



ISOLATION AND EVALUATION OF FOOD DYE FROM BUTTERFLY PEAS

¹Aishwarya S. Jaiswal, ²Kamlesh A. Kadam, ³Dr. Hemant H.gangurde, ⁴Muskan M. Mouraiya, ⁵Pratiksha A. Dhokane

¹Student, ²Assistant Professor, ³Professor, ⁴Student, ⁵Student

¹Department of Pharmacy, ²Department of Pharmacognosy, ³Department of Pharmaceutics, ⁴Department of Pharmacy, ⁵Department of Pharmacy

^{1,2,3,4,5}Shri Sant Gajanan Maharaj College of Pharmacy, Buldana, 443001, MS, India

^{2,3}Abasaheb Kakade College of B. Pharmacy, Bodhegaon, 414503, MS, India

Abstract: Folk remedies made from butterfly pea (*Clitoria ternata* Linn) are utilised. The seeds and roots are used as laxatives and digestive aids. The leaves and roots are used as anthelmintic, an antidote to animal sings, and a therapy for urogenital diseases. Butterfly pea flowers contain anthocyanins. Plant pigments called anthocyanins are what give plant blooms their red, violet, and blue hues. Six main anthocyanin sematins (A1, A2, B1, B1, D1 and D2). were identified as malonylated delphinidin 3.35-inglucosides with 3', 5'-sae chains and alternate D-glucose and p-coumaric acid.

A very limited variety of distinct pigments are responsible for the many colours of floral colour. The sole difference between these colours, which share the same carbon backbone, is the type of substituent groups they contain. Anthocyanins' colour stability is influenced by their chemical makeup, pH, temperature, oxygen content, light, and water activity. Anthocyanins tend to be red in extremely acidic solutions and blue in basic solutions. Butterfly pea is an example of a flower that contains anthocyanins, although its application has not yet been perfect. The purpose of this work was to identify the anthocyanins in the butterfly pea blossom and use an extract of it as an indicator in an acid-base titration based on anthocyanin characteristics.

Index Terms: Isolation of butterfly peas, Evaluation, Extractive value (Water), Extractive value (Alcohol), Total ash value, Acid insoluble ash value, Water soluble ash value, Chemical test, Oil and Fat test.

INTRODUCTION:

Butterfly pea:

Chemical composition from natural plants, like herbs, has been receiving significant public attention due to essential influences on human health. As a result, the extraction and use of natural colour compounds are becoming increasingly careful. Numerous studies have been carried out to determine the impact of the colour compound in food processing, such as the application of betalains by Deepti Dabas and George Kean. [2] Extracts by Ricardo F. R. da Silva et al. To use in croissants is elderberry (*Sambucus nigra* L.) anthocyanin [3]. Fernandez-López, J. examined the impact of natural rosemary, lemon, and orange extracts on beef meatball processing to assess their antibacterial and antioxidant [4].

One of the most organic colorants that was frequently utilized in food is anthocyanin. The most prominent water-soluble pigment in plants is anthocyanin, which comes in more than 540 different varieties in hues including orange, red, purple, and blue. Anthocyanins are found in all plant tissues, including flowers, fruits, stems, and roots. Some fruits and flowers, like, contain a lot of anthocyanin. Blood orange, aubergine,

raspberry and cherry. Considering this, the use of colorants from Anthocyanins are being researched for use in food and medicine [5]. In place of synthetic colorings, Butterfly Pea flower (BFP) anthocyanins were frequently utilized because they also have drug effects, anticancer properties, and antioxidant capacity. The butterfly pea flower, or *Clitoria ternatea* plants with flowers that belong to the legume family



Fig 1 plant of butterfly peas

The flower has a blue colour so their colour are applied for various decorative items and natural coloring agent. The extract from Butterfly Pea flowers has a blue hue. They can, however, turn pale pink when exposed to neutral or weakly acidic water. According to a prior study, butterfly pea has significant quantities of anthocyanin and other chemicals with free phenolic and flavonoid groups both have the ability to scavenge free radicals. BFP's primary anthocyanin is Defining their blue colour is the delphinine glycoside. The BFF extract was utilised in cosmetics as hair colour. Additionally, it is utilised in the food industry as a colouring for confections. However, The blue hue of BFP's extract is sensitive to pH, unlike anthocyanin extracts from other plants. Compared to other materials, they are less stable and more difficult to handle due to light and temperature degradation. due to artificial colorings. Malaysia [1,6]

