* 2010) [7] etc. In India, traditional medicines are used for preparation of various cosmetics, colours and beautification. Therefore researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs (Benkeblia, 2004) [8].

Almost all plant parts of jasmine *viz.,* leaves, flowers, stem and roots are having pharmaceutical properties. Dried leaves are used to treat indolent ulcers (Warrier, 1995) [9]. The jasmolactones A, B, C, D (Secoiridoid lactones), multifloroside, multiroside and secoiridoid glycosides have been isolated from *J. multiflorum* and these lactones and secoiridoid glycosides have cardiotropic activities (Shen *et al.,* 1990) [10]. *Jasminum* species is used to treat various skin diseases, leprosy and also acts as antidepressant, anti-inflammatory and analgesic. The jasmine leaves and flowers are generally used in traditional medicine to prevent and treat breast cancer (Kalaiselvi *et al.,* 2011) [11].

Extraction solvents are an important source in the itinerary of phytochemical processing for the discovery of bio-active constituents from plant materials. Selection of suitable extraction solvent is also important for production of herbal products. Therefore, the present study has been designed to screen the phytochemical components and to determine the antioxidant activity in leaves and flowers of *Jasminum multiflorum* (Pink Kakada) with the use of various solvents.

Material and methods

Plant material and sample preparation

Leaves and flowers of *Jasminum multiflorum* (Pink Kakada) (Fig 1.) were collected from department of floriculture and landscaping, Coimbatore during the month of September in 2018. These collected plant materials were cleaned up with tap water and followed by distilled water to remove the dust particles and were shade dried at room temperature for about five days. The dried samples were pulverized to a fine powder in a mechanical blender and stored at -21ºC for further use. 30 g of sample was packed in a muslin cloth and kept into a Soxhlet apparatus and extraction was carried out with 300 ml of different solvents at 55–60°C for 10 hrs. The solvents used for extraction were *viz.*, methanol, ethanol, ethyl acetate, chloroform, petroleum ether and aqueous extracts for phytochemical screening and methanol, ethanol, ethyl acetate and aqueous extracts for antioxidant analysis. The final extract was filtered through Whatman filter paper No. 1 and then the solvents were evaporated with the use of rotary evaporator. The crude extracts were stored at -21ºC for further experimental use.

Fig 1: *Jasminum multiflorum* (Pink Kakada)

Phytochemical screening

The extracts of leaves and flowers were tested for the presence of alkaloids**,** tannins, phlobatannins**,** saponins**,** carbohydrates**,** sterols and terpenoids, flavonoids**,** cardiac glycosides, proteins and amino acids as per the standard procedures described by Jamil *et al.*, 2012 [12]. The qualitative results are expressed as (+) Positive for the presence and (-) Negative for the absence of phytochemicals. Qualitative phytochemical analysis was carried out by using standard procedures as described by Singleton and Rossi, 1965 [13] and Liu *et al.*, 2008 [14].

Qualitative tests

Alkaloids 1. Mayor's test	4 ml of 1% HCl was added into 1 ml of extract and then it was filtered through Whatman filter paper No. 40 followed by addition of 6 drops of Mayor's reagent into filtrate. The development of creamish or orange precipitate indicated the presence of alkaloids.
Flavonoids 1. Shibita's test 2. Pew's test	5 ml of sodium hydroxide (20%) was added into 5 ml of extract and formation of yellow colour indicated the presence of flavonoids. Extract of 5 ml was mixed with 0.1 g of metallic zinc and 8 ml of concentrated sulphuric acid. The mixture was observed for red colour as indicative of flavonols.
Tannins 1. Ferric chloride test:	2 ml of extract and few drops of ferric chloride (10%) solution were added in to a test tube. The development of blackish blue or blackish green colour indicated the presence tannins.
Phlobatannins 1. HCl test	Dilute HCl was added into 2ml of extract and observed for red precipitate indicating the presence of phlobatannins.
Carbohydrates 1. Fehling's Test	Copper sulphate (6.932 g) was dissolved in distilled water and the make volume was made up to 100 ml (solution A). Potassium sodium tartarate (34.6 g) and sodium hydroxide (10 g) were dissolved in distilled water and the make volume was made up to 100 ml (solution B). Two solutions were mixed in equal volume prior to use. The final solution and extract (1ml each) were mixed and boiled. Formation of brick red precipitate indicated the presence of carbohydrates.
Sterols and terpenoids 1. Salkowski test	1ml of extract was mixed with few drops of concentrated sulphuric acid and allowed to stand for some time. The formation of red colour at upper layer indicated the presence of steroids and yellow colour appears at lower layer indicated the presence of terpenoids.
Cardiac glycosides 1. Keller–Kiliani test	2 ml of glacial acetic acid and few drops of 1% $FeCl_3$ were added to 1 ml of the extract. Then, it was underlayed with 1 ml of concentrated sulphuric acid. The appearance of green-blue colour indicated the presence of cardiac glycosides.
Proteins 1. Biuret test	2 ml of the extract, 5 drops of copper sulphate (1%) and 2 ml of sodium hydroxide (10%) were mixed. The development of violet colour indicated the presence of proteins.
Saponins 1. Frothing test	The extract of 3 ml was mixed with 10 ml of distilled water and shaken vigorously for about 5 min. It was allowed to stand for 30 min and observed for honeycomb froth, which was indicative of the presence of saponins.

