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Screening of phytochemical constituents in some ornamental flowers of Saurashtra region

Pooja Moteriya, Satasiya Rinkal and Sumitra Chanda

Abstract
Objectives: To determine various phytoconstituents present in some ornamental flowers of Saurashtra region.
Methods: Qualitative phytochemical analysis and fluorescence analysis was done by standard methods.
Results: Different flowers showed various levels of phytoconstituents like alkaloids, flavonoids, cardiac glycosides, triterpenes, phlobatanins, tannins, saponins and steroids in different amounts. Maximum amount of flavonoids and triterpenes were present in different flowers followed by tannins and phlobatanins. Saponins were absent in all.
Conclusions: The results of the present study suggest that even ornamental flowers have phytoconstituents that can act as a source of natural antimicrobics or antioxidants and can be used for the development of plant based drugs.

Keywords: *Alstonia scholaris, Calotropis procera, Cassia auriculata, Catharanthus roseus, Nerium indicum, Peltophoram pterocarpum*), *Rosa spps,* flower extracts, phytochemical analysis, fluorescence analysis.

1. Introduction
Medicinal plants are traditionally used for the treatment of various diseases in India and all over the world since the beginning of civilization. In fact, natural products are a source of synthetic and traditional herbal medicine. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times.
Medicinal plants have been playing a vital role in the health and healing of man since dawn of human civilization. There is a cure for every disease or disorder in nature and traditionally the plants are being used to treat them. The medicinal plants are reported to possess various pharmacological activities like antiinflammatory [1], antiulcer [2, 3], antibacterial [4, 5], antioxidant [6, 7], anticancer [8], antiurolithiatic [9], etc.
The two major health threats which mankind faces today are infectious diseases caused by bacteria and fungi and free radicals induced oxidative stress related diseases and disorders. Medicinal plants are sources of certain bioactive molecules which act as antioxidants and antimicrobial agents [10]. They can protect the human body against both cellular oxidation reactions and infections caused by various pathogens. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of a number of diseases. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential [11]. Flowers have been used since as far back as 50,000 years in funeral rituals. Flowers are mainly used for religious purposes, after which they are thrown away into the environment. In modern times, people have sought ways to cultivate, buy, wear, or otherwise be around flowers and blooming plants, partly because of their agreeable appearance and smell. The other most important use of flowers is their use in medicine and cosmetics. There are many reports of flowers extracts showing antibacterial, antioxidant, anti-cancer, hepatoprotective activities. Some of the flower extracts showing antioxidant and antibacterial activities, hepatoprotective and cytotoxicity are reported in Table 1. The flowers belonged to various families like Magnoliaceae, Leguminosae, Bignoniaceae, Geraniaceae, Punicaceae, Nymphaeaceae, Bromeliaceae, Lythraceae, Moringaceae, Urticaceae, etc.

Table 1: List of some flowers, their family and their various activities.

No.	Botanical Name	Family	Activity	References
1.	*Michelia Champaca* Linn	Magnoliaceae	Antioxidant Antimicrobial	[12]
2.	*Cassia fistula* Linn	Leguminosae	Antioxidant	[13]
3.	*Pyrostegia venusta (Ker Gawl)* Miers.	Bignoniaceae	Antioxidant	[14]
4.	*Geranium sanguineum* L.	Geraniaceae	Antioxidant Antimicrobial	[15]
5.	*Punica granatum var. Isfahan Malas*	Punicaceae	Antioxidant	[16]
6.	*Nymphaea alba*	Nymphaeaceae	Antioxidant	[17]
7.	*Neoglaziovia variegata*	Bromeliaceae	Antioxidant Antibacterial	[18]
8.	*Woodfordia fruticosa* Kurz	Lythraceae	Hepatoprotective; Cytotoxic, anti-inflammatory and analgesic	[19, 20]
9.	*Moringa oleifera* (Lam)	Moringaceae	Antioxidant	[21]
10.	*Urtica dioica* L. *Malva neglecta* Wallr.	Urticaceae	Antioxidant	[22]

In the present work, seven different flowers like *Alstonia scholaris, Calotropis procera, Cassia auriculata, Catharanthus roseus, Nerium indicum, Peltophoram pterocarpum, Rosa spps.* Were selected to determine their phytochemical constituents.

1.1 Plant description

The description of the flowers and the therapeutic uses are given below.

1.1.1 *Alstonia scholaris* R.Br.

