Defence Life Science Journal, Vol. 8, No. 1, January 2023, pp. 29-40, DOI : 10.14429/dlsj.7.18172

Preliminary Pharmacognostic, Physicochemical and Phytochemical Evaluation of *Plumeria-Obtuse* Seed Pods

Sunil Shewale[#], Vaishali Undale[#*], Maruti Shelar[#], Bhagyashri Warude[$], Mohini Kuchekar[%], Vrushali Bhalchim[#], Shital Satone[#], Shweta Lembhe[#] and Sachin Gundecha[#]

[#]*Department of Pharmacology, Dr. D.Y. Patil Institute of Pharmaceutical Sciences & Research, University of Pune- 411 018, Maharashtra, India*
[$]*Department of Pharmaceutical Chemistry, Rasiklal M. Dhariwal College of Pharmacy, University of Pune- 411 019, Maharashtra, India*
[%]*Department of Pharmacognosy, Modern College of Pharmacy, Nigdi. University of Pune- 411 002, Maharashtra, India*
[*]*E-mail: vaishali.undale@dypvp.edu.in*

ABSTRACT

Plumeria obtuse L. (Apocynaceae) is an ornate outdoor plant. The plant was traditionally used during accidental injuries. However, the pharmacognosy of this plant is very poorly explored. Therefore, we have conducted this study to assess the distinctive qualities of the *P. obtusa*. To investigate *P. obtusa* seed pods' preliminary pharmacognostic, physical-chemical, phytochemical, microscopic, and phytoconstituent potential. Initially, the shape and microscopic characteristics of plant seed pods were assessed. Physicochemical analysis was used for the standardization. Utilizing several chemical techniques, phytoconstituents were evaluated qualitatively. This was followed by quantitative estimation and analytical profiling of various phytoconstituents. The basic characteristics of the seed pod have been documented by macroscopy to be its brown color, sweet aroma, bitter flavor, coarse texture, and rough fracture. Microscopy showed the existence of vascular bundles, lignified fibers, calcium oxalate crystals and arteries. The results of the physicochemical analysis revealed no foreign organic matter, 2.8 % weight-average moisture content and a high total ash value of 14.80 compared to an acid insoluble ash value of 0.70, which indicated that there was less inorganic matter in the plant. The extractive values were 3.93, 6.03 and 10.16 % w/w for water soluble, alcohol soluble and hydro-alcoholic soluble extracts respectively. Flavonoids, glycosides, saponins, phenolic constituents, tannins and carbohydrates were found during early phytochemical analysis. Instrumental analysis has given an idea about functional groups present whereas GCMS technique helped in identification of phytoconstituents. The results of this study can be significantly used as a reference support for quality control and standardization of *P. obtusa* and preparation of a monograph of plant.

Keywords: *Plumeria obtuse;* Pharmacognostic; Phytochemical; Physicochemical Parameters

ABBREVIATIONS:

^{0}C	:	Degree celsius
Da	:	Daltons
EI	:	Electron ionization
FTIR	:	Fourier transform infra-red
G	:	gram
GAE	:	Gallic acid equivalent
GCMS	:	Gas chromatography-mass spectroscopy
HOD	:	Head of department
Hz	:	Hertz
LOD	:	Loss on drying
ml	:	Milliliter
µl	:	Microliter
mm	:	Millimeter
µg	:	Microgram
MHz	:	Megahertz
NIST	:	National Institute of Standards and Technology
Nm	:	Nanometer
NMR	:	Nuclear magnetic resonance
PVDF	:	Polyvinylidene difluoride
QE	:	Quercetin equivalent
Rf	:	Retention factor
Sp	:	Species
TLC	:	Thin layer chromatography
UV	:	Ultra-violet
Wt	:	Weight
WHO	:	World Health Organisation

Received : 31 May 2022, Revised : 11 October 2022
Accepted : 15 November 2022, Online published : 3 April 2023

1. INTRODUCTION

In many developing countries, herbs are used to treat various medical conditions. This awareness has been accepted and approved from one age bracket to another in rural communities.[1] In field of innovative allopathy drug research and development, a lot of bioactive components of plants was investigate through combinations with synthetic

and chemical chemistry.[2,3] Moreover, compared to synthetic counterparts, plant-based medications are generally safe, inexpensive and may have therapeutic benefits.[4] However, such medicines are limited in developed countries due to a lack of documented evidence regarding quality control and evaluation methods.[5] Hence, its standardization through assessing pharmacognostic, physicochemical and phytochemical parameters is crucial. This will further ascertain the reproducibility, safety and effectiveness of herbal medicines.

P. obtusa is geologically disseminated in northern Central America, Greater Antilles, Florida and southern Mexico.[6,7] *P. obtusa* is a member of the Apocynaceae family, with common names such as White frangipani, Melia, Champa, Singapore graveyard flower, Araliya and Temple tree.[8] The major constituents present in this plant are amyrin, beta-sitosterol, glycosides, lupeol, triterpenes etc. *Plumeria sp.* has several therapeutic applications such as rubefacient, purgative, haemostatic, febrifuge, emmenagogue, laxative, vermifuge and stimulant.[9,10] Its pharmacognostic and phytochemistry are poorly understood despite its many applications.

The seed pod of *P. obtusa* plant could also be useful in prevention of medical ailment. But, it has never been evaluated for any pharmacognosy and or pharmacological parameters earlier. Hence, current study could be the first step for any such evaluation. This study emphasizes on morphological, microscopical, physicochemical, and phytochemical analyses of seed pods of *P. obtusa*.

2. STUDY PROCEDURES

2.1 Gathering, Verification, and Drying of Plant Part

The plant's fresh leaves and seed pods were harvested in India's Maharashtra state in Tehsil-Akole [Mehenduri], District-Ahmednagar. The leaf and seed pods were washed under the faucet to get rid of the dust and debris. The plant's herbarium was created and forwarded for authentication at Botanical Survey of India [BSI], which a reputable taxonomic research association run by the Indian government. The seed pods were dried by shade drying, ground into a powder using an electronic grinder, sieved through mesh screen 20, and then placed in an airtight container for long-standing storage.

2.2 Chemicals

"Analytical-Grade" chemicals used were purchased from certified vendor from Mumbai. The study ensured use of highest purity chemicals for experimental work.

2.3 Pharmacognostic Analysis

2.3.1 Organoleptic Evaluation of Plant

Plant leaves and seed pods were evaluated for various organoleptic characteristics.[11]

2.3.2 Microscopic Evaluation of Plant Seed Pods

The plant seed pods were also evaluated for microscopic characteristics using small transverse section of seed pod which was further stained, mounted and examined under high resolution microscope.

2.3.3 Powder Microscopy

Powder microscopy of dried seed pods powder was performed according to procedures.[12]

2.4 Physicochemical Evaluation

We have studied various evaluation parameters including "total ash, acid-insoluble ash, water soluble ash, alcohol and water-soluble extractive values, foreign organic matter and humidity content" were determined as per standard procedures.

