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Research Article

COMPARATIVE QUANTITATIVE ASSAY OF APIGENIN AND LUTEOLINE STUDY OF DIFFERENT MARKET SAMPLES OF *BHARANGI* (*CLERODENDRUM SERRATUM* LINN) W.R.T. UPLC

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ABSTRACT

Bharangi (Clerodendrum Serratum Linn*)* is one of ancient drug used in many preparations for treatment of many pathological conditions like *Kasa, Shwasa, Jwara, Gulma, Aruchi*, etc. That's why there is need of pharmacognostical and phytochemical screening of different market samples of *Bharangi (Clerodendrum Serratum* Linn*)* with the help of UPLC study. **Objectives:** To undertake pharmacognostic and phytochemical screening of different market samples of roots of *Bharangi (Clerodendrum Serratum* Linn*)* and to compare the apigenin and Luteolin quantitatively in different market sample of root of *Bharangi* with the help of UPLC study. **Materials and Methods:** This was a laboratory-based study that included the analysis of different market samples of *Bharangi (Clerodendrum Serratum* Linn*)* in 3 phases, such as follows; a) Macroscopic and microscopic examinations with physio-chemical assessment involving the examination of ash value, pH water soluble extract, methanol - soluble external, acid insoluble ash and the extraction of volatile oil utilizing a clevenger apparatus. b) A qualitative phyto-chemical screening is conducted-demploying TLC method followed by phase 3 c) Which involves UPLC study. **Results:** The present showed that the market sample from Bengaluru had the highest quality of luteolin followed by Delhi and Rajasthan. All the three samples of *Bharangi (Clerodendrum Serratum* Linn*)* comprised both apigenin and luteolin with variations in both the quantity and this could be because of various factors such as soil properties, cultivation methods, environment, collection methods, extraction methods and age of plant. **Conclusion:** All the samples of *Bharangi (Clerodendrum Serratum* Linn*)* successfully fulfilled all the pharmacopoeia standards hence they are suitable for medicinal use.

INTRODUCTION

One of the oldest and still active traditions in India, Ayurveda is still very much in use in the twenty-first century. Ayurveda's *Dravya Guna* section focuses on plant research. The term *Dravya Guna*, which is made up of the phrases *"Dravya"* and *"Guna,"* refers to the systemic steady of *Dravya's Karma* (activity) and Guna (properties). [1]

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According to Ayurveda, it is a science that teaches about *Ayu* (life) by carefully examining *Ayushyani* and *Anayushyani Dravyani*, or the substances that have Properties and actions that support longevity and those that have the opposite effect.[2] *Bhishag* (doctor), *Dravya* (drug), *Upsthata* (attender), and *Rogi* (patient) are the four Ayurvedic components that are necessary for *Chikitsa.*[3] Consequently, one of the foundations for *Chikitsa* is *Dravya*.

Nowadays, there is a noticeable reliance on distributors for raw drugs, which leads to adulteration and substitution for financial gain. Therefore, determining whether a medicine is authentic is crucial. Brief information about drugs, including their identification, purity, actions, characteristics, and

various preparations for certain diseases, can be found in our classics. *Acharyas* mention *Bharangi* (*Clerodendrum serratum* Linn), one of the significant Ayurvedic plants. It works well for respiratory conditions. In India and other tropical and subtropical regions of the world, this shrub is extensively dispersed. In English, this perinneal plant is frequently referred to as "beetle killer," "blue bush," or "blue glory".[4] Gontubarangi in Kannada, Babhanaiti in Hindi, and *Bharangi* in Marathi and Sanskrit.[5] It is beneficial mostly for *Shwasa* (asthma), *Kasa* (cough), and *Peenasa*, according to Ayurvedic texts. [6-8]

In addition, it is grown and utilized for ornamental purposes in Assam and Sikkim to Tenasserium**.** [9] It is utilized in numerous significant Ayurvedic preparations or recipes, which raises medicinal demand and may result in a shortage of authentic drugs. It is necessary to investigate pharmacognostical and phytochemical screening of various market samples of *Bharangi (Clerodendrum Serratum* Linn*)* in order to compare the apigenine and luteolin quantitatively in various market samples of *Bharangi (Clerodendrum Serratum* Linn*)* roots because it is adulterated and substituted. The present has attempted to study and evaluate the same.