Total phenol content

Total phenol content of the leaves and flower extracts was estimated by using the Folin–Ciocalteu colorimetric method as described by Singleton and Rossi, 1965 [13] with certain modifications. 200 µl (1 mg ml^{-1}) of extract was mixed with 8.5 ml of distilled water and then 0.5 ml of Folin–Ciocalteu reagent was added and mixed thoroughly for about 5 min. After adding 20% sodium carbonate (1 ml), the test tubes were incubated in dark for 60 min at room temperature. The absorbance was measured at 760nm by using bio-

spectrophotometer. The calibration curve was prepared using Gallic acid equivalent and the results were expressed as mg per gram of Gallic acid equivalent.

Total flavonoid content

Total flavonoid content of the leaves and flower extracts were estimated as per the aluminium chloride method described by Liu *et al.,* 2008 [14]. Extract of 0.5 ml was added to 3 ml of sodium nitrate (5%) followed by 2.5 ml of distilled water. It was mixed thoroughly and incubated at room temperature for 3 min. Then 0.3 ml of 10% aluminium chloride was added. The test tubes were allowed to stand for 5 minutes and then 2 ml of sodium hydroxide (1M) was added. After 60 min, the final volume was made up to 10 ml and the absorbance was measured at 415nm by using bio-spectrophotometer. The standard curve was prepared using Quercetin and the results are expressed in milligram per gram (mg/g) Quercetin equivalent.

Antioxidant activity

1. DPPH radical scavenging activity

The DPPH radical scavenging activity assay was determined according to the method given by Brand- Williams *et al*., 1995 [15] with some modifications. The stock solution was prepared by using 100 ml of methanol along with 24 mg of DPPH and was stored at 20ºC until needed. The working solution was prepared by diluting DPPH solution with methanol to obtain an absorbance of about 0.980 (±0.02) at 517 nm using bio-spectrophotometer. The varying concentrations (100– 500 µg/ml) of 100 µl fractions were prepared by mixing 3 ml aliquot of this solution. Then it was shaken well and incubated in dark for 15 min at room temperature. After 15 minutes the absorbance was measured at 517 nm by using bio-spectrophotometer. Ascorbic acid standards were used as positive controls. The percentage of DPPH radical scavenging activity was calculated based on the following formula.

$$\% \text{ Inhibition} = \left(\frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}}\right) \text{x } 100$$

The antioxidant capacity of test samples was expressed as EC_{50}, the concentration necessary for 50% reduction of DPPH.

1. ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity was determined based on the procedure given by Re *et al.*, 1999 [16] with some slight modifications. ABTS radicle cation mixture was prepared by mixing potassium persulphate (2.45mM) with ABTS (7mM). The solution is kept in dark for overnight to release ABTS radical cations. The working solution is then diluted with 50 % methanol to get an absorbance of 0.700 (±0.02). 300 µl aliquot of this solution was taken and 100µl of the extract at different concentration (100 – 500 µl/ml) was added. Their absorbance at 734 nm was measured using bio-spectrophotometer after 6 minutes of incubation. Ascorbic acid was used as standard and the antioxidant capacity of the extract was expressed as EC_{50} *i.e.* the concentration required to inhibit 50% of ABTS free radicles. The percent inhibition was measured using the following formula.

$$\% \text{ Inhibition} = \left(\frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}}\right) \text{x } 100$$

2. Chelating potential

The ability of the extract to chelate Iron (II) was estimated using the procedure described by Liu *et al*., 2008[14]. 200 µl aliquot of each sample was mixed with 100 µl of $FeCl_2.2H_2O$ (2.0 mM) and 900 µl of methanol. The test tubes were incubated in dark for 5 min; the reaction was initiated by the addition of 400 µl of ferrozine (5.0 mM). After 10 minutes of incubation, the absorbance was measured by using bio-spectrophotometer at 562 nm. Ascorbic acid was used as standard. The chelating power of the extract was expressed as EC_{50} i.e. effective concentration that chelates 50% of iron (II). The chelating activity (%) was calculated using the following equation.

$$\text{Chelating potential } \% = \left(\frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}}\right) \text{x } 100$$

Results and discussion

Phytochemical analysis

The present study revealed that the various alcoholic and aqueous extract of leaf showed presence of some secondary metabolites like alkaloids, tannins, carbohydrates, sterols and terpenoids, flavonoids, cardiac glycosides, proteins and amino acids and the secondary metabolites like phlobatannins and saponins were not detected (Table 1). Among the different solvents used in the extraction, the ethanol extract of leaves showed maximum number of phytoconstituents *viz.*, alkaloids, tannins, flavonoids, cardiac glycosides, proteins and amino acids followed by methanol extract, which exhibited tannins, sterols and terpenoids, flavonoids and cardiac glycosides. The extract of other solvents - Ethyl acetate (alkaloids, flavonoids and cardiac glycosides), chloroform (flavonoids, carbohydrates, sterols and terpenoids) petroleum ether (tannins, flavonoids, proteins and amino acids) and aqueous (tannins, flavonoids and sterols and terpenoids) showed minimum number of components.

Regarding flower extracts the maximum number of components was detected in ethanol extract (alkaloids, tannins, flavonoids, cardiac glycosides, proteins and amino acids) followed by methanol (alkaloids, tannins, cardiac glycosides, proteins and amino acids) and petroleum ether extracts (carbohydrates, sterols and terpenoids, flavonoids, proteins and amino acids). Comparatively less number of components were observed in flower extract of ethyl acetate (flavonoids, cardiac glycosides, proteins and amino acids), chloroform (sterols and terpenoids, flavonoids, proteins and amino acids) and aqueous extract (tannins, carbohydrates and flavonoids) (Table 1). The above results of present experiment are in accordance with the findings of Akash *et al*., 2011 [17] who reported that the presence of terpenoids, flavonoids, steroids, glycosides, tannins and saponins in solvent extract of leaves and flowers of *Jasminum arborescence.* It is also similar to the findings of Mittal *et al.*, 2016 [18] in *Jasminum auriculatum.*

Table 1: Qualitative phytochemical analysis in leaves and flowers of *Jasminum multiflorum* (Pink Kakada)

Chemical constituents		Leaf extract						Flower extract					
		Met.	Eth.	EA	Chl.	Pet.	Aqu.	Met.	Eth.	EA	Chl.	Pet.	Aqu.
Alkaloids (Mayer's test)		-	-	+	-	-	-	+	+	-	-	-	-
Tannins (Ferric chloride test)		+	+	-	-	+	+	+	+	-	-	-	+
phlobatannins (HCl test)		-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	1. Shibita's reaction test	-	+	+	+	-	-	-	+	+	-	+	-
	2. Pew's test	-	+	+		+	+	-	+	-	+	+	+
Carbohydrates (Fehling's Test)		-	-	-	+	-	-	-	-	-	-	+	+
Sterols and terpenoids (Salkowski test)		+	-	+	+	-	+	-	-	-	+	+	-
Saponins (Frothing test)		-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides (Keller Kiliani test)		+	+	+	-	-	-	+	+	+	-	-	-
Proteins and amino acids (Biuret test)		-	+	-	+	+	-	+	-	+	+	+	-