2.5 Phytochemical Examination

2.5.1 Preparation of Plants Extract

The dried powder of seed pods were extracted using a reflux method with hydroalcoholic solvents viz; water and ethanol (H_2O: C_2H_2OH - 40: 60 units). Whatman filter (No: 42) paper was used for filtration of crude solution and after that an excess solvents was removed., The concentrated solution was placed over a water bath. The extract was kept at 4 °C until the remainder of the study was completed before analysis.

2.5.2 Initial Qualitative Phytochemical Screening

The extracted plant material was phytochemical screening of was performed as recommended by standard method.[12,13,14,15] These tests were conducted to determine the presence or absence of "carbohydrates, proteins, fats, fixed oils, flavonoids, glycosides, alkaloids, tannins, and saponins".

2.5.3 Fluorescence Analysis

By subjecting the powdered drug and its extracts to various reagents and examining them under Ultraviolet (UV) radiation under different wavelength[16,17] along with that fluorescence properties of the substance were examined. It is capable of often converting into fluorescent derivatives, which it then radiates in a variety of colors. Consequently, it is viewed as a crucial factor in pharmacognostic evaluation.[18]

2.5.4 Thin Layer Chromatography (TLC) of Extract

For confirmatory qualitative assessment of several secondary metabolites, pre-coated TLC plates were employed. Using a capillary tube and an amount of 1 to 101, ethanol was used for extract dilution and positioned to a TLC plate 2 cm from the bottom. The plate was then stored in a glass chamber with a TLC solvent saturated in it. The mobile phase then passed through the adsorbent phase and moved upward as a result. Each compound's retention factor (Rf) was calculated as it moved over the TLC plate. TLC plate was examined under UV cabinet for development of colors, some of them were exposed to the hot air and others were sprayed with different spraying reagents. The Rf expressed the movement of the analyte.[19]

2.5.5 Quantitative Phytochemical Analysis

2.5.5.1 Assessment of Total Phenolic Constituents

The Folin-Ciocalteu reagent was employed in the current investigation to gauge total phenolic content of extracts. The addition of extract (0.5 ml), Folin-Ciocalteu reagent (2.5 ml, Dilution-1:10) and Na2CO3 (2 ml, 7.5 % w/v) were done. The mixture was then incubate at 32 ^{0}C for 15 min. A UV-Vis spectrophotometer was used to measure the absorbance at 750 nm. Results obtained was given as the mg of GA/g of extract (gallic acid equivalent) was used to express the amount of phenolics in the extracts.[20, 21, 22]

2.5.5.2 Assessment of Total Flavonoid Content

By using colorimetry, total flavonoid content of extract was ascertained using technique. In a volumetric flask, extract and standard solutions of quercetin (10 to 60 g/ml) were added to distilled water. After every 5 minute break, 5 % Sodium nitrate (0.3 ml), 10 % aluminium chloride (0.3 ml), and 1M Sodium hydroxide (2 ml) were added to the flask, respectively, before a volume of 10 ml of distilled water was added. At 510 nm, an absorbance of this solution was measured in comparison to a control. The outcome were represented as mg of extract/g of QE equivalent.[22]

2.6 Analytical Profile of Phytoconstituents

2.6.1 Ultra-Violet Spectroscopic Analysis

A UV spectrometer [V-630 model] was used to determine UV spectrum of extract. The hydro-alcoholic extract of *P. obtusa* seed pods showed a maximum absorption when the UV spectrum's absorption of light as a function of wavelength was presented. It distinguishes herbal medicines by being typical of a particular functional group that is present in substance by accepted standards.

2.6.2 Fourier Transform Infra-Red(FTIR) Spectroscopic Analysis

10 mg of dried extract and 100 mg of KBr pellets were used to get translucent discs. Then it was run under "FTIR spectroscopy" ["Shimadzu, IR Affinity 1, Japan"] through a scan range of 400-4000 cm-1, resolution of 4 cm-1.

2.6.3 Gas Chromatography-Mass Spectroscopy(GCMS) Analysis

The sample was examined using the GCMS/EI mode on an Agilent GCMS triple quadruple system 7010B [HS 7697A, GC 8890 and ALS 7693A]. The ionisation energy in the positive electron ionization mode was -70 eV. Solvent lag was found to be 0–3 minutes. A scanning period of 0.5 s was set up with bits ranging from 50 Da to 500 Da. 2500 C was the temperature setting. Dichloromethane was added to a test tube with a stopper after 20 mg of the sample was taken out. 1 minute of vortexing Following a 30-minute sonication, allow it to cool to room temperature. The solution was then put into the vial, passed through a 0.45 m PVDF filter, and then injected into the GCMS. The average peak area was compare with overall area to determine the proportional percentage amount of each component. MS Workstation 8 manages chromatograms and mass spectra. The chemical components were ascertained using the "National Institute of Standards and Technology" (NIST) Version 2.0 library database.

2.6.4 H Nuclear Magnetic Resonance (NMR) Based Spectroscopic Analysis

The extract was dissolved in Deuterated methanol (600 µl). It was first vortexed, then centrifuged and transferred to NMR tube (5 mm). The NMR spectrum was recorded using Bruker Avance 500 MHz at 300 K. A pulse sequence of the noesygppr1d was used for 297 scans at a time of 3.27 s and a spectral width (10,000 Hz) to record each spectrum. Text/PDF formats were used to export the 1H NMR spectrum from the Bruker instrument.

2.7 Flow Properties of Powder (Rheology)

Rheology related with flow of matter. Different rheology parameters were evaluated for coarsely powdered seed pods of crude drug.

3. RESULTS

3.1 Authentication of Plant

Based on herbarium, the BSI verified the plant's authenticity and issued an authentication certificate (BSI/WRC100-1/TECH/2019/62). It verified that the plant species submitted was *P. obtusa* L., which is a member of the Apocynaceae family.

3.2 Organoleptic Evaluation

P. obtusa is mostly an outdoor decorative plant. A shrubby, angiosperm, terrestrial plant, growing either as a small shrub or tree between 0.9-6.1 meters high. The leaves are thick and leathery, dark green, a little shiny, have tertiary veins, obovate with blunt ends, and are found in groups around tips of the branches petiolate, obovate to oblong-obovate about 6 to 22 cm long and 2 to 7 cm wide. The fruits are dry follicles that split along one side to let loose winged seed pods. Flowers appear in clusters, mostly at branch tips. Each blossom measures about 5 cm in diameter. It is creamy-white and has an orange center. The calyx is 5 lobed, with equal to sub-equal lobes[23]. The dry soil, moderate water, and full or semi-shade are the plant preferences for growth. The fresh plant, dried and powdered seed pod is specified in Figure 1. Organoleptic characteristics of a plant are presented in Table 1.