Anthocyanin is an antioxidant chemical that inhibits heart ischemia-reperfusion injury, reduces the incidence of colon cancer, has anti-inflammatory properties, and helps people stay healthy by preventing diabetes and obesity. As a result, adding anthocyanin to food increases both the nutritional value and the sensory component of the final product. The range of anthocyanins from BFP is also broad (from red-orange to purple-blue), allowing for easy product diversification in action.

However, prior studies showed that the anthocyanin content in pH, temperature, light, and solvent extraction all have an impact on butterfly extract [5].

BRIEF INTRODUCTION OF BUTTERFLY PEAS:

Family: Fabaceae

Buttefly pea

It is also known as Asian pigeonwings or bluebell vine. this plant is seen most in southeast Asian countries Vietnam, Malaysia, and Thailand, but it's also found throughout India, China, central south America along with the east and west India

The morphological characteristics of the genus are as follows:

Leaves:

It has trifoliate leaves. These compound leaves are alternate, stalked & have three leaflets over an inch long, with the terminal leaflet on a long stalk

Flower:

It is a large wild flower with one to three showy purple flowers that are up to 2 inches long

Uses:

Culinary use: Flower which is use as a natural food coloring.

Traditional medicine: use as Memory enhancing, Nootropic , Antistress ,Anxiolytic

Textile use: use to dye natural fibers and is used by traditional societies

Literature Review:

Li Hsien Chen et al:The ability of the butterfly pea and flower fermentation solution to scavenge free radicals, have high concentrations of reducing power, moisturise, and whiten.demonstrated that the butterfly pea and flower fermentation solution not only reduces skin redness, itching, allergies, and irritation, but also has antioxidative qualities, encourages moisture retention, and has whitening benefits. The results get better as the concentration rises.Therefore, butterfly bean flowers might be appropriate as a raw material for natural cosmetics.

Angkana Tantituvanont et al: One of the most intriguing sources of natural colour used in food and cosmetics is the butterfly pea. The primary pigments in its petals, anthocyanins, are readily removed with water. The stability of the colour aqueous extract from butterfly pea petals was discovered to be influenced by the pH of the medium, temperature, and light. Acidity and alkalinity of the solvent affected the stability of the colour as well as the color's shade. The colour was most stable in pH 4 solution when it was dark and least stable in pH 7 solution when it was exposed to UV radiation. The colour loss increases with increasing temperature. In this study, a microparticulated system made using the spray drying process was used to try to increase the colour stability.

Gelatin and hydroxypropyl methylcellulose (HPMC) were employed as carrier polymers. Using a 23-factorial design, the operating condition resulting in the best production yield was identified. % solid in the feed solution, inlet temperature, and solution feed rate were the determining factors. For both HPMC and gelatin, the ideal conditions were 5% w/w solid in the feed solution, 130 C inlet temperature, and 10 ml/min solution feed rate. Under heat and UV light, the colour stability of the microparticulated particles was investigated. Compared to HPMC microparticulated system and aqueous colour solution, gelatin microparticulated system offered higher UV protection. Therefore, when creating the microparticulated particles, the type of polymer should be carefully chosen. However, neither the gelatin nor the HPMC microparticulated systems showed any protection against thermal degradation.

Nyi Mekar Saptarini et al:Anthocyanins are found in the flowers of butterfly pea (*Clitoria ternatea* Linn). Anthocyanins' colour varies depending on the pH of the solution.The purpose of this study is to establish the usefulness of butterfly pea extract as an acid-base titration indicator. The results revealed that the butterfly pea extract's refractive index ranges from 1.382+ 0.25 to 1.390+ 0.30, its specific gravity ranges from 0.975 + 0.20 to 0.993 + 0.25, its maximum wavelength is between 572 and 614 nm, and its discolouration changes from violet to blue at pH 4 to blue to green at pH 9 to green to yellow at pH 12, according to the data. We concluded that the butterfly pea extract can be used as an acid- base titration indicator.