Family: Apocynaceae
Habit: 12-18m tree with bitter milky juice, glabrous.
Distribution: Wildly cultivated thought in India. Also cultivated in Sri Lanka, Java, and tropical Africa.
Vernacular name: Saptaparni
Constituents: Bark, alkaloid, essential oil.
Action/Uses: The bark is bitter, astringent, acrid, thermogenic, digestive, laxative, depurative, stomachic, cardio tonic and tonic. The flower and fruits are used in fever, malaria, leprosy, skin diseases, tumours, chronic and foul ulcers, asthma, and bronchitis.

Reported activities
Anti-inflammatory and analgesic activities from leaf [23]
Antibacterial activity from bark [24]

1.1.2 *Calotropis procera* (Ait.) R.Br.

Family: Asclepiadaceae
Habit: 1-2m erect shrub having young parts clothed with white cottony.
Distribution: North, western and central India from sind of Punjab, upper Bengal.
Vernacular name: Akado
Constituents: Latex, juice contains active substance.
Action/Uses: Roots of wide variety is used in poisonous snake bite. The milky juice is used as blistering agent. Flowers are used in cholera and asthma.

Reported activities
Anti-oxidant activities of flowers [25]
Anti-mitotic activities of latex [26]

1.1.3 *Cassia auriculata* L.

Family: Ceasalpiniaceae
Habit: 1-2 m, erect bushy shrub, Bark; smooth, reddish brown.
Distribution: Sri Lanka, Indo –china, Malaya, Australia, Grain wild in South India and Sri Lanka.
Vernacular name: Awal
Constituents: Stem bark, Tannin, Ash.
Action/Uses: Used in fever, urinary diseases. The shrub is especially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. Its bark is used as an astringent, leaves and fruits anthelmintic, seeds used to treat in eye troubles and root employed in skin diseases, these are also used for the treatment of ulcers, leprosy and liver disease.

Reported activities
Antihyperlipidemic activity from flowers [27]
Antimicrobial activity from flowers [28]

1.1.4 *Catharanthus roseus* G. Don.

Family: Apocynaceae
Habit: 20-40 cm, perennial under shrubs
Distribution: Commonly found throughout India
Vernacular name: Baramasi
Constituents: Roots; ajmalcime, serpentine and reserpine. Flower: vincristine, vinblastine Action/Uses: Leaves are used in diabetes. The plant is recognized to control major diseases such as leukemia and diabetes.

Reported activities
Anti-cancer activities from flowers [29]
Antibacterial activities from different parts [30]

1.1.5 *Nerium indicum* L.

Family: Apocynaceae
Habit: 4-8 feet, perennial under shrubs.
Distribution: Commonly found throughout India
Vernacular name: Karen
Constituents: Cardiac aglycones and glycosides.
Action/Uses: *Nerium indicum* has been used in the treatment of asthma, Cardiac illness diabetes mellitus. Leaves are used in making medicine. The flowers and leaves of *Nerium indicum* (Arali) have been used to stimulate cardiac muscles, relieve pain and eliminate blood stasis. It is reported to have antibacterial and antidiabetic activities.

Reported Activities
Antitumor activity of oil extracted from flowers [31]
Antioxidant, antibacterial and antitumor from leaf and flowers [32]

1.1.6 *Peltophorum pterocarpum* (DC.)

Family: Fabaceae
Habit: Small to medium-size tree, 5-10 m tall
Distribution: Commonly found throughout India and Sri Lanka.

Vernacular name: Peltophorum
Constituents: phenolic and analgesic activity
Action/Uses: The tree is widely grown in tropical regions as ornamental flowers. Young leaves and pods are eaten by livestock. The timber can be used for furniture. In traditional medicine, *Peltophorum pterocarpum* flowers are used as an astringent to cure or relieve intestinal disorders after pain at childbirth, sprains, bruises and swelling or as a lotion for eye troubles, muscular pains and sores.

Reported activities
Antioxidant and antiglycemic activity from different parts [33]
Antimicrobial, antioxidant, and cytotoxic activities from flowers [34]

1.1.7 *Rosa spp.*

Family: Rosaceae
Habit: Deciduous shrub growing up to 2.2 m.
Distribution: Commonly found throughout India.
Vernacular name: Gulab.
Constituents: Flavonoids and tannins
Action/Uses: Its flowers are reported to have astringent, analgesic, anti-inflammatory, antidepressant, antibacterial, diuretic and anti-HIV activity. Its flowers are commonly used in traditional Chinese medicine.