3.3 Microscopic Evaluation (Transverse Section)

The transverse section of seed pods showed occurrence of a dermal tissue system, ground tissue system and vascular tissue system, and is displayed in

Table 1. Organoleptic characteristics of *P. obtusa* seed pods and leaves

S. No.	Organoleptic character	Observation	
		Seed pod	Leaves
1.	Color	Brown to dark brown	Glossy green
2.	Odor	Strong sweet	Slight
3.	Taste	Pungent	Sweet-soul
4.	Shape	T shape with double pod	Obovate rounded at tip
5.	Size	10-30 cm	60-75 cm
6.	Texture	Course	Smooth
7.	Fracture	Rough	Rigid

Figure 2. The outer layer of dermis tissue comprises of dilated, thin, flattened, cylindrical epidermal cells. A bunch of xylem elements with very thin phloem tissue was seen. This vasculature was comprised of a heavy mass of homogenous parenchymatous cells called chlorenchyma, comprises of very small intercellular spaces. chlorenchyma cells are particularly concentrated with chloroplast, useful for photosynthesis and energy storage. Each vascular bundle was subtended by a layer of long, narrow, and thick-walled sclerenchymatous sheath containing abundant lignin, making a hard cell wall. Lignified peripheral fibers surrounded the ring of the vascular bundle. Rhomboidal prisms of calcium oxalate crystals were also found in plant. There were four subsidiary cells lying on the lateral sides of the guard cells, surrounding cyclocytic stomata. The size of subsidiary cells was even or slightly uneven. A wide stomatal aperture characterized guard cells. Sunken stomata were also observed in a small pit, preserving water by decreasing water loss.

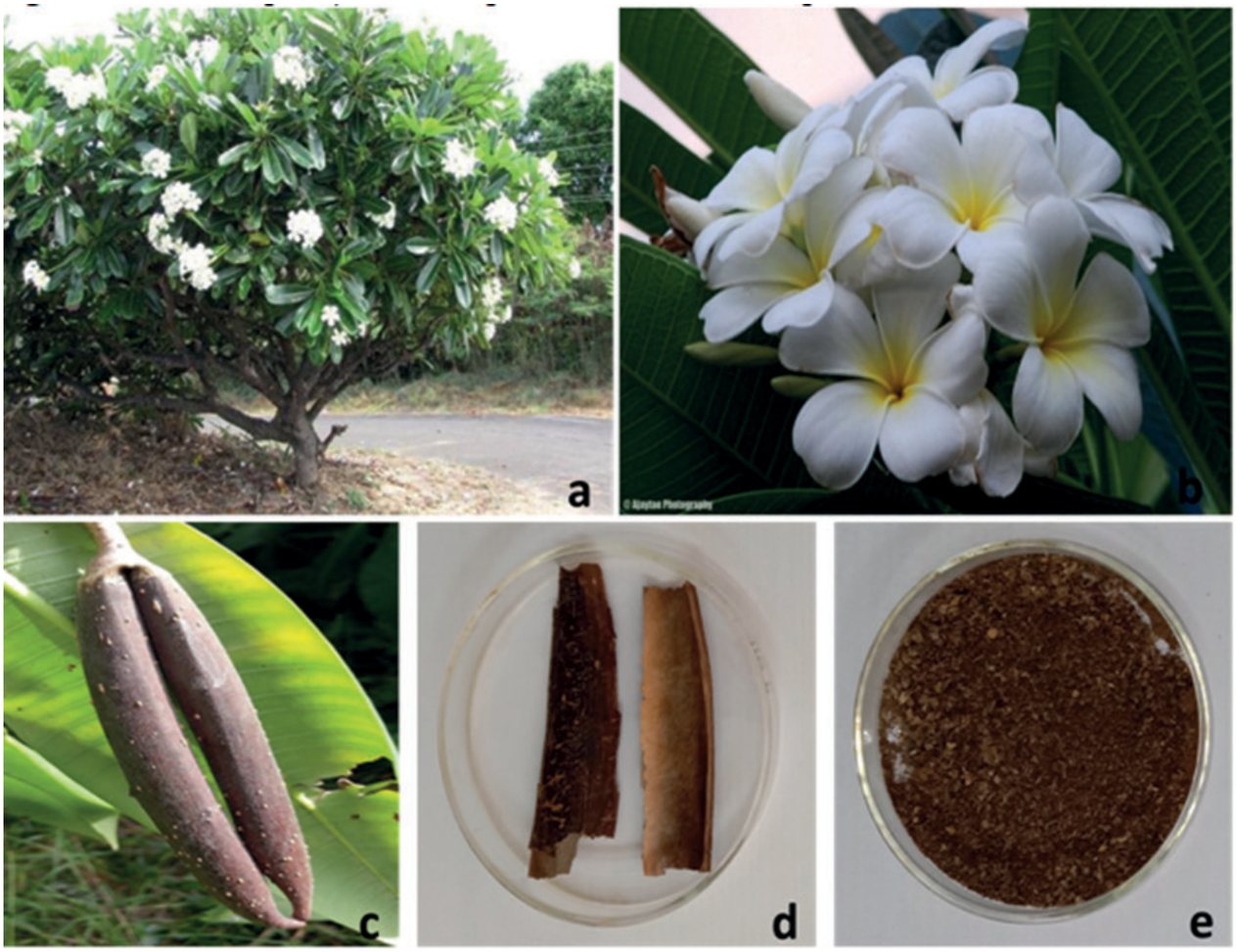


Figure 1. The fresh plant, dried and powdered form of seed pod.

Note: a) Fresh P. obtusa (PO) plant b) Flowers of PO plant c) PO seed pods d) Dry form of PO seed pod e) Powder form of PO seed pods