Georgianna K. Oguis et al: The only leguminous plant species now known to produce a group of ultrastable cyclic plant defence peptides known as cyclotides is the butterfly pea (*Clitoria ternatea*). Cyclotides have gained considerable interest for agricultural applications, which has led to the recent approval of a butterfly pea extract as an environmentally benign insecticide (SeroX®). In this study, we sought to identify the differences between butterfly pea accessions from throughout the world in terms of cyclotide expression and toxicity towards insect cells. We demonstrate significant differences in cyclotide expression in 23 butterfly pea accessions from 11 different nations using peptide extracts from those plants. The cyclotide Cter M, which is normally the one most abundantly expressed in vegetative butterfly pea tissues, is lacking in some accessions.

These accessions contained CterM-like precursor genes that contained missense mutations that were probably causing the lack of Cter M expression, according to genomic and transcriptomic sequencing. One accession does not produce detectable quantities of the following cyclotides: cliotide T1, cliotide T4, Cter A, and Cter Q, according to peptide profiling results. When cytotoxicity was measured against Sf9 (*Spodoptera*

frugiperda) cells, it was discovered that Cter M is not necessary for cytotoxicity because accessions lacking this peptide also exhibited cytotoxicity. Overall, the findings of this study offer fundamental knowledge regarding the traits that should be considered when selectively developing butterfly pea with improved insecticidal qualities.

Kim Ngan T. Nguyen et al.: Because of their exceptional stability and promise as peptide therapies, cyclotides are cyclic miniproteins produced from plants with three overlapping disulfide links. In this research, we characterise and examine the biological activity of the cyclotides from the medicinal plant *Clitoria ternatea* (butterfly pea). We identified 41 unique cyclotide sequences, which we called cliotides, using a combined proteomic and transcriptomic approach, making *C. ternatea* one of the richest cyclotide-producing plants to date. With minimal inhibitory concentrations as low as 0.5 μM , certain members of the cationic cliotides exhibit strong antibacterial activity specifically against Gram-negative bacteria. Surprisingly, they also have strong immunostimulatory action.

Nguyen Minh Thuy et al.: Although butterfly pea flowers have a strong sensory appeal, they are not yet widely used in Vietnam. Butterfly pea flower extracts are a convenient source of natural blue food colouring. In this study, UPLC in conjunction with a UV and mass spectrometer device was used to identify the anthocyanin chemicals in butterfly pea flowers. The anthocyanin compounds' chromatograms and spectra from positive and negative ion electrospray MS/MS analysis were determined.

Five anthocyanins were found in the butterfly pea flower extract by examining the chromatograms and spectra for each ion.

These were delphinidin-3-(600 -p-coumaroyl)-rutinoside, cyanidin 3-(600 -p- coumaroyl)-rutinoside, and delphinidin-3-(p-coumaroyl) both the cis- and trans-isomers of glucose, Delphinidin-3-pyranoside and cyanidin-3-(p-coumaroyl-glucoside). Additionally, it was found that cyanidin-3-(p-coumaroyl-glucoside), delphinidin-3-(600 -p-coumaroyl)- rutinoside, cyanidin-3-(p-coumaroyl-glucoside), and delphinidin-3-pyranoside were the four anthocyanins with the highest abundance. The butterfly pea flower extract used in this investigation contained cyanidin derivatives, which were not found in the extract used in earlier experiments.

Michael Gomez et al.: *Clitoria ternatea*, also known as butterfly pea, is a versatile forage legume. In addition to providing bioactive chemicals for medical application, it serves as a cover crop and decorative plant. It can adjust to a broad range of temperature, precipitation, and altitude. The extremely appealing fodder legume known as the butterfly pea is typically chosen by animals over other legumes. It is perfect for forage and hay production because to its slender stem, big leaves, lack of bloat, and nontoxic nature. This plant is a good candidate for waste land development due to its robust growth, resistance to frost and dry spells, and strong grazing pressures. This legume's cultivation and use in animal production will ensure appropriate nutrient availability and lessen grazing strain on natural ranges. This study examines plant distribution, agronomic traits, genetic variation, and butterfly pea's use as a medicine, its chemical makeup, and its role in raising animals.