(+) Positive for presence and (-) Negative for absence
Met. -Methanol, Eth. -Ethanol, EA -Ethyl acetate, Chl. -Chloroform,
Pet. -Petroleum ether and Aqu. – Aqueous extract

The detection of phytochemical compounds in plants is known to have medicinal importance as detailed below

Secondary metabolites	Biological activity
Alkaloids	Antimalarial (Dua *et al*., 2013) [19], anti-inflammatory (Augusto *et al*., 2011) [20], antimicrobial (Benbott *et al.,* 2012) [21], antispasmodic, cytotoxicity and pharmacological effects (Thite *et al.,* 2013) [22].
Steroids	Cardiotonic effect, antibacterial and insecticidal properties (Alexei *et al*., 2009) [23]
Tannins	Antibacterial (Hisanori *et al.,* 2001) [4], antitumor and antiviral activities (Kumari and Jain, 2012) [25].
Cardiac glycosides	At against congestive heart failure and cardiac arrhythmia (Vladimir and Ludmila, 2001) [26].
Flavonoids	Anti-inflammatory activity (Lee *et al.,* 2003) [27].
Other secondary metabolites also have some biological activities such as antidiabetic (Upaganlawar, 2003) [3], antitumor (Radu and Kqueen, 2002) [4], antimicrobial (Hussaini and Mahasneh, 2009) [5], antioxidant (Latif *et al*., 2010) [6], anti-acne activities (Harisaranraj *et al*., 2010) [7] etc.	

Total phenol and total flavonoid content

The total phenol and flavonoid compounds from plants are classified as secondary metabolites are responsible for many roles in biological system. They occur in three forms: conjugated, soluble and insoluble form. In humans, the phytochemicals reduce the DNA oxidation, protect collagen and prevent platelet accumulation, thus preserving the plasticity of blood vessels and reduce the risk of cancer, neurodegenerative diseases and cardiovascular diseases. In the present study, the total phenol and flavonoid content was found maximum in the ethanol extract of leaves (31.58 ±1.61 mg/g and 13.54 ±0.69 mg/g) and minimum was in petroleum ether (12.48 ±0.54 mg/g and 6.47 ±0.28 mg/g), while the aqueous extracts showed higher phenol and flavonoids content (16.84 ± 0.84 mg/g and 7.21 ± 0.36 mg/g) than that of petroleum ether (Table 2).

In case of flower extract, the total phenol and flavonoid content was found maximum in the ethanol extract (25.98 ±1.32 mg/g and 11.89 ± 0.61 mg/g) and minimum was in petroleum ether (15.37 ± 0.66 mg/g and 5.43 ± 0.23 mg/g), while it was 18.68 ± 0.93 mg/g and 6.04 ± 0.30 mg/g in aqueous extract. The present study clearly elucidate that leaf extract has high total phenol and flavonoid content than that of flower extract. The results were in line with the findings of Dubey *et al.,* 2016 [28] who also reported that the total flavonoid content of leaves (30 mg g^{-1}) was higher than that of flowers (19 mg g^{-1}) of *Jasminum officinale*

Table 2: Total phenol and total flavonoid content of the different extract of *Jasminum multiflorum* (Pink Kakada)

Solvents	Total phenol (mg/g Gallic acid equivalent)		Total flavonoid (mg/g Quercetin equivalent)	
	Leaf	Flower	Leaf	Flower
Methanol	28.32 ± 1.44	23.74 ± 1.21	12.26 ± 0.63	10.32 ± 0.53
Ethanol	31.58 ±1.61	25.98 ± 1.32	13.54 ± 0.69	11.89 ±0.61
Ethyl acetate	25.64 ± 1.27	20.56 ± 1.02	9.36 ± 0.46	8.52 ± 0.42
chloroform	20.42 ±1.45	15.74 ± 1.12	8.85 ±0.63	7.86 ± 0.56
Petroleum ether	12.48 ±0.54	15.37 ± 0.66	6.47 ±0.28	5.43 ± 0.23
Aqueous	18.68 ± 0.93	16.84 ±0.84	7.21 ± 0.36	6.04 ±0.30

