Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 2983-2988
Received: 13-01-2018
Accepted: 14-02-2018

Chudasama RG
Department of Biotechnology, Shree. M. & N. Virani Science College, Kalawad Road, Rajkot, Gujarat, India

NJ Dhanani
Department of Biotechnology, Shree. M. & N. Virani Science College, Kalawad Road, Rajkot, Gujarat, India

RM Amrutiya
Department of Biotechnology, Shree. M. & N. Virani Science College, Kalawad Road, Rajkot, Gujarat, India

R Chandni
Department of Biotechnology, Shree. M. & N. Virani Science College, Kalawad Road, Rajkot, Gujarat, India

G Jayanthi
Coastal and Marine Ecology Division, Gujarat Institute of Desert Ecology, Bhuj - Kachchh, Gujarat, India

K Karthikeyan
Environmental Laboratory Division, Gujarat Institute of Desert Ecology, Bhuj - Kachchh, Gujarat, India

Correspondence
Chudasama RG
Department of Biotechnology, Shree. M. & N. Virani Science College, Kalawad Road, Rajkot, Gujarat, India

Screening of selected plants from semi-arid region for its phytochemical constituents and antimicrobial activity

Chudasama RG, NJ Dhanani, RM Amrutiya, R Chandni, G Jayanthi and K Karthikeyan

Abstract
The main aim of the study was to investigate phytochemical screening of the various plant parts (leaves, fruits, flowers) of native plant species *viz*., *Nerium oleander, Lawsonia inermis, Pithecellobium dulce, Euphorbia tithymaloides, Punica granatum, Caesalpinia pulcherrima, Plumeria obtusa, Carica papaya, Cassia fistula, Cordia dichotoma, Euphorbia prostrate and Cyanthillium cinereum* collected from the Garden of GUIDE institute, Kachchh, Gujarat. Qualitative phytochemical screening of plants showed the presence of alkaloids, steroids, cardiac glycosides, flavonoid, reducing sugars, saponins, terpenoids, tannins, coumarin, phenols, emodin, anthocyanin, quinones, anthraquinones, protein and amino acid, phlobatanin and volatile oil which exhibit positive result with negligible variations in selected plants. The Antimicrobial activity on aqueous plant extract of *P. obtusa* indicates that the showed significant inhibitory zone in antimicrobial activity against human pathogens *viz*., *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis* and *Staphylococcus aureus* by agar diffusion method. These verdict, however, support the traditional use of *P. obtusa* for the treatment of the various ailments.

Keywords: medicinal plant, phytochemical screening, human pathogens, antimicrobial activity

Introduction
Countries like India have been using crude plants as medicine ever since Vedic period. The foremost part of the total population in developing countries still uses traditional folk medicine attained from plant resources. In recent years this interest to evaluate plants possessing antimicrobial activity for various diseases is growing. Plants containing antimicrobial activity are used medicinally in different countries as a resource of many potent and powerful drugs [1]. Some medicinally important plant contains bioactive organic compounds like alkaloids, steroid, tannins, cardiac glycosides, reducing sugars, phlobatanin, coumarin, terpenoids and flavonoid which produced particular physiological action on human health [2]. Most of the plants are alleged to be important source of new bioactive compounds with potential therapeutic purpose [1]. The secondary metabolites of many plants could be directly used for the production of new potent drugs. In the recent primary healthcare system of the developing countries like India, traditional medicine would be able to play essential role. Plants have vast significance on the human health and many of these plants are used as a food or food ingredients [2]. The plant *Plumeria obtusa* L. belong to Apocynaceae commonly known as Gulechin, Graveyard Tree or Frangipanier [3, 4] is a small, much-branched, evergreen or partly deciduous tree [5]. It is commonly found in West Indies including Bahamas; southern Mexico, Belize, Guatemala, Florida and in some region of India. The plant has been used in the administration of diabetes mellitus, asthma, gonorrhea and constipation and also as a contraceptive, expectorant and anthelmintic [6, 7, 8]. As compared to modern synthetic drugs, the natural medicines obtained from plants are more satisfactory for human health. So, the most vital factor required to study the maximum benefit from the traditional system of medicine for providing sufficient healthcare service to rural people or undeveloped area.