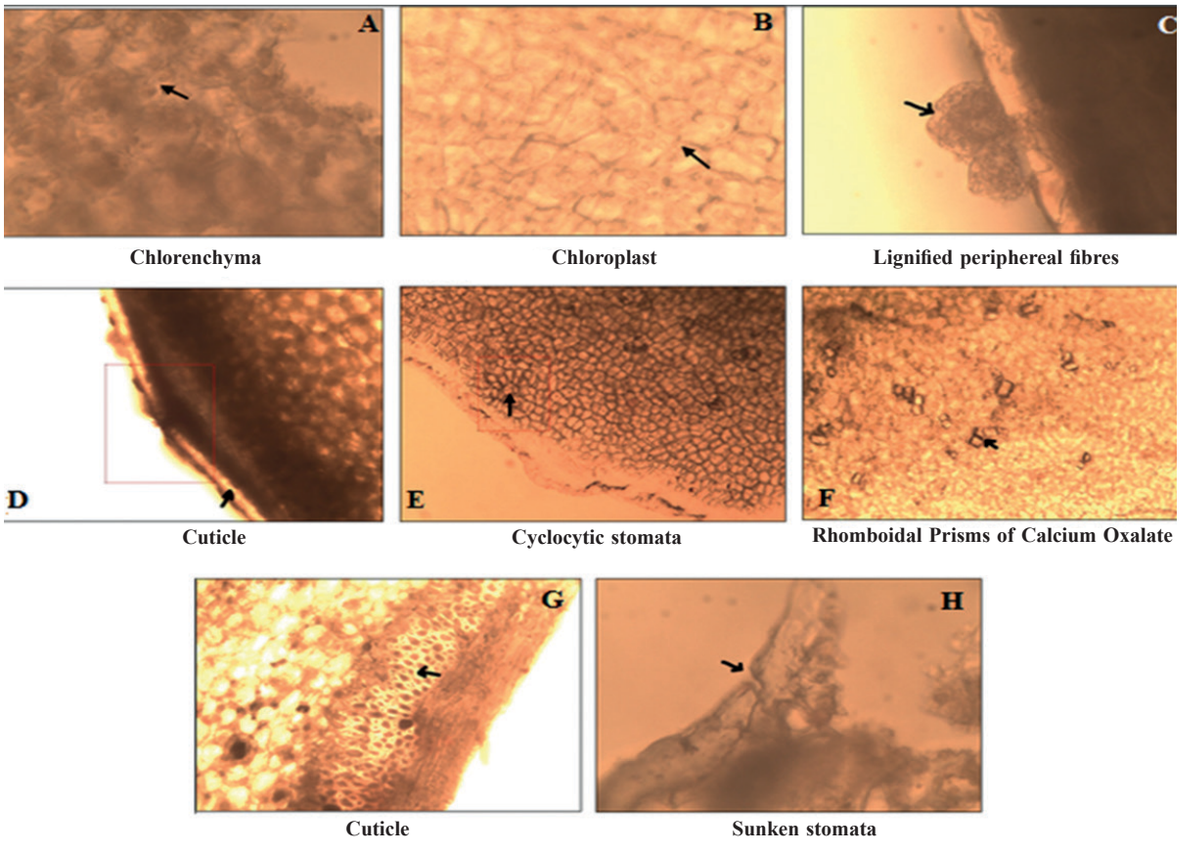


Figure 2. *Transverse section of P. obtusa seed pod.* ***A:*** *Chlorenchyma,* ***B:*** *Chloroplast,* ***C:*** *Lignified peripheral fibers,* ***D:*** *Cuticle,* ***E:****Cyclocytic stomata,* ***F:*** *Rhomboidal Prisms of Calcium Oxalate,* ***G:****Sclerenchymatous sheath,* ***H:*** *Sunken stomata.*

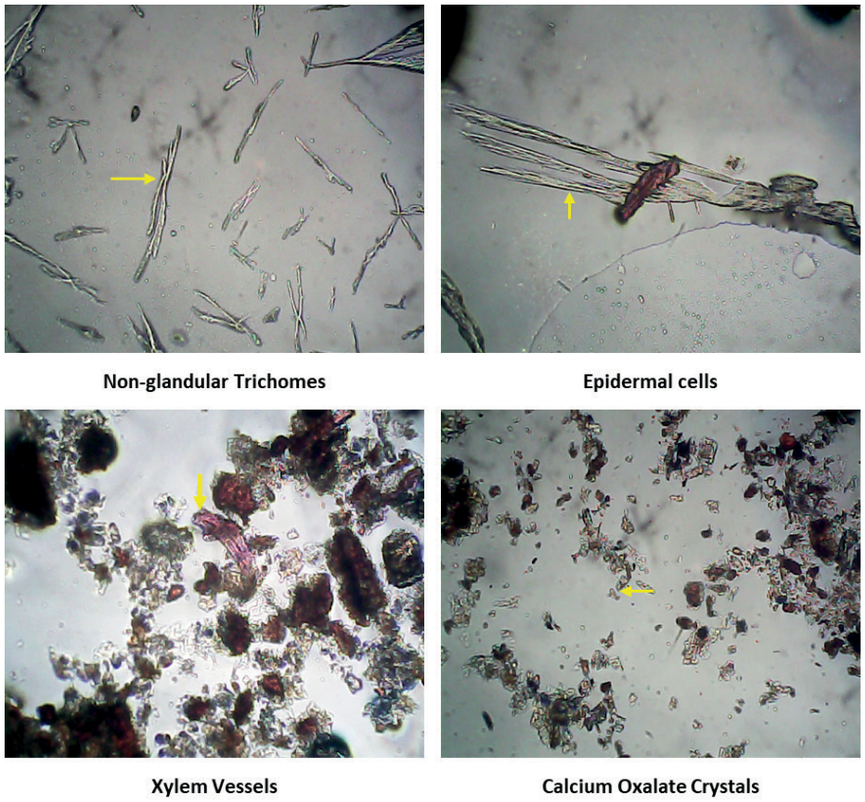


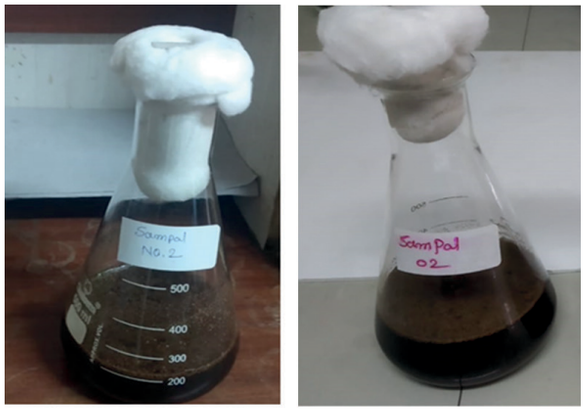
Figure 3. Powder microscopy of *P. obtusa* seed pods.

3.4 Powder Microscopy

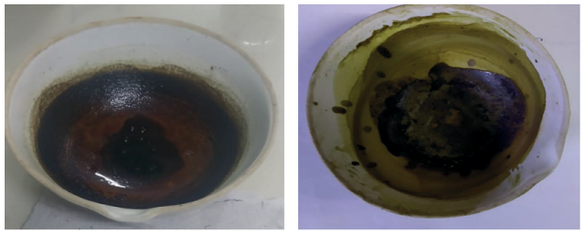
All parts of the plant included many rhomboidal calcium oxalate crystals, according to the powder microscopy. The seeds contained several broken pieces of non-glandular unicellular and uniseriate multicellular trichomes. In surface view, epidermal cells were asymmetrical and either rectangular or polyhedral in shape. In the pulverized seeds, there was also an annular and scalariform thickening of lignified xylem vessels. Figure 3 shows the microscopic characteristics of the powder.

3.5 Physicochemical Evaluation

In comparison to total ash value (14.8 %) and water-soluble ash value (12.30 %), acid insoluble ash value (0.70 %) was less. 2.8 % of material was damp, but there were no foreign organic materials. The hydro-alcoholic extractive value 10.16 % was obtain, while extractive values for substances, which are soluble in water and alcohol were reported as 6.03 % and 3.93 %, respectively. The water-soluble extract had a reddish-brown color and a non-sticky consistency. The hydro-alcoholic extracts and alcohol had loosened nature and appeared deep brown to greenish. The procedure of extraction value determination is shown in Figure 4. Table 2 lists the findings of the physicochemical analysis.



a) In process- Determination of water soluble extractive value & alcohol soluble extractive value of *Plumeria Obtusa (Sample 2)*



Plumeria Obtusa after avaporation to dryness for water soluble extract

Plumeria Obtusa after avaporation to dryness for alcohol soluble extract

Figure 4. In-process determination of extractive values.

3.6 Phytochemical Analysis

3.6.1 Preliminary Phytochemical Tests

Preliminary phytochemical screening reported presence of glycosides, flavonoids, alkaloids, tannins and steroids as shown in Table 3.