METHODOLOGY

Materials

The *Sangraha Desha* (place of collection) of different parts are required to be analysed under the light of modern scientific methodology. To evaluate *Bharangi (Clerodendrum Serratum* Linn*)* collected from different *Desha*, this study was carried out in four steps. The material utilized and the methodologies adopted are present here under: a) Procurement of plant from nature source, b) Organoleptic study of sample collected from nature habitat and different market sample, c) Physico-chemical evaluation collected samples and d) Phyto-chemical study for quantification. Samples of *Bharangi (Clerodendrum Serratum* Linn*)* were collected from different states of market sample i.e. Sample A - Bengaluru (Karnataka), Sample B - Najafgarh (Delhi) and Sample C - Bharatpur (Rajasthan). The drug thus obtained was duly authenticated at department of research analytical lab of RGESAM & H, Ron. Plant identification was done mainly on the basis of morphology and family characters of plant and microscopic features of the drug.

Analytical study

Microscopic study, physico-chemical & phyto-chemical analysis of *Bharangi (Clerodendrum Serratum* Linn*)* and physico-chemical analysis of *Bharangi (Clerodendrum Serratum* Linn*)* was done at Research analytical laboratory of RGESAMC & H, Ron. All the samples of *Bharangi (Clerodendrum Serratum* Linn*)* were collected and foreign matters like, leaves, stalk etc. were removed. It was washed below tap water, dried in shade, under normal environment condition and then very fine powder was prepared which was used for study. Around 100-200 grams of the *Bharangi (Clerodendrum Serratum* Linn*)* were collected from different cities and states. 50 grams of *Bharangi (Clerodendrum Serratum* Linn*)* was kept apart for organoleptic study. Then the drug was made into course powder and preserved in airtight container for phyto-chemical and physico-chemical analysis.

The aim of drug standardization was to ensure the uniformity thought and practice so that the characteristic feature of entity is understood in same manner by each and everyone. *Acharya Charaka* has detailed the guidelines for standardization which are still relevant in today's era in *Charaka Vimana Sthana.* Moreover, WHO has also derived comprehensive monogram which is similar to guidelines of *Acharya Charaka*. Study was conducted at Poornayu Research Centre and Lab, 23B, 3rd floor Marilingappa extension, Bengaluru, Karnataka.

Pharmacognostic Study

- Organoleptic evaluation- Colour, odour, taste, size and shape of all three samples of *Bharangi (Clerodendrum Serratum* Linn) root were studied. *Panchabhautika Parikshana* was done by *Shabda, Sparsha, Roopa, Rasa, Gandha Parikshana*.
- Morphological study: Macroscopic characters of three samples of *Bharangi* (*Clerodendrum Serratum* Linn) root were studied by 6x magnifying glass.
- Microscopic study: Microscopic characters of three samples of *Bharangi* (*Clerodendrum Serratum* Linn) root as well as powder were studied.

Microscopic Examination

- **Requirements:** Razor blade, forceps, needlles, brush, watch glass, glass sliders, cover slips, blotting paper, pholoroglucinol, HCI, water, dropper, compound microscope.
- **Procedure:** Little quantity of *Bharangi* (*Clerodendrum Serratum* Linn) root was soaked in water mixed with 5ml of glycerine and left for overnight. On next day, the *Bharangi* (Clerodendrum *Serratum* Linn) root was taken for microscopic examination. The *Bharangi* (Clerodendrum *Serratum* Linn) root and razor blade was made wet with water. The razor blade was taken in right hand and held at right angle. Section was done by moving razor blade over the *Bharangi* (Clerodendrum *Serratum* Linn) root. The sections present on the razor blade were now

transferred with a brush into the water in watch glass.

- Staining and mounting: A thin uniform and entire section were taken and transferred with a brush to middle of clean glass slide and drop of Phloroglucinol was added. Section was allowed to stain or few minutes and then the excess of stain was washed off using water. Then section was mounted by placing 1-2 drops of HCL by dropper and covered with a clean, thin cover glass. Excess of HCL was removed by blotting paper and observed under microscope. Microscopic features were observed; photographs were taken and compared with given description of API.

Powder Microscopy

Fine powder of the roots of *Bharangi* (*Clerodendrum Serratum* Linn) were soaked in water and 2-3 drops of chloralhydrate for few minutes. The soaked drugs were taken to the slide with a spatula and spread neatly. A drop of 1% Safranine stain (1g safranine in 50% alcohol) was put and left for minutes. Excess stain was removed by carefully washing with water. Then the cover slip was placed. Excess water was removed with the help of blotting paper and the slide was observed under the microscope. The same procedure was followed for the evaluation of powder microscopy in other sample of *Bharangi* (*Clerodendrum Serratum* Linn) roots powder.

Physico-Chemical Evaluation

This was done by making the drugs free from moulds, insects, animal faeces matter and other contamination such as earth, stones and extraneous materials. With the drug and digital balance, 100-500 grams of the drug sample was examined and the minimum quantity prescribed in the monograph was spread it out in a thin layer. The foreign matter was detected by inspection with the unaided eye or using a lens (6x). Later it was separated and weighed and the percentage was calculated which was not more than 2%.