Materials and Methods
Collection of plant materials
Twelve different plants parts (leaves, fruits, flowers) were collected from various plants *viz*., *Nerium oleander, Lawsonia inermis, Pithecellobium dulce, Euphorbia tithymaloides, Punica granatum, Caesalpinia pulcherrima, Plumeria obtusa, Carica papaya, Cassia fistula, Cordia dichotoma, Euphorbia prostrata* and *Cyanthillium cinereum* in the garden of GUIDE campus, Bhuj (Gujarat). The plants were identified in the digital herbarium of GUIDE institute.

Preparation of plant extract [9]

The collected samples were collected in large quantity and washed with normal tap water to remove dust particles and other impurities. After drying, they were air dried under room temperature to retain its nutrients/constituents and was powdered in an electric grinder to increase surface area. About 20 grams of plant materials (leaves, flowers and fruit) was mixed with 150 ml of distilled water to prepare aqueous crude extract. The final extract preparations were done using rotavapor for further study.

Extraction of Bio-active Constituents

Cold aqueous maceration method was used to obtain the plant extracts. This was achieved by blending the air-dried powdered plant sample into 500cm^3 of distilled water. The blended suspension was then filtered and the extracts were evaporated to dryness on a water bath, cooled and transferred into the containers labelled and kept for phytochemical and antimicrobial test.

Screening of phytochemical components [10, 11]

1. Detection of Alkaloids

Wagner's test: Take 2 ml of filtrate with 1% HCl and heat gently. Then add 3 drops of Wagner's reagent (iodine in potassium iodide). Formation of red/brown precipitate indicates the presence of alkaloids.

2. Detection of steroids

Take 2 ml of plant extract, add 10 ml chloroform and add equal volume (10 ml) of Concentrated H_2SO_4. Appearance of upper red acid layer with green fluorescence indicates the presence of steroids.

3. Detection of cardiac glycosides

Keller killani test: Add 2ml of filtrate in 2.5ml glacial acetic acid and add 1ml of ferric chloride ($FeCl_3$). Then add 1ml Conc. H_2SO_4. Appearance of upper red acid layer with green fluorescence indicates the presence of cardiac glycosides.

4. Detection of flavonoid

NaOH test: Take 2ml extract and add 5 drops of diluted NaOH, followed by few drops (5 drops) of diluted HCl. Formation of a yellow solution with NaOH, turn colourless with diluted HCl and indicate the presence of flavonoids.

5. Detection of reducing sugar

Fehling test: Add 5ml of diluted H_2SO_4, 1ml extract in a test tube and boil for 15 min., cool it and neutralize with 10% NaOH to pH 7 and add 5ml fehling solution. Formation of brick red precipitates indicates the presence of reducing sugar.

6. Detection of saponins

Frothing/foam test: Take 0.5ml of filtrate, 5ml of distilled water and shake well. Formation of persistence of frothing /foam indicates the presence of saponins.

7. Detection of terpenoids

Salkowski test: Add 2.5ml of extract, 1ml chloroform ($CHCl_3$) & 1.5ml of concentrated H_2SO_4 in test tube. Formation of yellow colour / reddish brown colour indicates the presence of terpenoids.

8. Detection of tannins

Braemer's test: 2ml of 10% alcoholic $FeCl_3$ will be added to 1ml of extract. Formation of dark- blue or gray colouration of solution indicates the presence of tannins.

9. Detection of coumarin

Add 3ml of 10% NaOH in 2ml of extract. Formation of yellow colour indicates the presence of Coumarin.

10. Detection of phenol

$FeCl_3$ test: Take 2ml extract and add 4 drops of $FeCl_3$. Formation of bluish black colour indicates the presence of phenol.