Table 2. Physicochemical parameters studied for seed pods of *P. obtuse*

S. No.	Parameters	Observation (% w/w)
1.	Moisture content	2.8
2.	Total ash	14.80
3.	Acid insoluble ash	0.70
4.	Water soluble ash	12.30
5.	Foreign organic matter	Nil
6.	Water soluble extractive value	6.03
7.	Alcohol soluble extractive value	3.93
8.	Hydro-alcoholic extractive value (Water: Ethanol-40:60)	10.16

3.6.2 Fluorescence Analysis

When the drug powder, different extracts, and drug powder treated with various reagents and/or chemicals were studied under visible and UV light, the typical fluorescence colors such as dark brown, yellowish-green, and dark green were observed (254 and 365 nm wavelengths). In Supplementary Table S1, fluorescence data details are provided.

3.6.3 Qualitative Thin Layer Chromatographic Analysis

Table 4 lists the outcomes of the thin-layer chromatographic analysis. Figure 5 depicts the secondary metabolite spots. A light green precipitate appeared after spraying Mayer's reagent on the plate, indicating the presence of alkaloid (Rf 0.79). When examined under a UV transilluminator, flavonoid compounds produced a green and yellow fluorescence and were discovered with Rf values of 0.85 and 0.88. The hydro-alcoholic extract included phenolic and tannin components, which were validated by the spot's pale green and brownish hues after $FeCl_3$ spray, respectively. Tannin was found to have an Rf value of 0.80 and phenols, 0.78.

3.6.4 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis performed using standard curves for gallic acid and quercetin correspondingly, revealed in Supplementary Figure S1, quantitative estimation of total phenolic and flavonoid content was determined. The extract's phenolic concentration was 79.69 g GAE/mg, while its flavonoid content was 37.97 g QE/mg. This demonstrates that phenolic compounds are abundant in *P. obtusa* seed pods.

3.6.5 Analytical Profiling of Phytoconstituents

3.6.5.1 UV Spectroscopy Analysis

According to UV spectroscopy of the plant, many functional groups, including ketone, phenol, arene, etc., were present in the range of 220 to 285 nm wavelengths. Detailed information is provided in Supplementary Table S2.

Table 3. Phytochemical analysis study of *P. obtuse* extracts

			Positive (+) / Negative (-)		
S. No.	**Parameters**	**Method**	**Water extract**	**Hydro-alcoholic extract**	**Ethanol extract**
		Molish test	+	+	-
1.	Carbohydrates	Fehling solution test	-	-	-
		Benedict's	-	+	-
2.	Amino acids	Ninhydrin test	-	-	-
3.	Proteins	Biuret	-	-	-
		Shinoda test	-	+	+
4.	Flavonoids	Zn. Hydrochloride test	-	-	-
		Lead acetate test	-	-	-
		Dragendroff's test	+	+	+
5.	Alkaloids	Mayer's test	+	+	-
		Hager test	-	+	+
		Wagner's test	+	+	-
		Borntrager's test	-	-	-
6.	Glycosides	Keller Killani test	-	+	+
7.	Volatile oil	Stain test	-	-	-
8.	Fixed oils & fats	Spot test	-	-	-
		Libermann Buchard test	-	+	-
9.	Steroids	Salkowski test	-	-	+
10.	Saponins	Foaming test	-	-	-
		FeCl3 test	-	+	+
11.	Tannins & phenols	Potassium dichromate test	-	+	+

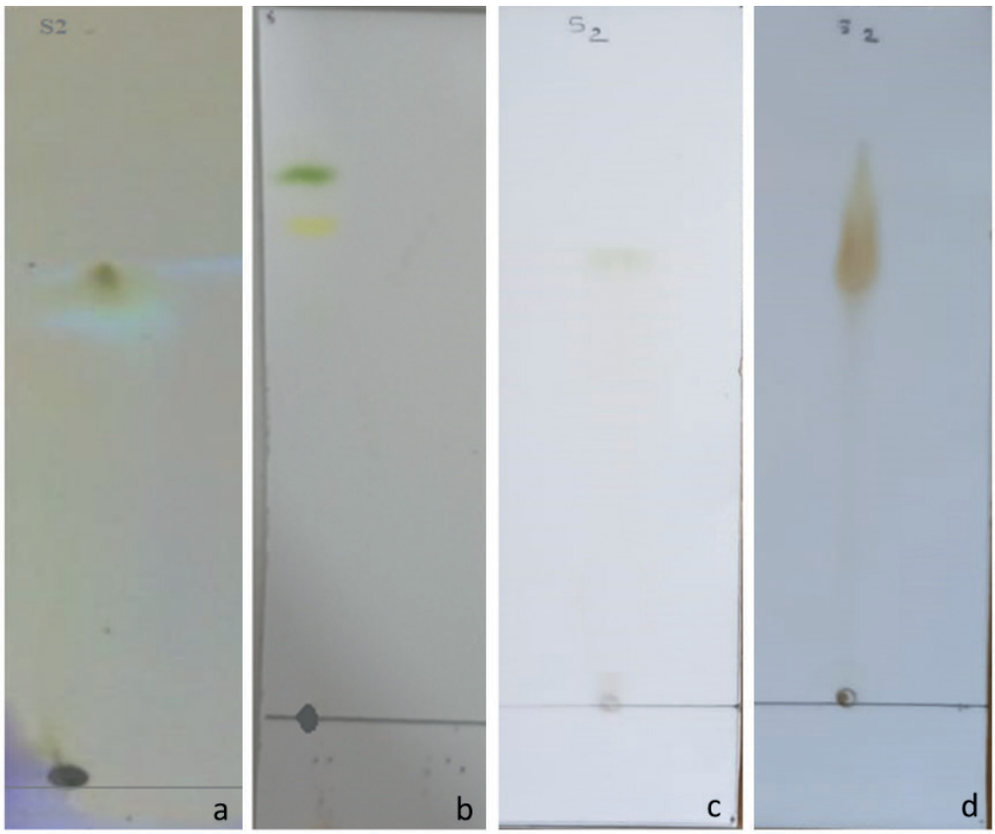


Figure 5. Thin layer chromatographic analysis of a plant extract.

Table 4. Confirmatory tests

TLC Plate	Phytoconstituents	Solvent system used	Confirmatory test	Rf value
a.	Alkaloids	EA: Chloroform: Water (5:3:1)	Mayer's reagent spray	0.79
b.	Flavonoids	N Butanol: EA: Water (5:10:15)	3 % boric acid + 10 % oxalic acid spray	0.85, 0.88
c.	Tannins	Chloroform: Water (6:4)	FeCl3 spray	0.80
d.	Phenols	Methanol: water (6:3)	FeCl3 spray	0.78

3.6.5.2 FT-IR Analysis

The results of FT-IR analysis inveterate existence of O-H, C=C, COOH, NH2, C-H, C-O and CH3 functional groups. The FT-IP spectrum is given in Supplementary Figure S2.

3.6.5.3 GCMS Analysis

Supplementary Figure S4 provides the produced GCMS spectrum with the identification of seven phytoconstituents from the extract with retention times ranging from 11.25 to 24.10. The chromatogram shows that Cyclopropanetetradecanoic acid, 2 octyl-methyl ester has the lowest peak area (14.6 %) and Octaethylene Glycol Monododecyl Ether has the biggest peak area (54.6 %). The hydro-alcoholic extract's phytoconstituents and other compounds identified by FTIR and GCMS are listed in Table 5.