Determination of Moisture by Air Methods

In this procedure, first, weight of the empty aluminum dish was recorded (W1). Then 2g of sample was added into weighted dish and weight (W2) was noted down again. The dish was kept open in oven maintained at said temperature for fixed time (100% for 5 hrs.). Then the dish was removed, cooled and weighed the dish again (W3) and calculation was done. Percentage of moisture content with reference to the air-dried drugs was calculated with the calculation - % of moisture = W2-W3 / W2-W1 X 100.

Determination of Ash Value

In determination of total ash value, the carbon was removed at temperature (450℃) because Alkali chlorides, which may be volatile at high temperature, would otherwise be lost. If carbon was still present after heating at a moderate temperature, the water-soluble ash may be separated and the residue again ignited as described in the early, or the ash may be broken up, with the addition of alcohol and ignited again. The total ash consisted mainly of carbonates, phosphates, silicates and silica. If the total ash be treated with diluted HCl, the % of acid insoluble ash may be determined. This usually consist mainly of silica and mainly of acid-insoluble ash in drugs such as senna, cloves, liquor ice, valerian, and tragacanth indicate contamination with earthy material. Senna leaf, which may be used directly as the powdered drug, is required to have low acid- insoluble ash (2.5%).

Estimation of pH value

The pH value of a liquid was determined by glass electrode in pH meter. pH meter was standardized by standard pH solution then electrode was dipped in the aqueous solution and pH noted.

Estimation of Total Ash Content: A clean silica crucible was taken in the muffle furnace at 400℃ for 30 min. Cooled it in desiccator. Empty weight of crucible (W1) was noted down and 2g of sample (W2) was added. The sample was ignited in the crucible on burner by heat till the sample smoke ceases. The crucible was placed in muffle furnace for hours at 550℃ (wait till it reach 50℃ to open). It was then cooled and noted down the weight (W3). Calculation was done using the formula=Total ash % W3-W1/Weight of sample X 100.

Estimation of Acid insoluble ash content

Reagent: 10 % HCl solution.

Procedure: To ash add 25ml HCl and boil for 5 min. Filter through ash less filter paper. Then paper was washed with hot water transfer paper with the residue to crucible (W1). Heated over a burner till smoke cease and transferred to muffle furnace (4 hours 550 ℃) (W4).

Calculation: Acid insoluble ash % = W4-W1 / Weight of Sample X 100

Extractive Value

Aim: determination of hydro-alcoholic soluble extractive which is used as a means of evaluating drugs, the constituents of which are estimated by continues extraction process.

Materials required: Clevenger's apparatus, rotary evaporator, lypholizer.

Procedure: Dry powder of *Bharangi* (*Clerodendrum Serratum* Linn) roots was placed inside a thimble made from thick filter paper (Whatman's filter paper No.1). The thimble was placed in Soxhlet distillation unit, which contains an extraction chamber, which was

suspended above a flask containing the solvent-Ethanol and below a condenser. The extraction chamber was designed so that when the solvent surrounding the sample exceeds a certain level it is automatically emptied by siphon side arm, which the solvent running back down to the boiling distillation flask. This cycle was allowed to repeat many times, over hours or days till the extraction becomes colorless. During each cycle, a portion of the extractable compound dissolves in the solvent.

After many cycles the desired compound was concentrated in distillation flask. After extraction the solvent was evaporated by reduced pressure in a rotary evaporator and then further lyophilized, which created a very low temperature and low-pressure environment in which aqueous solvents will sublime, leaving only solute behind. This technique was a good way to dry down or remove solvent and can also use to dehydrate samples. To have this work the most efficiently, samples should be shell frozen to maximize surface area. By these methods active principle of the sample was preserved.

Phytochemical Tests

Test for carbohydrates

Test for Reducing Sugar

Benedict test: Equal volume of Bendict's reagent and test solution was mixed in the test tube and heated in boiling water bath for 5 minutes. Solution appeared green, yellow or red depending on amount of reducing sugar present in test solution.

Test for Monosaccharides: Equal volume of Barfoed's reagent and test solution was mixed in test tube and heated in boiling water for 1-2 minutes and cooled. Red ppt observed confirms the test.