11. Detection of Emodin

Take 2ml extract, 2ml of NH_4OH and 3ml of benzene solution in test tube. Formation of red colour indicates the presence of Emodin.

12. Detection of anthocyanin

In 2ml extract add 2ml of 2N HCl and Ammonia. Formation of pink-red turn blue-violet indicates the presence of anthocyanin.

13. Detection of quinones

Add 2ml of diluted NaOH in 1ml of extract. Formation of blue green or red colour indicates the presence of quinine.

14. Detection of phlobatanin

In this test, add 2ml of extract and then boiled with 2ml of 1% HCl. Formation of red precipitates indicates the presence of phlobatanin.

15. Detection of volatile oil

In this test, add 2ml of extract, 0.1ml of diluted NaOH and small quantity of diluted HCl. Then shake the solution. Formation of white precipitate indicates the presence of volatile oil.

16. Detection of protein and amino acid

Xanthoprotein test: Take 2ml extract and add 2 drops of HNO_3. Formation of yellow colour indicates the presence of protein and amino acids.

17. Detection of anthraquinones (Borntrager's test): Add 1ml of diluted (10%) ammonia and 2ml of extract. Formation of pink red colour in the ammonical lower layer indicates the presence of anthraquinones.

Test organisms

Various human pathogens viz., *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* maintained on nutrient agar slants at refrigerated condition. The collected microorganisms was pre-cultured and enriched in LB (Luria bertani) broth for activation of pathogens.

Antimicrobial activity

The crude extract of leaf and flower of *P. obtusa* was tested by agar well diffusion method [12]. The overnight grown LB broth culture of the test organisms was seeded over the MH (Muller Hinton) agar plates using sterile cotton swab for lawn growth of microbes. The wells were made over the MH agar plates using sterile cork borer. Different volume of crude extract (50µl, 100µl, 150µl, 200µl) used to check antimicrobial activity. Control plates also prepared for each pathogen. The plates were incubated at 37°C for 16-18 hrs. The zone of the inhibition surrounding each well after the incubation period, confirms the antimicrobial activity of the respective crude extract. The diameters of the inhibition zones were measured in millimetres (mm) to determine the antimicrobial activity of crude extract.

Results and Discussion

Phytochemical studies on all the plant samples revealed the presence of various medicinally active components. The phytochemical constituents of twelve plants investigated are summarized in Table-1. Among the twelve plants, *Plumeria obtusa* possess more phytochemical constituents compared to other plants *viz*., presence of alkaloids, steroids, cardiac glycosides, saponins, terpenoids, tannin, coumarin, phenol, emodin, anthocyanin, quinones, phlobatannin and volatile oil. Phytochemical screening of various extracts of *P. obtusa* leaves yielded sterols, alkaloids, favonoids, terpenoids, glycosides [13]. Alkaloids have biological activities such as anti-oxidant, anti-carcinogenic and anti-artherosclerosis activities. Phenolic compounds contribute to anti-microbial and antioxidant activities [14]. Glycosides can reduce the cholesterol levels in the body [15]. These findings have proved that the local variety of *P. obtusa* can be used for treating different ailments and advanced studies would expand the potential of applying these phytochemicals in production of efficient and safe drugs.

In recent times, there has been considerable interest in the use of plant parts as an alternative method to control human pathogens and mechanism of plant products has been shown to be specially targeted against resistant pathogens. The problem of multidrug resistant strain is a severe warning. The cost of chemotherapeutic drug is costly for the developing countries like India. This work showed that the percentage yield of phytochemical component in leaf and flower extract of *Plumeria obtusa* was 59%. The leaves of *N. oleander* possess flavonoids, alkaloids, cardiac glycosides and tannins [16]. High quantity of polyphenols is present in the leaves of *N. oleander* [17]. Similarly in present study, *Nerium oleander* consists of alkaloids, saponins, tannin, coumarin and phenol are present in leaf and flower. Steroids are present only in fruit. Cardiac glycosides and emodin are present in leaf, fruit and flower. Terpenoid are present in fruit and flower. Quinones are present in leaf and fruit.