3.6.5.4 NMR Analysis

By eliminating noise, solvent, impurity peaks, and other widely negotiable signals from the H1 NMR spectrum displayed in Supplementary FigureS3, a database of spectra was produced. H1 NMR was used to determine the quantity of protons in each chemical as well as their electronic state. Detailed information is provided in Supplementary Table S3.

3.7 Assessment of Powder Rheology

It's crucial to do a preliminary evaluation of the powder's properties prior to formulation. The *P. obtusa* seed pod powder was assessed for a number of criteria in this study. While the tapped density was 0.19 (g/ml), the bulk density was 0.5 (g/ml). According to this analysis, powder sample has low bulk and tapped densities, which gives it a fair chance to flow and rearrange itself under

Table 5. Phytoconstituents & other compounds identified in the hydro-alcoholic extract of *P. obtusa* seed pods by FTIR and GCMS

S. No.	Peak Value	Compounds	S.No.	Peak Value	Compounds
		Fourier Transfoma Infra-Red (FTIR)			
1.	3941.79	R-OH(Alcohols), C_6H_5OH (Phenol)	12	3711.33	R-OH(Alcohols) C_6H_5OH (Phenol)
2.	3930.22	R-OH(Alcohols), C_6H_5OH (Phenol)	13	3689.16	R-OH(Alcohols) C_6H_5OH (Phenol)
3.	3891.65	R-OH(Alcohols), C_6H_5OH (Phenol)	14	3675.66	R-OH(Alcohols) C_6H_5OH (Phenol)
4.	3882	R-OH(Alcohols), C_6H_5OH (Phenol)	15	3648.66	R-OH(Alcohols) C_6H_5OH (Phenol)
5.	3863.68	R-OH(Alcohols), C_6H_5OH (Phenol)	16	3618.77	R-OH(Alcohols) C_6H_5OH (Phenol)
6.	3853.08	R-OH(Alcohols), C_6H_5OH (Phenol)	17	3587.91	R-OH(Alcohols) C_6H_5OH (Phenol)
7.	3826.08	R-OH(Alcohols), C_6H_5OH (Phenol)	18	3396.03	RNH_2 (Amine) and RC(=O)NR'R (Amide),
8.	3777.87	R-OH(Alcohols,) C_6H_5OH (Phenol)	19	2358.52	C-N, COOH (Carboxyl acid)
9.	3744.12	R-OH(Alcohols,) C_6H_5OH (Phenol)	20	1623.77	Alkynes
10.	3734.48	R-OH(Alcohols,) C_6H_5OH (Phenol)	24	1385.6	ROR'(Ether), C_6H_5OH (Phenol)
11.	3723.87	R-OH(Alcohols,) C_6H_5OH (Phenol)	22	1099.23	R-OH (Alcohols), COOH (Carboxylic Acid), RCOOR' (ester), ROR' (ether)

compression. Similar to that, it has a low Carr's index of 9.82 % and a high Hausner's ratio of 1.23, both of which indicate low cohesiveness and good flowability.

4. DISCUSSION

Numerous bioactive substances derived from plants are the foundation of the contemporary medical system. Setting standards and quality control requirements for crude drug as medicine or herbal formulation prior to its use is of utmost important However, there aren't any adequate or reliable quality control procedures available for the Ayurvedic industry.[24] So, in order to guarantee the quality and therapeutic application of any medicine, standardisation and a complete pharmacognostic evaluation are crucial.[25]

There are numerous proven medicinal advantages of *P. obtusa*. In the current study, its seed pods' physicochemical and pharmacognostic characteristics are evaluated. Before conducting any testing, an organoleptic and histological analysis of medicinal plant is recommended by the WHO as the first step in confirming its identity and purity.[26] According to the macroscopic analysis, the plant was angiosperm, with leathery, dark green leaves and T-shaped seed pods.[27,28] An important instrument for studying plants at the microscopic level is supported by numerous historical and contemporary advances in cell biology.[29] Additionally, when employed in powder form, these qualities can be used to standardize drugs, create plant monographs, and lower adulteration. When identifying the cellular and structural characteristics of plants to establish their botanical origin and to differentiate between species with comparable morphological characteristics, powder microscopy is helpful.[30] *P. obtusa* seed pod's distinctive microscopic features show occurrence of established tissue systems made up of xylem and phloem components, vascular bundles and lignified fibers. The xylem channels, trichomes and calcium oxalate crystals are the diagnostic characteristics of powder microscopy. Similar microscopic characteristics of additional *Plumeria* spp. have been described in published literature by Kalantri, *et al*.[31]

It can be very helpful to evaluate purity and quality of crude drugs using their physicochemical properties. Ash values indicate quality and purity. Ash levels are used to identify impurities, earthy material, and inorganic substances.[32,33] Determination of extractive values are supportive for evaluating the chemical components contained in crude medications and figuring out whether specific compounds are soluble in a given solvent.[34]

According to the physicochemical parameters, water-soluble ash and plant medicine had the highest total ash values. The material had less siliceous contaminants and inorganic compounds because the acid-insoluble ash content was the lowest (0.70 %). According to reports, the proportion of the hydro-alcoholic extract was larger than the percentages of the water and alcoholic extracts. Due to the moisture content, crude medicines degrade either chemically or by microbial growth[35]. Using the LOD technique, the moisture content of *P. obtusa* seed pods was determined to be 2.8 %. Since there was no foreign organic stuff in the powder, the drug's purity was quite high.

Conventional phytochemical tests remain the best choice for preliminary screening as they are economical, easy and require few resources.[36] Secondary metabolites may be responsible for therapeutic properties of medicinal plants.[37] Various phytoconstituents such as tannins, alkaloids, phenols and flavonoids were detected in hydro-alcoholic and other extracts. These findings are supported by study conducted by Kamran, *et.al*. on *P. obtusa* plant with a limitation of use of leaves and flowers in the study.[23] Fluorescence examination revealed a distinctive colour in the current investigations, which most likely indicated presence of flavones, terpenoids, sterols etc.[38] Through qualitative TLC profiling, the more accurate identification and determination of comparable phytoconstituents were confirmed. The quantitative estimation of phenols and flavonoids was used to support this data. It was discovered that *P. obtusa* seed pods may possess strong antioxidant properties[39] due to their high phenol and flavonoid contents.

Spectrometric techniques can be used for initial screening of medicinal plant to ascertain their biological activity.[40] The functional groups of active chemicals in extract were identified by the UV spectra. Both FTIR and gas chromatography-mass spectrometry (GCMS) have been widely employed to confirm functional groups and identify several bioactive compounds.[41,42,43] Thus, FTIR and GCMS techniques were used to conduct a more thorough investigation of significant plant constituents. The outcome of this investigation produced trustworthy data that will aid in the development and marketing of novel medications. This study, which has found that *P. obtusa* seed pod powder has good flow ability, evaluates numerous rheological characteristics.

5. CONCLUSION

According to the study's findings, pharmacognostic analysis of *P. obtusa* seed pods will assist to establish a standard for its authenticity and identification. The discovery of its bioactivity, toxicity profile and the proof of its safety and effectiveness in clinical tests will be made easier by subsequent research made possible by this fundamental information. However, there are still many opportunities to investigate other hidden characteristics of this plant's seed pods, including the identification of additional significant phytoconstituents utilizing newly discovered methods.