1) **Test for pentose sugar:** Equal volume of test solution and dilute HCl was mixed in test-tube heated and then some crystal of phlorophicinol were added. Appearance of red colours confirms the test.
2) **Test for non-reducing sugar:** Test solution does not give response to Benedict test and Fehling's test.
3) **Test for Tannins:** Few drops of FeCl solution were added to 1 ml of test solution. Appearance of black precipitate shows the presence of Tannins.
4) **Test for Alkaloids:** Few drops of Dragendroff's reagent when added to 1ml test solution it gives brown, orange brown precipitate, which confirms the test.
5) **Test for steroids:** Salkowaski reaction: To 2ml of extract, 2ml of chloroform and 2ml conc. H-SO, was added and shaken, chloroform layer appears red and acid layer shows greenish yellow fluorescence.
6) **Test for Glycosides**

A. Test for saponin glycosides: The test solution was shaken vigorously, foam persists for at least 1 minute, it confirms the presence of saponin.

B. Test for cardiac glycoside/deoxy sugar/Keller-Killiani test: To the 2ml solution Glacial acetic acid, 1 drop 5% FeCl, and conc. H:50, was added. Reddish brown colour appeared at the junction of two liquid layers and upper layer appears blue green.

C. Test for Anthraquinone glycosides/Bontrager's test: To 3ml extract, dil. H:SO, was added, boiled and filtered. To cold filtrate qual volume of chloroform was added, Shaked well and ammonia was added. Ammoniacal layer turns pink or red.

Test for proteins

Millions test: To 3ml test solution 5ml Millions reagent was added. Presence of white precipitate confirmed the test. When warmed it turns into red brick or ppt dissolves giving red colored solution.

Test for Amino acids

Test for Tyrosine- 3ml test solution and 3 drops Millions reagent was heated in test tube. Dark red color of the solution confirms the test.

Test for Cysteine - To 5ml test solution few drops of 40% NaOH _ 10% Lead acetate solution was added and boiled. Black ppt of lead sulphate is formed.

Test for Starch: One drop of (N/10) iodine solution was added to 1ml of test drug. Black precipitate confirms the presence of starch.

Test for Salts: Ash of drug material was prepared. 50% HCl v/v or 50% HNO, vv was added to ash and kept for 1 hour. The solution was filtered, and following tests were performed:

A) Test for carbonate - Ash of the test drug gives CO; fumes when 1 drop conc. H_2SO_4 is added.

B) Test for Nitrate- If red fumes come while adding conc H_2SO_4, to ash then it confirms the presence of nitrate in the ash.

C) Test for Sulphate- To 1ml test solution 1ml lead acetate was added, while ppt comes. While NaOH dissolves the white ppt and makes it transparent. This confirms the test.

Test for chloride- To 3ml test solution prepared in HNO, a few drops of lead acetate was added. While ppt confirms the test.

D) Test for Calcium- Ammonia 1 drop when added to the test solution gives white ppt shows the presence of calcium.

E) Test for Potassium- Ash when subjected to direct flame, if gives out violet flame, it confirms the test.

F) Test for Sodium- Ash when subjected for the direct flame if gives out golden yellow flames then it confirms the presence of sodium in the ash.

G) Test for Iron- to 5ml of test solution few drops of 5% ammonium thiocyanate was added, solution turns blood red.

Powder Behavioral Study

The fine powder of the drug was treated with different chemicals viz- conc. HCl, comc. HNO, conc. H_2SO, water, ethanol and methanol. The change in colour of the powder was observed and compared with its natural colour.

Fluorescence study

The appearance of drug powder in UV chamber under normal light Le, white light, in short wave light (250nm) and in long wave light (365nm) is observed, and then is compared with its natural colour or appearance of drug powder in natural light. All the samples of the powder were observed and compared with its natural colour.

Determination of Terpenoids

Salkowski test was used to detect terpenoids. Extract (5ml) was mixed with chloroform (2ml) and concentrated sulfuric acid (3ml) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for presence of terpenoids.

Determination of Volatile oil

Materials: Water, air dried drug, porcelain chits, conical flask, heating mantle and distillation apparatus.

Procedure: 30gm of air-dried drug together with 300ml of water was taken in one litre distilling flask and a few pieces of porous porcelain chits were added in the distilling flask, which is then connected to the steel head. Before attaching the condenser, water was run into the graduated receiver, keeping the tap T open until the water overflows. Any air bubbles in rubber tubing a-b were carefully removed by pressing the tube. The tap is then closed and then the condenser was attached. The contents of the flask were now heated and stirred by frequent agitation until ebullition commences. The distillation was continued to a rate which keeps the lower end of the condenser cool. The flank was rotated occasionally to wash down any material that adheres to its sides. At the end of 3 hours, heating was discontinued, the apparatus was allowed to cool for 10 minutes and tap was opened and the tube L1 was lowered slowly; as soon as the layer of the oil completely enters into the graduated part of receiver the tap was closed and the volume was read. The tube L1 was then raised still the level of the water in it was above the level of B. When the tap T was slowly opened to return the oil to the bulb. The distillation was again continued for another hour and the volume of the oil was again read, after cooling the apparatus as before. The content of volatile oil in drug was calculated by taking the reading levels in the graduated tube.