L. inermis are also reported to contain flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, coumarins and also it possesses anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antioxidant and anticancer properties [18]. In present study, the leaf of *L. inermis* comprises of alkaloids, steroids, cardiac glycosides, flavonoids, saponins, terpenoids, tannins, coumarin, phenol and emodin. The presence of alkaloids, terpenoids, flavonoids, glycosides, phenol, tannins and saponins was determined in the leaf of *P. dulce*. The presence of these components in these plants supports the traditional and folkloric usage in treating chronic diarrhoea and inflammations and also it contributes to the number of biological activities [19]. In present study, *P. dulce* was observed to contain alkaloids, steroids, cardiac glycosides, flavonoids, saponins, terpenoids, tannins, coumarin, phenol, emodin in the leaf.

In *E. tithymaloides*, the phytoconstituent such glycosides are present and other constituents such as alkaloids, phenols and steroids were to be absent [20]. Similarly in present study, *E. tithymaloides* contains cardiac glycosides, flavonoids, saponins are present in stem. While alkaloids, steroids, terpenoids, tannins, coumarin, phenol, emodin, reducing sugar, emodin, anthocyanin, quinones, phlobatanin, volatile oil and anthraquinones are absent. The bioactive compounds like alkaloids, saponin, coumarins, proteins, steroids and tannins are present in *P. granatum* [21]. In the present study, *P. granatum* found to contain alkaloids, cardiac glycosides, tannins, phenol, emodin and quinines in leaf, fruit and flower. Saponin and terpenoid are present in leaf and fruit. Steroid, protein and aminoacid are present in flower. Flavonoids, reducing sugar, coumarin, anthocyanin, phlobatanin, volatile oil and anthraquinones are found to be absent. Phenols, tannins, coumarins, quinines are found in *C. pulcherrima*. Similarly in the present study, *C. pulcherrima* contains alkaloids, cardiac glycosides, tannins, phenol, quinines, saponin, terpenoid, steroid, protein and aminoacid, coumarin, phlobatanin. Whereas, flavanoids, reducing sugar, steroid, emodin, anthocyanin, volatile oil and anthraquinones are found to be absent. Preliminary phytochemical screening of *P.obtusa* revealed the presence of sterols, alkaloids, terpenoids and glycosides [22]. Similarly in the present study, the *P. obtusa* contains alkaloids, steroids, cardiac glycosides, saponins, terpenoids, tannins, coumarin, phenol, emodin, anthocyanin, quinines are present both in leaf and flower and was absent for flavonoids and reducing sugar. Phlobatanin and volatile oil are present only in leaf whereas anthraquinones, protein and aminoacid are present only in flower.

The Phytochemical analysis of the leaves of *C. Papaya* showed that the leaves contained saponins, cardiac glycosides, and alkaloids [23]. In present study, *C. Papaya* found to contain Cardiac glycosides, saponins and terpenoids are present both in leaf and fruit. Reducing sugar, steroids, emodin, anthocyanin, phlobatanin, volatile oil and anthraquinones are found to be absent. Alkaloid, tannin, coumarin, phenol, protein and aminoacid are present in leaf and not in fruit. Flavonoids and quinones are present in fruit and not in leaf of *C. Papaya.* Phytochemical testing with the aqueous extract of *C. fistula* confirmed for the presence of higher concentration of reducing sugars, tannins, flavonoids, terpenoids and saponins [24]. In present study, *C. fistula* contains alkaloid, steroids, cardiac glycosides and terpenoids are present and it was found to be absent for reducing sugar, flavanoids, anthocyanin, phlobatanin and anthraquinones in leaf, fruit and flower. Saponins, coumarin, protein and aminoacid are present both in leaf and flower. Tannins, phenol, quinones, are present in leaf and fruit. Saponin was present in leaf and fruit. Whereas, Volatile oil is present in fruit and flower. The phytochemical screening of *C. dichotoma* revealed the presence alkaloids, carbohydrates, phenolic compounds, tannins, flavonoids, steroids and saponins [25]. In present study, *C. dichotoma* contains alkaloid and steroids in fruit. Tannins, emodin, volatile oil is present in flower. Cardiac glycosides, terpenoids, coumarin and phenol are present both in leaf and fruit. Preliminary phytochemical studies of *E. prostrata* revealed the presence of flavonoids, tannins, glycosides and saponins [26]. Similarly in the present study, *E. prostrata* contains alkaloid, saponins, cardiac glycosides, terpenoids, coumarin, tannin, phenol, quinines, protein and aminoacid in leaf. Alkaloids, phenols, saponins and phlobtannins are the compounds present in leaf extract of *C. cinereum*. In present study, *C. cinereum* found to possess saponins, cardiac glycosides, coumarin, quinones, volatile oil, protein and aminoacid in leaf.