ACKNOWLEDGMENTS

We acknowledge the excellent assistance provided by Dr. Bhushan Pimple, Head of the Department of Pharmacognosy at Modern College of Pharmacy in Pune, during the microscopic analysis of plant material. Additionally, we would like to thank Ms. Akshata Kawaste for her assistance with the experimental work for this study.

REFERENCES

1. Butt, M.A.; Ahmad, M.; Fatima, A.; Sultana, S.; Zafar, M.; Yaseen, G.; Ashraf, M.A.; Shinwari, Z.K. & Kayani, S. Ethnomedicinal uses of plants for the treatment of snake and scorpion bite in Northern Pakistan. *J. ethnopharmacol.,* 2015, **168**, 164-181. doi: 10.1016/j.jep.2015.03.045.
2. Abhishek, B.; & Saini, A. Evaluation of physiochemical screening and standardization on the root of rotula aquatica lour. *Indian J. Pharm. Biol. Res.,* 2013, **1**, 63-68. doi: 10.30750/ijpbr.1.1.6.
3. Arunakumar, S. & Muthuselvam, M. Analysis of phytochemical constituents and antimicrobial activities of Aloe vera L. against Clinical Pathogen. *World J. Agric. Sci.,* 2009, **5,** 572-576.
4. Erkan, N.; Ayranci, G. & Ayranci, E. Antioxidant activities of rosemary (Rosmarinus Officinalis L.) extract, blackseed (Nigella sativa L.) essential oil, carnosic acid, rosmarinic acid and sesamol. *Food Chem.,* 2008, **110,** 76-82. doi: 10.1016/j.foodchem.2008.01.058.
5. Venkateswarlu, G. & Ganapathy, S. Preliminary pharmacognostic and phytochemical study on Argyreiacymosa root. *J. Anal. Pharm. Res.,* 2018, **7,** 494-498. doi: 10.15406/japlr.2018.07.00273.
6. Herbal & Natural Medicine. The most complete medicinal herbs database backed by science. 2020, Available online from: https://www.herbal-organic.com/en/herb/20274(accessed date: August 2021).
7. Nabi, J. & Narwariya, P. Lalit Comprehensive overview of *Plumeria obtusa*. *World J. Pharm. Res.,* 2017, **6,** 664-676. doi: 10.20959/wjpr20174-8212.
8. Bihani, T.; Tandel, P.; & Wadekar, J. *Plumeria obtusa* L.: A systematic review of its traditional uses, morphology, phytochemistry and pharmacology. *Phytomed. Plus.,* 2021, **1,** 100052. doi: 10.1016/j.phyplu.2021.100052.
9. Dogra, N.K. Phytochemical Analysis and In vitro antioxidant studies of *Plumeria obtusa* L. Leaves. *Indian J. Pharm. Sci.,* 2016, **78,** 169-171. doi: 10.4103/0250-474x.180256.
10. Shinde, P.R.; Patil, P.S. & Bairagi, V.A. Phytopharmacological review of *Plumeria* species. *Sch. Acad. J. Pharm.,* 2014, **3,** 217-227.
11. World Health Organization. Quality control method for herbal materials. 2011, Available online from: http://apps.who.int/iris/bitstream/handle/10665/44479/9789241500739_eng.pdf;jsessionid=6FE146542F12CB793D8C63FBE

86041E2?sequence=1. Accessed date: May 2020.
12. Khandelwal, K.R. Handbook of practical pharmacognosy: Techniques and experiments. 19th (Ed.). Nirali Prakashan, India, 2008, pp. 1-8, 149-156.
13. Harborne, J.B. Phytochemical methods; A guide to modern techniques of plant analysis. 3rd (Ed.). Chapman & Hall, London, 1998, pp. 107-138, 187-227.
14. Wagner, H. & Bladt, S. Plant drug analysis: A thin layer chromatography Atlas. 2nd (Ed.). Springer-Verlag, Berlin, 1996, pp.3-51, 99-123.
15. Kokate, C.K.; Purohit, A.R.; Gokhale, S.B. Pathway to screen phytochemical nature of natural drugs. Pharmacognosy, 42nd (Ed.). Nirali Prakashan, Pune, 2008, pp. 56-61
16. Gayathri, V. & Kiruba, D. Fluorescence analysis of two medicinal plants – P sidium Guajava L and Citrus Aurantium. *Int. J. Pharm. Sci.,* 2015, **6,** 1279-1282. doi: 10.13040/IJPSR.0975-8232.
17. Alam, F. & Us Saqib, Q.N. Pharmacognostic study and development of quality control parameters for fruit, bark and leaf of Zanthoxylumarmatum (Rutaceae). *Anc. Sci. Life.,* 2015, **34,** 147-155. doi:10.4103/0257-7941.157159.
18. Madhavan, V.; Gajendra, ST SN.; Yoganarasimhan, S.N. & Gurudeva, M.R. Pharmacognostical Studies on Flickingeria nodosa (Dalz) Seidenf. Stem and Pseudobulbs- A botanical source of the Ayurvedic drug Jivanti. *Indian J. Nat. Prod. Resour.,* 2010, **1,** 22-28
19. Biradar, S. & Rachetti, B. Extraction of some secondary metabolites & Thin layer chromatography from different parts of Acacia farnesiana L. *IOSR J. Pharm. Biol. Sci.,* 2013, **7,** 44-48.
20. Noreen, H.; Semmar, N.; Farman, M. & McCullagh, J.S.O. Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopusdidymus. *Asian Pac. J. Trop. Med.,* 2017, 10, 792-801. doi: 10.1016/j.apjtm.2017.07.024.
21. Shewale, S.; Undale, V.; Shelar, M.; Bhalchim, V.; Kuchekar, M.; Warude, B.; Wawale, V. Preliminary pharmacognostic, physicochemical and phytochemical evaluation of *Sansevieria cylindrica* leaves. *J. Pharm. Negat Results*, 2022, **13**, 1253-1271.
22. Maswada, H. Assessment of total antioxidant capacity and antiradical scavenging activity of three Egyptian wild plants. *J. Med. Sci.,* 2013, **13,** 546-554. doi: 10.3923/jms.2013.546.554.
23. Kamran, R.M.; Khaliq, H.A. & Uzair, M. Pharmacognostic and phytochemical studies on *Plumeria obtusa* L. *J. Phytopharmacol.,* 2020, **9,** 120-124. doi: 10.31254/phyto.2020.9208.
24. Verma, S. & Singh, S.P. Current and future status of herbal medicines. *Vet. World.,* 2008, **2,** 347. doi: 10.5455/vetworld.2008.347-350.
25. Singh, P.; Khosa, R.L.; Srivastava, S.; Mishra, G.; Jha, K.K.; Srivastava, S.; Sangeeta Verma, R. & Tahseen, Md. Pharmacognostical study and establishment of quality parameters of aerial parts of Costusspeciosus-a well known tropical folklore medicine. *Asian Pac. J. Trop. Biomed.,* 2014, **4,** 486-491. doi: 10.12980/APJTB.4.2014C1103.
26. Rakholiya, K.; Chanda, S. Pharmacognostic, physicochemical and phytochemical investigation of Mangiferaindica L. var. Kesar leaf. *Asian Pac. J. Trop. Biomed.,* 2012, **2,** S680-684. doi: 10.1016/S2221-1691(12)60295-0.
27. Lim, T.K. Edible medicinal and non-medicinal plants. Dordrecht: Springer, Netherlands, 2012, pp. 80-81.
28. Singh, R.; Vinchurkar, K.; Dhokhane, S. & Mishra, D. Pharmacognostical and phytochemical evaluation of *Plumeria obtusa* (Linn.) leaves. *Pramana Res. J.,* 2019, **9,** 198-206. doi:16.10089.PRJ.2019.V9I8.19.3821.
29. Mineyuki, Y. & Otegui, M.S. For microscopy special issue on 'Plant Science'. *Microscopy,* 2019, **68,** 3. doi: 10.1093/jmicro/dfz006.
30. Aeri, V.; Anantha Narayana, D.B.; Singh, D. Powdered crude drug microscopy of leaves and barks. 1st (Ed.)., 2019, pp. 1-4. doi: 10.1016/C2018-0-02740-1.
31. Kalantri, M.R. & Aher, A.N. Pharmacognosy, physicochemical and preliminary phytochemical study on flower of *Plumeria rubra*. *Inter. J. Bot. Stud.,* 2021, **6,** 558-562.
32. Mandal, M.; Misra, D.; Ghosh, N.N. & Mandal, V. Physicochemical and elemental studies of Hydrocotyle javanica Thunb. for standardization as herbal drug. *Asian Pac. J. Trop. Biomed.,* 2017, **7,** 979-986. doi: 10.1016/j.apjtb.2017.10.001.
33. Prasanth, D.; Rao, A.S. & Yejella, R.P. Assessment of pharmacognostic, phytochemical and physicochemical standards of Aralia racemosa (L.) root. *Indian J. Pharm. Edu. Res.,* 2016, **50,** S225–S231. doi:10.5530/ijper.50.3.33.
34. Azad, A.K.; Awang, M.; Rahman, M.M. Phytochemical and microbiological evaluation of a local medicinal plant Bacopa monnieri (l.) *Penn. Int. J. Curr. Pharm. Rev. Res.,* 2012, **3,** 66-78.
35. Shah, B. & Seth, A. Textbook of Pharmacognosy and Phytochemistry. eBook Edition. Elsevier, New Delhi, 2010, pp. 110-114.
36. Shaikh, J.R. & Patil, M.K. Qualitative tests for preliminary phytochemical screening: An overview. *Inter. J. Chem. Stud.,* 2020, **8,** 603-608. doi: 10.22271/chemi.2020.v8.i2i.8834.
37. Paliwal, P.; Pancholi, S.S. & Patel, R.K. Pharmacognostic parameters for evaluation of the rhizomes of Curcuma caesia. *J. Adv. Pharm. Technol. Res.,* 2011, **2,** 56-61. doi: 10.4103/2231-4040.79811.
38. Kasturi, O.R. & Ramesh, B. Physicochemical and fluorescence analysis of leaves of Alternanthera brasiliana (L). Kuntze and Alternanthera bettzickiana (Regel) Voss. *Int. J. Pharm. Sci. Rev. Res.,* 2018, **51,** 66-71.
39. Aryal, S.; Baniya, M.K.; Danekhu, K.; Kunwar, P.;