HPLC STUDY

Materials and Reagents: API standard Apigenin and Luteolin were purchased from Bionova Supplies, (Bangaluru, India). HPLC grade methanol, acetonitrile, orthophosphoric acid, acetic acid glacial extra pure and Millipore water were used for HPLC analysis is procured by Poornayu Research Labs (Bengaluru, India), from their standard vendors.

Sample preparation

Chromatographic conditions	
HpLC system	Shimadzu prominence
Software	Lab solution
HplC column	Hypersil ODS C18 (250mm X 4.6 mm, 5µm)
Flow rate	1 mL/mim
Injection volume	20 µL
Column thermostat temperature	25°C
autosampler temperature	Ambient
Elution	Isocratic
Detector	352nm
Mobile preparation	
Methanol: Acetonitrile: Acetic acid: Orthophosphoric acid: Water (40:20:0.05:0.05:40) mixed and sonicate for 10min and filter with 0.45µM filter	
Standard preparation	Weighed accurately 10mg of apigenin and luteolin standards in to 100ml vf dissolved and make up to the mark with ethyl acetate.

Sample preparation
Weighed 1.0gm of the plant materials were macerated with 70% ethanol at room temperature for 24hr. The solution was centrifuged, supernated liquid was evaporated and the residue obtained was extracted with ethyl acetate and then volume was adjusted to 50ml with ethyl acetate.

RESULTS

Histological Study

The prime root was tetrarch to Pentarch and cork cambium Aries in second layer of cortex giving rise to stratified cork, 16-20 cells thick xylem fibers root is characterized by stratified cork. Secondary phloem interspersed with sclereids and ring porous xylem. Starch grains were found in medullary rays and xylem parenchyma and are similar to those found in phloem parenchyma. Articular crystal of calcium oxalate was scattered in medullary rays and xylem parenchyma cells. xylem occupied the central area and consist of vessels, tracheid's, fibers, xylem rays and xylem parenchyma. Medullary rays mostly biseriate. Prismatic and acicular crystals of Ca oxalate, simple and compound starch grains with hilum found throughout the section.

Organoleptic Characters

Table 2: Comparative Organoleptic characters

Characteristics	Sample A	Sample B	Sample C
Shabda - Granular/ Fibrous	Fibrous	Fibrous	Fibrous
Sparsha - Snighdha/Ruksha	*Ruksha*	*Ruksha*	*Ruksha*
Roopa - Colour	Light brown	Greenish yellow	Light brown
Ras	*Katu, Tikta*	*Katu, Tikta*	*Katu, Tikta*
Gandha	Aromatic	Aromatic	Aromatic

Table 3: Comparative Microscopic characters

Characteristic	Sample A	Sample B	Sample C
Colour	Light brown	Greenish yellow	Light brown
Odor	Aromatic	Aromatic	Aromatic
Taste	Bitter and Astringent	Astringent	Astringent
Shape	Oblong	Oblong	Oblong
Size	2-4 cm	L- 8-10 cm	L- 3-4 cm
Texture	Rough	Rough	Rough

Physio-Chemical Analysis

Table 3: Physio-chemical Analysis Results

Test	Test method	Value of API [78]	Sample A	Sample B	Sample C
Total acid number	Titration		7.32%	7.54%	7.88%
Ash Value (%w/w)	Gravimetric	< 11%	2.05%	1.22%	1.61%
Acid insoluble ash (%w/w)		< 1%	0.01%	0.45%	0.23%
Water soluble extract (%w/w)	Chemical tests	> 12%	16.56%	5.71%	10.99%
Alcohol soluble extract (%w/w)		> 6%	2.07%	1.23%	5.34%