Reported activities:
Antioxidant activity from flowers [35]
Antioxidant activity from flowers [36]

2. Materials and Methods

2.1 Plant Collection

Seven different flowers like *Alstonia scholaris* R. Br., *Calotropis procera* (Ait.) R. Br., *Cassia auriculata* L., *Catharanthus roseus* G.Don., *Nerium indicum* L., *Peltophoram pterocarpum* (DC.), *Rosa spps.* Were collected in the month of September 2013 from the campus of Saurastra University, Rajkot, Gujarat, India. Then the flowers were washed thoroughly with tap water, shade dried and crushed to fine powder and stored in air tight bottles.

2.2 Phytochemical analysis

2.2.1 Qualitative phytochemical analysis

The crude powder of different flowers was subjected to qualitative phytochemical analysis [37].

2.2.1.1 Flavonoids

Alkaline reagent test was performed for checking the presence of flavonoids. The crude powder of flower was treated with a few drops of diluted sodium hydroxide (NaOH) separately. Formation of intense yellow colour which turned colourless on addition of a few drops of diluted HCl indicated the presence of flavonoids.

2.2.1.2 Tannins

The crude powder of flower was treated with alcoholic ferric chloride ($FeCl_3$) reagent. Blue colour indicated the presence of tannins.

2.2.1.3 Phlobatanins

The crude powder of flower was boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence of the presence of phlobatanins.

2.2.1.4 Saponins

The presence of saponins was determined by Frothing test. The crude powder of flower was vigorously shaken with distilled water and was allowed to stand for 10 minutes and classified for saponin content as follows: no froth indicates absence of saponins and stable froth of more than 1.5 cm indicated the presence of saponins.

2.2.1.5 Steroids

Liebermann-Burchard reaction was performed for checking the presence of steroids. A chloroformic solution of the crude powder of flower was treated with acetic anhydride and a few drops of concentrated H_2SO_4 were added down the sides of the test tube. A blue green ring indicated the presence of steroids.

2.2.1.6 Cardiac glycosides

Keller-kiliani test was performed for checking the presence of cardiac glycosides. The crude powder of flower was treated with 1.0 ml mixture of 5% $FeCl_3$ and glacial acetic acid (1:99 v v^{-1}). To this solution, a few drops of concentrated H_2SO_4 were added. Appearance of greenish blue colour within few minutes indicated the presence of cardiac glycosides.

2.2.1.7 Triterpenes

Chloroform extract of the crude powder of flower was treated with concentrated sulphuric acid (H_2SO_4). Appearance of reddish brown ring indicated the presence of triterpenes.

2.2.1.8 Alkaloids

The crude powder of flower was dissolved in 2 N HCl. The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with a few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent and the other portion was treated with equal amount of Wagner's reagent. The creamish precipitate, orange precipitate and brown precipitate indicate the presence of respective alkaloids. A (+) score was recorded if the reagent produced only a slight opaqueness; A (++) score was recorded if a definite turbidity, but no flocculation was observed and A (+++) score was recorded if a heavy precipitate of flocculation was observed.

2.2.2. Fluorescence analysis

Fluorescence study of flower powder was performed as per Kokashi *et al.*, [38]. A small quantity of the flower powder was placed on a grease free clean microscopic slid and 1-2 drops of

freshly prepared reagent solution were added, mixed by gentle tilting of 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 long 365 nm) ultra violet radiations. The colours observed by application of different reagents in different radiations were recorded.

3. Results and Discussion

Plant products have been part of phytomedicine since time immemorial. These can be derived from any part of the plant i.e. any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers [39-41]. In the present work, the dried flower powders of seven plants were evaluated for the presence of flavonoids, tannins, phlobatannins, saponins, steroids, cardiac glycosides, triterpenes and alkaloids.

The preliminary qualitative phytochemical investigation of *Alstonia scholaris* flower was performed which showed the presence of Flavonoids, Tannins, Steroids, Cardiac glycosides, Triterpenes, Alkaloids (Table 2). The flower was devoid of Phlobatannins and Saponins.

The preliminary qualitative phytochemical investigation of *Calotropis procera* flower was performed which showed the presence of Flavonoids, Phlobatannis, Triterpenes, Alkaloids by Mayer's reagent (Table 2). The flower was devoid of Saponins, Tannins, Steroids, Cardiac glycosides and alkaloids by Dragondroff's and Wagner's reagent.

The preliminary qualitative phytochemical investigation of Cassia *auriculata* flower was performed which showed the presence of Flavonoids, Tannins, Triterpenes, Alkaloids by Dragondroff's and Wagner's reagent (Table 2). The flower was devoid of Saponins, Phlobatannis, Steroids, Cardiac glycosides and Alkaloids by Mayer's reagent.