Gurung, R. & Koirala, N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants (Basel),* 2019, **8,** 96. doi:10.3390/plants8040096.

40. Konappa, N.; Udayashankar, A.C.; Krishnamurthy, S.; Pradeep, C.K.; Chowdappa, S. & Jogaiah, S. GC-MS analysis of phytoconstituents from Amomum nilgiricum and molecular docking interactions of bioactive serverogenin acetate with target proteins. *Sci. Rep.,* 2020, **10,** 16438. doi: 10.1038/s41598-020-73442-0.
41. Satapute, P.; Paidi, M.K.; Kurjogi, M.; Jogaiah, S. Physiological adaptation and spectral annotation of Arsenic and Cadmium heavy metal-resistant and susceptible strain Pseudomonas taiwanensis. *Environ. Pollut.,* 2019, **251,** 555-563. doi: 10.1016/j.envpol.2019.05.054.
42. Fan, S.; Chang, J.; Zong, Y.; Hu, G. & Jia, J. GC-MS Analysis of the Composition of the Essential Oil from Dendranthema indicum Var. Aromaticum Using Three Extraction Methods and Two Columns. *Mol.,* 2018, **23,** 576. doi:10.3390/molecules23030576.
43. Ndika, E.V.; Chidozie, U.S. & Ikechukwu, U.K. (2019) Chemical modification of cellulose from palm kernel de-oiled cake to microcrystalline cellulose and its evaluation as a pharmaceutical excipient. *Afr. J. Pure Appl. Chem.,* 2019,**13,** 49-57. doi:10.5897/AJPAC2019.0787.

CONTRIBUTORS

Mr. Sunil Shewale, obtained his M Pharmacy from Savitribai Phule University of Pune, Maharashtra, India in Quality Assurance Techniques.
He is pursuing his PhD in Pharmacology from Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Pune.
He was involved in conceptualization, study design, experimental work, manuscript preparation, data curation.

Dr. Vaishali Undale, is a HOD, Department of Pharmacology at Dr. D. Y. Patil Institute of Pharmaceutical Sciences, & Research, Pune. Savitribai Phule University of Pune. Maharashtra (India).
She was involved in critical review for intellectual content, reviewing and editing.

Dr. Maruti Shelar, is an Associate Professor, Department of Pharmacognosy at Dr. D. Y. Patil Institute of Pharmaceutical Sciences, & Research, Pune. Savitribai Phule University of Pune. Maharashtra (India).
He was involved in study design, supervision, manuscript preparation, reviewing and editing.

Ms. Bhagyashri Warude, is an Assistant Professor, Department of Pharmaceutical Chemistry at Rasiklal M. Dhariwal College of Pharmacy, Pune. Savitribai Phule University of Pune. Maharashtra (India).
She was involved in critical review for intellectual content, reviewing and editing.

Dr. Mohini Kuchekar, is an Assistant Professor, Department of Pharmacognosy at Modern College of Pharmacy, Nigdi. Savitribai Phule University of Pune. Maharashtra (India).
She was involved in supervision, experimental work, reviewing and editing.

Ms. Vrushali Bhalchim, obtained her M. Pharmacy from Savitribai Phule University of Pune, Maharashtra, India in Pharmacology. She is pursuing her PhD in Pharmacology from Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Pune.
She was involved in experimental work and data curation.

Ms. Shital Satone, obtained her M Pharmacy from Savitribai Phule University of Pune, Maharashtra, India in Pharmacology.
She was involved in study design, experimental work.

Ms. Shweta Lembhe, obtained her M Pharmacy from Savitribai Phule University of Pune, Maharashtra, India in Pharmacology.
She was involved in study design, experimental work.

Mr. Sachin Gundecha, obtained his M Pharmacy from Savitribai Phule University of Pune, Maharashtra, India in Pharmaceutics. He is pursuing his PhD in Pharmaceutics from Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Pune.
He was involved in supervision, reviewing and editing.