Phyto-Chemical Analysis

Table 4: Phyto-chemical Analysis Results

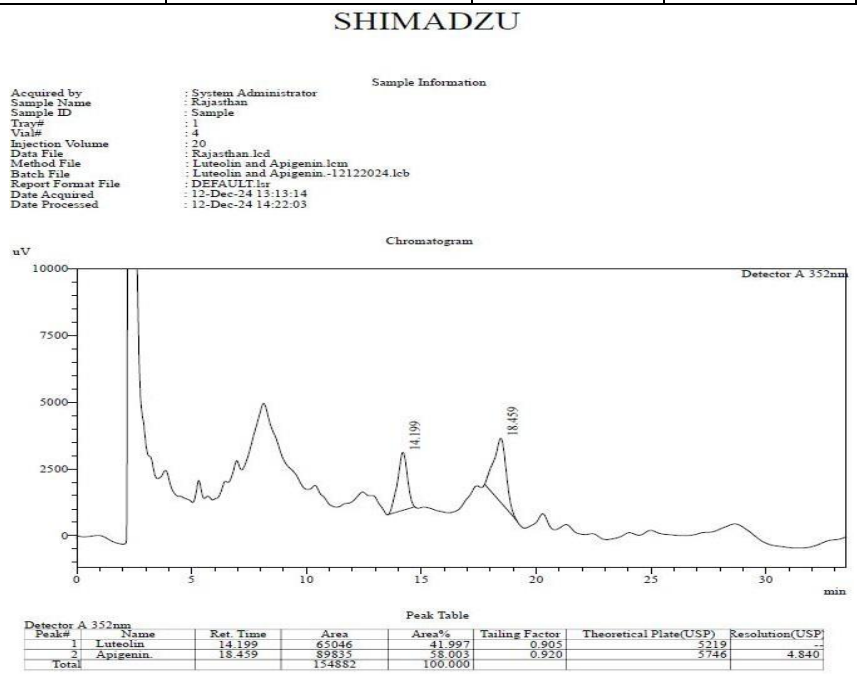
Test	Sample A		Sample B		Sample C	
	Aqueous extract	Alcohol extract	Aqueous extract	Alcohol extract	Aqueous extract	Alcohol extract
Tannin	Absent	Absent	Present	Absent	Absent	Present
Alkaloid	Present	Present	Present	Absent	Present	Present
Steroid	Present	Present	Present	Present	Present	Present

Glycosides	Absent	Present	Absent	Present	Absent	Present

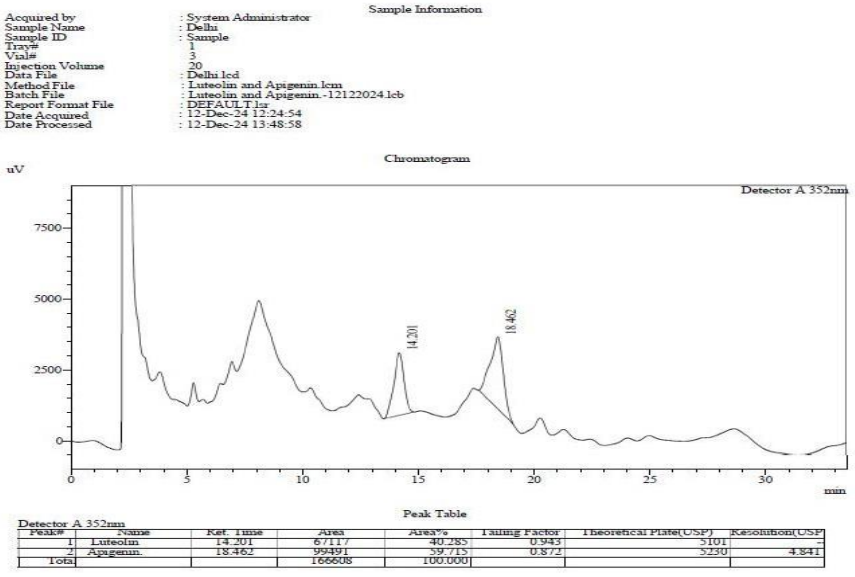
HPLC STUDY

Table 5: HPLC Analysis Results

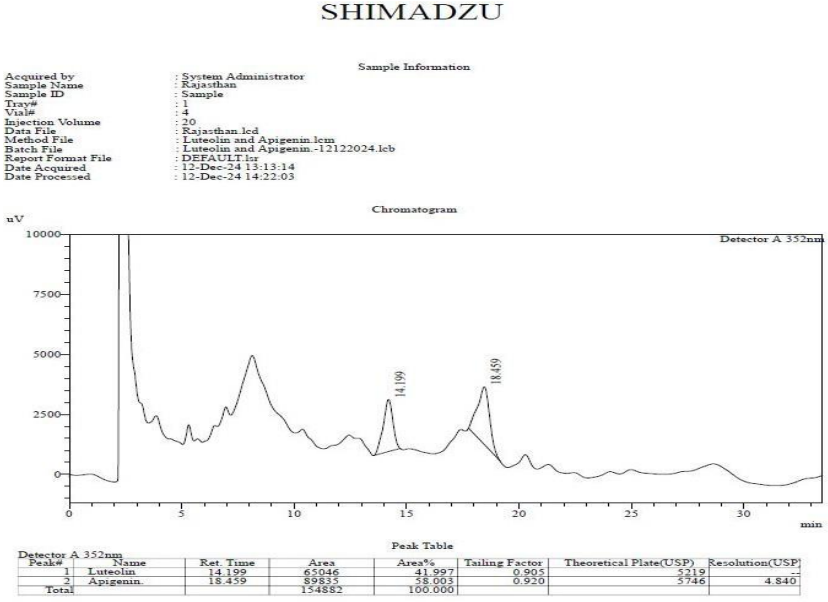
Sl. No.	Test Parameters	Test Method	Results (mg/g)		
			Sample A	**Sample B**	**Sample C**
1	Apigenin	Assay by HPLC	4.64	6.19	5.59
2	Luteolin		10.62	7.26	7.03