Antioxidant activity

DPPH (2, 2 - diphenyl -1- picryl hydroxyl) radicle scavenging activity has been extensively used to determine the antioxidant potential of plant extracts. DPPH potential was calculated based on EC_{50} value and it was presented in Table 3. In the present study, it was revealed that the leaf and flower extracts of *Jasminum multiflorum* possess inhibiting activity but the maximum inhibiting activity was found in ethanolic extract of leaves (141.2 ±1.24 µg/ml) and minimum in aqueous extract (524.6 ±2.35 µg/ml), while in flower extracts, the maximum reduction of free radicles was found in ethanolic extract (252.4 ±2.41 µg/ml) and minimum in aqueous extract (556.6 ±1.51 µg/ml). Compared to flower extracts, the maximum reduction was found in leaf extract. Bhagat *et al.,* 2010[29] in *Jasminum arborescence* reported that the DPPH scavenging activity showed 40 to 80 % and in *Jasminum auriculatum* the range was from 22 to 70 % (Srivastava *et al.,* 2014) [30].

ABTS radicle scavenging activity clearly implied that the samples inhibit or scavenge the radical in a concentration dependent manner Table 3. The leaf extract has the highest ABTS radicles scavenging activity than the flower extract. The antioxidant activity of ethanolic extract of leaves and flowers (149.3 ± 2.41 µg/ml and 101.4 ±2.35 µg/ml) was

higher compared to all other solvent extracts, but lower than standard *i.e.* ascorbic acid.

An important mechanism of antioxidant activity is the ability to chelate or deactivate transition metals. Hence, screening of iron (II) chelating ability of extract is considered very important. The chelating potential of the leaf and flower extracts were expressed in Table 3. The ethanol extract of leaf and flowers showed the highest chelating potential (28.90 ±1.26 µg/ml and 59.64 ± 0.98 µg/ml) when compared to other extracts. Ascorbic acid standard showed the highest chelating potential (7.17± 0.08µg/ml) than the samples. Similar findings has been reported by Sulaiman and Prasad, 2015[31] in *Jasminum auriculatum* and *Jasminum garandiflorum*, Bhagath *et al.*, 2010 [32] in *Jasminum arborescence* and Dubey *et al.*, 2016[28] in *Jasminum officinale.*

Table 3: Antioxidant effect (EC_{50}) on DPPH radicals, ABTS radicals and chelating potential of *Jasminum multiflorum* (Pink Kakada)

Solvents	Scavenging ability on DPPH radicals (EC_{50} µg/ml)		Scavenging ability on ABTS radicals (EC_{50} µg/ml)		Chelating potential (EC_{50} µg/ml)	
	Leaf extract	Flower extract	Leaf extract	Flower extract	Leaf extract	Flower extract
Methanol	177.21 ± 2.81	437.83 ± 1.23	163.2 ± 3.28	178.5 ± 2.15	46.76 ± 2.51	65.77 ± 2.12
Ethanol	141.26 ± 1.24	252.42 ± 2.41	149.3 ± 2.14	101.4 ± 2.35	28.90 ± 1.26	59.64 ± 0.98
Ethyl acetate	310.25 ± 1.52	455.54 ± 2.54	255.21 ± 0.89	205.9 ± 0.97	126.81 ± 2.41	103.52 ± 1.95
Aqueous	524.64 ± 2.35	556.62 ± 1.51	293.50 ± 0.94	243.7 ± 0.76	160.13 ± 2.35	105.24 ± 1.25
Standard (Ascorbic acid)	54.23 ± 2.10		81.26± 0.81		7.17± 0.08	

Conclusion

In the present study the *Jasminum multiflorum* (Pink Kakada) leaf and flower extracts were used to determine various activities. The phytochemical screening showed the presence of alkaloids, tannins, carbohydrates, sterols and terpenoids, flavonoids, cardiac glycosides, proteins and amino acids. Among the different solvents used, number of bio active components, total phenol, total flavonoid content and antioxidant activity were found maximum in case of ethanolic extract of both leaves and flowers. These secondary metabolites (phytochemicals) identified in the leaf and flower extracts are responsible for various biological activities in plants in order to protect themselves from biotic and abiotic stresses. Hence, therefore study in this area may reveal the potential thrust of *Jasminum multiflorum* to be used in the pharmaceutical industry.

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