Table 1: Qualitative analysis of phytochemical constituents of different plant samples

Sr No.	Name of the plant species	Plant parts	Phytochemical constituents screened																% positive results
			Alkaloids	Steroids	Cardiac glycosides	Flavonoid	Reducing sugar	Saponins	Terpenoids	Tannins	coumarin	phenol	Emodin	anthocyanin	quinones	phlobatanin	volatile oil	protein and amino acid	anthraquinones	
1	*Nerium Oleander*	Leaf	+	_	+	_	_	+	_	+	+	+	+	_	+	_	_	_	_	47
		Fruit	−	+	+	_	_	_	+	_	_	_	+	_	+	_	_	_	_	53
		Flower	+	+	+	_	_	+	+	+	+	+	+	_	_	_	_	_	_	29
2	*Lawsonia inermis*	Leaf	+	+	+	+	_	+	+	+	+	+	+	_	_	_	_	_	–	59
3	*Pithecellobium dulce*	Leaf	+	+	+	+	–	+	+	+	+	+	–	–	–	–	–	+	–	59
4	*Euphorbia Tithymaloides*	Stem	_	_	+	+	_	+	_	_	_	_	_	_	_	_	_	_	_	18
5	*Punica granatum*	Leaf	+	_	+	_	_	+	+	+	_	+	+	_	+	_	_	_	_	41
		Fruit	+	_	+	_	_	+	+	+	_	+	+	_	+	_	_	_	_	47
		Flower	+	+	+	_	_	_	_	+	_	+	+	_	+	_	_	+	_	47
6	*Caesalpinia Pulcherrima*	Leaf	+	_	+	_	_	+	+	+	+	+	_	_	+	+	_	_	_	53
7	*Plumeria obtusa*	Leaf	+	+	+	_	_	+	+	+	+	+	+	+	+	+	+	_	_	76
		Flower	+	+	+	_	_	+	+	+	+	+	+	+	+	-	_	+	+	59
8	*Carica Papaya*	Leaf	+	_	+	_	_	+	+	+	+	+	_	_	_	_	_	+	_	47
		Fruit	_	_	+	+	_	+	+	_	_	_	_	_	+	_	_	_	_	29
9	*Cassia fistula*	Leaf	+	+	+	_	_	+	+	+	+	+	_	_	+	_	_	+	_	52
		Fruit	+	+	+	_	_	_	+	+	_	+	+	_	+	_	+	_	_	59
		Flower	+	+	+	_	_	+	+	_	+	_	_	_	_	_	+	+	_	47
10	*Cordia dichotoma*	Leaf	_	_	+	_	_	_	+	+	+	+	+	_	_	_	+	_	_	35
		Fruit	+	+	+	_	_	+	+	_	+	+	_	_	_	_	_	_	_	41
11	*Euphorbia prostrate*	Leaf	+	_	+	_	_	+	+	+	+	+	_	_	+	_	_	+	_	53
12	*Cyanthillium Cinereum*	Leaf	_	_	+	_	_	+	_	_	+	_	_	_	+	_	+	+	_	35

Table 2: Antibacterial Activity of *Plumeria obtusa* on various human pathogens.