Graph 6: HPLC Sample A



Graph 7: HPLC Sample B



Graph 8: HPLC Sample C

DISCUSSION

Urbanization and increased habitat loss have made people more reliant on raw drug suppliers. Authentic drugs are in short supply as a result of this reliance. It raises questions about the potential for this supply to provide inferior, contaminated, or replaced drugs. Variations in the active ingredients of medicinal plants are largely caused by biodiversity. It is often accepted that the amount of these active ingredients determines how effective a medicine is. Using a subpar medicine can result in insufficient potency. The World Health Organization (WHO) has attempted to address this problem by publishing the International Pharmacopeia, which gives priority to medicines of major, global, public health importance, focuses on medicines important for WHO health programmes around the world and develops standards for medicines that are not covered by monographs in other pharmacopeias. [10]

This particular substance holds substantial importance in the culinary world. Due to its therapeutic and commercial value, there is high demand of standard drug. Report from public health Institution have identified common adulterant or substituent of *Bharangi* (*Clerodendrum Serratum* Linn), including the bark of *Gardenia turgid* Roxb sold as a bark of *Bharangi* (*Clerodendrum Serratum Linn*). *Premna herbacea, G. Lantifolia, G. resinifera, G. turgida* plant root sometimes mixed with *Clerodendrum Serratum Linn* & solanum Xanthocarpum used substitute to *Clerodendrum Serratum Linn.* Such instances of low quality frug in the market is for either ignorance or profit-seeking motives. Given prevalence of substitution and adulteration in *Bharangi* (*Clerodendrum Serratum* Linn), it is imperative to conduct pharma-cognostical and phyto-chemical assessment of various different market samples. The practice of adulteration and substitution to meet growing market demands is pressing issue today. This study should also delve into specification of *Bharangi* (*Clerodendrum Serratum* Linn*)* in terms of habitat, irrigation methods, environmental conditions and unique cultivation and collection techniques. Understanding these factors is crucial for ensuring the production of highest quality of *Bharangi*.

Discussion on Drug Review

The literature review of the drug was done from lexious, scientific research papers, *Samhita* and previous work done. There are references in *Brihattrayi* and *Laghutrayi* regarding *Bharangi* (Clerodendrum *Serratum* Linn*) Narahari Pandit* in his commentary of *Raj Nighantu* has mentioned *Bharangi* (Clerodendrum *Serratum* Linn) as a *Katu*, *Tikta* and *Ushna veerya*. According to API *Katu, Tikta, Kashaya* and *Ushna veerya* and *katu vipaka* has mentioned. [11] In *Samhitas Acharya Charaka* mentioned *Bharangi* (*Clerodendrum Serratum* Linn) in *Pipalyadi gana*. *Acharya Vagbhata* in *Arkadi, Sursadi* and *Vatsakadi gana*. As per *Acharya* and *Nighantu*, it is described and advocated for asthma, fever, tumor, rhinitis and cough. It is also used in anti-poisonous treatment. It has many synonyms like *Phanji, Padma, Angaravalli, Gardabhashak, Yashti, Bhruguja* and many more. A long list of synonyms shows it is well known plant all over India and world. [12,13]

Discussion on Collection of Samples

Proper identification, collection and storage should be done by the marketing agencies to avoid the loss of chemical constituents from collected samples.

For this study, natural habitat samples were collected from Konkan region and Maharashtra state. Three samples were collected from three different states Delhi, Rajasthan and Karnataka. The different samples shown a mild range of variations, Sample A- Bengaluru -150Rs/100gm, Sample B- Delhi-200Rs/200gm Sample C- Bharatpur -300Rs/200gm. Price of sample B was less compared to other samples, it could be because of poor quality.

Discussion on Trade Importance

The trade of *Bharangi* (Clerodendrum *Serratum* Linn) holds significant importance for several reasons,

Culinary- *Bharangi* (*Clerodendrum Serratum* Linn) leaves was used as a vegetable in western ghats of Maharashtra, Konkan and other Adivasi areas of India.