The preliminary qualitative phytochemical investigation of *Catharanthus roseus* flower was performed which showed the presence of Flavonoids, Tannins, Triterpenes, Phlobatannis, Steroids, Alkaloids by Dragondroff's reagent, Wagner's reagent and Mayer's reagent (Table 2). The flower was devoid of Saponins and Cardiac glycosides.

The preliminary qualitative phytochemical investigation of *Nerium indicum* flower was performed which showed the presence of Flavonoids, Tannins, Triterpenes, Phlobatannis (Table 3). The flower was devoid of Saponins, Cardiac glycosides, Steroids and Alkaloids.

The preliminary qualitative phytochemical investigation of *Peltophoram pterocarpum* flower was performed which showed the presence of Flavonoids, Tannins, Triterpenes, Alkaloids by Dragondroff's reagent (Table 3). The flower was devoid of Saponins, Cardiac glycosides, Phlobatannis, Steroids and Alkaloids by Wagner's reagent and Mayer's reagent.

The preliminary qualitative phytochemical investigation of *Catharanthus roseus* flower was performed which showed the presence of Flavonoids, Tannins, Triterpenes and Phlobatannis (Table 3). The flower was devoid of Steroids, Saponins, Cardiac glycosides and Alkaloids.

From the results of preliminary phytochemical analysis, it can be concluded that different flowers possessed different phytoconstituents in different amounts. Flavonoids and triterpenes were 100% present followed by tannins (86%) and phlobatannins (57%). Alkaloids with different reagents showed about 50%, while other phytoconstituents were present in very less amount like steroids (29%) and cardiac glycosides (14%). Saponins were not present in any of the flower sample.

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. They are widely distributed as secondary metabolites in the plant kingdom [42]. Flavonoids in biological systems are ascribed to their antioxidant abilities, capacity to transfer electrons, quenching of free radicals and chelating abilities, activate antioxidant enzymes, reduce alpha tocopherol radicals and inhibit oxidases [43]. Plants rich in phytoconstituents like alkaloids, flavonoids, tannins, terpenoids and steroids have antibacterial properties [44]. The phytochemicals are also well known for many medicinal and physiological activity [45], anti-inflammatory effects [46]. Plants rich in flavonoids and tannins are reported for their antibacterial activities [47]. This is accomplished by inactivating bacterial enzymes. In addition, secondary metabolites such as tannins and other compounds of phenolic nature are also classified as active antimicrobial compounds [48]. Alkaloid enriched extract from *Prosopis juliflora* pods showed good antibacterial activity [49]. Thus, such studies in the initial stage of research will give an idea of what activity the plant might possess. There are many reports of such phytochemical screening and pharmacological activity [50, 51].

Table 2: Qualitative phytochemical analysis of flower powder

No.	Test	*Alstonia scholaris*	*Calotropis procera*	Cassia *auriculata*	*Catharanthus roseus*
1	Flavonoids	+++	+	++++	++++
2	Tannins	++	-	+++	++
3	Phlobatannis	-	++	-	++
4	Saponins	-	-	-	-
5	Steroids	+	-	-	++
6	Cardiac glycosides	++	-	-	-
7	Triterpenes	++	++	++	+++
8	Alkaloids				
	(1)Mayer's reagent	++	++	-	++
	(2)Dragondroff's reagent	+++	-	++	+
	(3)Wagner's reagent	++	-	++	+++

Table 3: Qualitative phytochemical analysis of flower powder

No.	Test	*Nerium indicum*	*Peltophoram pterocarpum*	*Rosa spps.*
1	Flavonoids	+++	++	++
2	Tannins	+++	++	+++
3	Phlobatannis	++	-	+++
4	Saponins	-	-	-
5	Steroid	-	-	-
6	Cardiac glycosides	-	-	-
7	Triterpenes	+++	++	+++
8	Alkaloids			
	(1)Mayer's reagent	-	-	-
	(2)Dragondroff's reagent	-	++	-
	(3)Wagner's reagent	-	-	-

3.1 Fluorescence analysis

The fluorescence character of powdered drug plays a vital role in the determination of quality and purity of the drug material. In the present study dried powder treated with various reagents showed characteristic fluorescence at 254 nm and 366 nm wavelength (Tables 4 to 10). Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs [52]. Similar fluorescence analysis is reported for other plants [53, 54].