Volume of crude extract (µl)	Diameter of Zone of Inhibition (mm)							
	E. coli		***K. pneumoniae***		***S. aureus***		***B. subtilis***	
	Flower	**Leaf**	**Flower**	**Leaf**	**Flower**	**Leaf**	**Flower**	**Leaf**
50	16±0.5mm	15±0.5mm	13±0.5mm	15±0.5mm	14±0.5mm	-	11±0.5mm	12±0.5mm
100	18±0.5mm	19±0.5mm	16±0.5mm	17±0.5mm	16±0.5mm	15±0.5mm	13±0.5mm	14±0.5mm
150	18±0.5mm	20±0.5mm	16±0.5mm	17±0.5mm	19±0.5mm	19±0.5mm	15±0.5mm	17±0.5mm
200	19±0.5mm	20±0.5mm	17±0.5mm	19±0.5mm	20±0.5mm	19±0.5mm	16±0.5mm	20±0.5mm

Among various plants screened, maximum occurrence of phytochemical constituents are found in *Plumeria obtusa*. Hence *P. obtusa* was subjected to antimicrobial activity. The results obtained in the present study showed that the crude extract of leaf and flower of *Plumeria obtusa* contains potential antibacterial activity *against E. coli*, *Proteus* sp, *Klebsiella* sp and *S. aureus*. The leaf extract of plant *P. obtusa* showed significant activity against all tested human pathogens in flower extract (Table-2). The maximum inhibition was recorded against *S. aureus* (20 mm±0.5mm) and *E. coli* (20 mm±0.5mm) followed by *Klebsiella* sp (19 mm±0.5mm) and *Proteus* sp (19mm±0.5mm) using 200 µl of crude leaf extract. The aqueous extract shows inhibitory effect and similarly the traditional healers uses water for their extraction.

Antibacterial activity of flower extract of *P. obtusa* showed significant activity against *Proteus* sp (20mm±0.5mm), *E. coli* (19mm±0.5mm) followed by *Klebsiella* sp (17mm±0.5mm) and *S. aureus* (16mm±0.5mm) in 200 µl of crude flower extract. The lowest antibacterial activity was recorded in *S. aureus* (12mm±0.5mm), *Klebsiella* sp. (15mm±0.5mm) and *E. coli* (15mm±0.5mm) in 50µl of crude leaf extract. The zone of inhibition was not recorded against *Proteus* sp. in 50µl of crude leaf extract, it may be due to minimum inhibitory concentration is greater than 50µl, possibly it may be 100µl. The lowest antibacterial activity was recorded in *S. aureus* (11mm±0.5mm) and *Klebsiella* sp. (13mm±0.5mm) followed by *Proteus* sp. (14mm±0.5mm) and *E. coli* (16mm±0.5mm) in 50µl of crude flower extract. Thus the significant activity of *P. obtusa* leaves against *S. aureus* and *E. coli* are due to the presence of bioactive compound and secondary metabolites.

Conclusion

Medicinal plants produce a wide range of metabolic compounds and research on such constituents will afford brightness on their therapeutic properties enlightening the activity of plants. Our pharmaceutical industry persistently probe for a new lead molecule having enhanced therapeutic action and fewer side effects. Phytochemical analyses of medicinal plants have gained interest in the development of new drug. Further studies should be made to authenticate for its action in treatment of an assortment of ailments. Various phytochemical constituents from the above medicinal plants can be subjected to pharmacological screening to get enhanced curative value for treatment of various diseases. The extracts of *P. obtusa* could be seen as a fine source for effectual medicine. Many of these constituents revealed as a result of screening to step forward towards the health status and may be considered as essential for healthy living in near future *P. obtusa* was suggested for further research on isolation, purification and characterization of the novel bioactive component responsible for pharmacological action.

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