Cultural & Traditional use - The roots of *Bharangi* (*Clerodendrum Serratum* Linn) was used for diseases of respiratory system and anti-inflammatory. The flowers of *Bharangi* (*Clerodendrum Serratum* Linn) were used for ornamental purpose in several parts of India and Nepal.

Medicinal use - As per our Samhitas, it is used as ingrains in some important preparations like *Kantakari ghrita, Amritprasha ghrita, Agastyaharitaki, Jivantyadi lehyam, Dashamooladi taila* and *Saindhavadi taila* etc. That is the reason for high demand of drugs.

Economical fraud - Fake *Bharangi* (Clerodendrum *Serratum* Linn) drug may be a part of broader scheme, involving economic fraud. This could false or poor-quality drug.

Market Manipulation - False drug or poor quality is used for manipulation of market. For example, a certain group in market if believes there is shortage of drugs leads to increase in price.

Discussion on Material and Methods

Materials for study were collected from 3 different market of 3 different states. A genuine sample was collected from Maharashtra state in Konkan region that was identified by local people. This was a laboratory-based study that included analysis of different market samples of *Bharangi* (Clerodendrum *Serratum Linn)*.

Pharmacognostical study: It is the science that deals with the identification, characterization and evaluation of medicinal plant and their parts. The primary aim of pharmacognostical study is to establish authenticity, quality and purity of plant materials used in herbal medicines and naturals products. It involves following aspects.

Pharmacognostical tests of all the three samples showed that most of parameters were similar and difference in some parameters may be due to soil properties, age of plant while collection, environment and method of collection.

Macroscopic examination: This includes visual examination of the plant material without the use of microscope. It entitles observing and recording, the size, shape, colour, texture, colour and taste of different plant parts, such as roots, stems, leaves, floors, fruits and seeds. Macroscopic features helped in the proper identification and differentiation of plant species. Macroscopic features of all three samples are slightly different, sample C bigger in length and width as compared to Sample A and B. It could be because of age of collected plant or due to mixing of bark of *Bharangi* or other substitute drugs.

Microscope examination - This involves the study of thin section of plant material under a microscope. Microscope examination allows the observation of cellular structure, tissue and other microscope features. It helps in verifying the authenticity of plant material and detecting adulteration and substitution. Microscopic features of all three samples are almost same.

Powder microscopy- For herbal drugs sold in powdered form, powder microscopy is employed to examine the size and shape of particles presence of specific cells or cell fragments and other characteristic that aid in the identification and quality assessment.

Organoleptic Evaluation- This refers to the assessment of the sensory properties of plant material such as taste, odour, appearance, Organoleptic evaluation is particularly important in traditional medicine system, which determines the medicinal properties of plants.

Physio-Chemical evaluation- Physio-chemical tests of all the three samples shows most of parameters comparable to APIs except Sample B which shows very less water-soluble extract as compared to API, parameters difference may be due to soil properties, age of plant while collection, environment and method of collection.

Phyto-Chemical evaluation- It is the branch of science that focuses on chemical composition of plant materials and the isolation, identification and quantification of bio-active compounds present in them. Phyto-Chemical studies provide valuable information about the chemical constituents responsible for the medicinal properties of medicinal plants and natural products. This process included; Extraction, Isolation, Identification and Quantification. As per phyto-chemical study the sample A comes as the best sample compared to Sample B and C.

HPLC profile of *Bharangi* (*Clerodendrum Serratum Linn*)

The HPLC analysis shows that the market sample from Bengaluru had the highest quality of Luteolin followed by Delhi and Rajasthan. The market sample from Delhi showed highest quantity of Apigenin followed by Rajasthan and Bengaluru. All the three samples of *Bharangi (Clerodendrum Serratum Linn)* comprised both apigenin and luteolin with variations in both the quantity and this could be possible because of several factors such as oil properties, cultivation methods, environment, collection methods, extraction methods and the age of plant.

CONCLUSION

The present showed that the market sample from Bengaluru had the highest quality of Luteolin followed by Delhi and Rajasthan. The market sample from Delhi showed the highest quantity of Apigenin followed by Rajasthan and Bengaluru. All the three samples of *Bharangi (Clerodendrum Serratum Linn)* comprised both apigenin and luteolin with variations in both the quantity and this could be because of various factors such as soil properties, cultivation methods, environment, collection methods, extraction methods and age of plant. All the samples of *Bharangi (Clerodendrum Serratum Linn)* successfully fulfilled all the pharmacopoeia standards hence they are suitable for medicinal use.

Recommendations for further study

The future researcher can focus on *Bharangi (Clerodendrum Serratum Linn)* to find out if it is available out of India and the level of Apigenin and Luteolin available in the samples.

Limitations of the Study

Proper identification of the drugs needs careful attention.

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