Table 4: Fluorescence analysis of *Alstonia scholaris* flower powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365 nm)
1 N NaOH (aq)	Green	Blank	Light green
1 N NaOH (alc)	Green	Blank	Light green
Ammonia	Light brown	Black	Light green
Picric acid	Light green	Black	Black
Petroleum ether	Green	Black	Light green
50% HCl	Light brown	Black	Green
50% H_2SO_4	Light brown	Black	Green
Ethyl acetate	Green	Black	Yellowish green
Ethyl alcohol	Green	Black	Light green
Methanol	Green	Black	Light green

Table 5: Fluorescence analysis of *Calotropis procera* flower powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365 nm)
1 N NaOH (aq)	Green	Black	Light green
1 N NaOH (alc)	Yellow	Black	Light green
Ammonia	Brown	Black	Green
Picric acid	Yellow	Black	Black
Petroleum ether	Brown	Black	Light green
50% HCl	Yellow	Black	Green
50% H_2SO_4	Brown	Black	Dark green
Ethyl acetate	Yellow	Black	Light green
Ethyl alcohol	Yellow	Black	Light green
Methanol	Yellow	Black	Light green

Table 6: Fluorescence analysis of *Cassia auriculata* flower powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365 nm)
1 N NaOH (aq)	Brown	Black	Llght green
1 N NaOH (alc)	Green	Black	Light green
Ammonia	Brown	Green	Light green
Picric acid	Yellow	Black	Black
Petroleum ether	Brown	Black	Light green
50% HCl	Brown	Green	Light green
50% H_2SO_4	Brown	Green	Light green
Ethyl acetate	Brown	Black	Light green
Ethyl alcohol	Brown	Black	Light green
Methanol	Brown	Black	Light green

Table 7: Fluorescence analysis of *Catharanthus roseus* flower powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365 nm)
1 N NaOH (aq)	Dark green	Black	Light green
1 N NaOH (alc)	Light brown	Black	Light green
Ammonia	Yellow	Black	Light green
Picric acid	Yellow	Black	Dark blue
Petroleum ether	Light brown	Black	Yellow
50% HCl	Light brown	Black	Light green
50% H_2SO_4	Light brown	Black	Light green
Ethyl acetate	Light brown	Black	Light green
Ethyl alcohol	Light brown	Black	Light green
Methanol	Light brown	Black	Light green

Table 8: Fluorescence analysis of *Nerium indicum* flower powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365 nm)
1 N NaOH (aq)	Dark green	Black	Black
1 N NaOH (alc)	Brown	Black	Light green
Ammonia	Green	Black	Green
Picric acid	Pinkish brown	Black	Black
Petroleum ether	Pinkish brown	Black	Green
50% HCl	Brownish pink	Black	Brown
50% H_2SO_4	Brownish pink	Black	Brown
Ethyl acetate	Brown	Black	Green
Ethyl alcohol	Pinkish brown	Black	Green
Methanol	Pinkish brown	Black	Light green

Table 9: Fluorescence analysis of *Peltophorum pterocarpum* flower powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365 nm)
1 N NaOH (aq)	Light brown	Black	Green
1 N NaOH (alc)	Yellow	Black	Light brown
Ammonia	Brown	Black	Green
Picric acid	Yellow	Black	Green
Petroleum ether	Brown	Black	Light green
50% HCl	Yellow	Black	Green
50% H_2SO_4	Dark brown	Black	Green
Ethyl acetate	Brown	Black	Brown
Ethyl alcohol	Brown	Black	Light brown
Methanol	Brown	Black	Light brown

Table 10: Fluorescence analysis of *Rosa spps.* Flower powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long wavelength (365 nm)
1 N NaOH (aq)	Dark brown	Black	Black
1 N NaOH (alc)	Pinkish brown	Black	Green
Ammonia	Dark green	Black	Black
Picric acid	Brown	Black	Black
Petroleum ether	Pinkish brown	Black	Light brown
50% HCl	Pinkish brown	Black	Black
50% H_2SO_4	Brown	Black	Black
Ethyl acetate	Brown	Black	Black
Ethyl alcohol	Pinkish brown	Black	Black
Methanol	Brown	Black	Black

4. Conclusions

The ornamental flowers of *Alstonia scholaris, Calotropis procera, Cassia auriculata, Catharanthus roseus, Nerium indicum, Peltophoram pterocarpum, Rosa spps* were rich in phytoconstituents like flavonoids, triterpenes an tannins. Hence, they can be utilized for preparation of drugs which can act as antimicrobics and antioxidants. They are commonly grown plants with lot of flowers almost all the year around, so availability and affordability is easy with no impingement on the environment. However, more stringent studies are required to identify their role and exact mechanism to be medicinally useful. The results do suggest that even flowers have promising compounds to act as antimicrobics and antioxidants and cannot be overlooked in potential drug development.

5. Conflict of Interest
We declare that we have no conflict of interest

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