Table 1 Summary of literature review of *garcinia indica*

PARTS OF PLANT	CHEMICAL CONSTITUENT	PHARMACOLOGICAL ACTIVITY
FRUIT	Rutin, Ternatin B2, Ternatin D2.	Anti-oxidant
LEAVE	Protein, carbohydrat, steroids, phenols.	Analgesic
FLOWER	Ellagic acid	Anti-inflammatory

ROOT	Cyanidin	Diuretic activity
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AIM AND OBJECTIVE:

1. Pharmacognostic studies on flower
2. To isolate and evaluation of food dye form butterfly pea flower
3. To characterized the isolated constituents qualitatively and quantitatively using advanced instruments.

- Hot air oven
- Spray dryer
- Muffle Furnace

4. To study the presence of chemical constituent.

PLAN OF WORK:

The dissertation work has been planned to carry out in the following systematic scheme

Pharmacognostic studies:

- Collection, authentication, processing and storage of flower
- Macroscopic characters
- Microscopic characters.

Standardization of plant material:

- Determination of extractive values
- Determination of moisture content / loss on drying (L.O.D.)
- Determination of ash values.

Phytochemical investigations:

- Extraction methodology (continuous extraction using Soxhlet extractor)
- Successive extraction based on solvent polarity
- Petroleum Ether (60-80°C)
- Methanol
- Fractionation of methanolic extract with ethyl acetate.

Characterization of flower extracts for various chemical constituents by chemical methods:

Test for carbohydrate

Fehling`s test

Barford`s test

Hexose test

Test for protein

Test for fat and oil (Fixed oil)

Oil and fat test

Test for flavonoids

4. PLANT PROFILE: -

Botanical name: *Clitoria ternatea*

Taxonomical Classification

Kingdom: Plantae

Division: Angiospermae
Class: Magnoliopsida, Magnoliophyta

Order : Fabales
Genus : Clitoria

Species: *C Ternate L.*

Family: Fabaceae,
Locality: In village (Ajisipur)
Habit: Climber

Vernacular name:

Hindi: Aparajita

Marathi: Gokurna

Malayalam name: Bhirind, kokam

Sanskrit: Aparajita, Girikarnika, Vishnukrantha.

English name: Butterfly peas

Kannada name: Shankhapushpa

Botanical name: *clioria ternatea*

Botanical name: *clioria ternatea*

Family: Fabaceace

Plant type: Middle climber



Habitat: This plant is native to equatorial Asia, including locations in South Asia and Southeast Asia but has also been introduced to Africa, Australia, and the America

Leaves: The leaves are pinnate, bearing 5-7 elliptical, 3-5 cm long leaflets.

Flowers: The flowers are fleshy, deep Blue or pure white, solitary or paired, about 4cm Brod .

Fruit pods: Flattened 4 to 13 cm long and 0.8 to 1.2 cm wide with margins thickened, sparse hair when mature and pale brown. When fruit pod is unmaturred their colour is green.

Uses

Butterfly peas flower is a common ingredient in many herbal teas, mixed drinks and cosmetics products.

It is rich in antioxidants and may be linked to several health benefits, including increased weight loss, better blood sugar control, and improvements in hair and skin health.

Butterfly peas flower are also used in food dye.

Flower which is used as natural food coloring.

Traditional medicine used as a memory enhancing nootropic, antistress, anxiolytic.

Textile used to dye.

Used to dye natural fibers and is used by traditional societies

5. PHARMACOGNOSTIC INVESTIGATION:

Collection, authentication, processing and storage of flower material – Collection, authentication, processing and storage were done for the flower material

Collection: Plant specimen was collected from Ajisipur, Dist- Buldhana Maharashtra, India in the month of January 2023

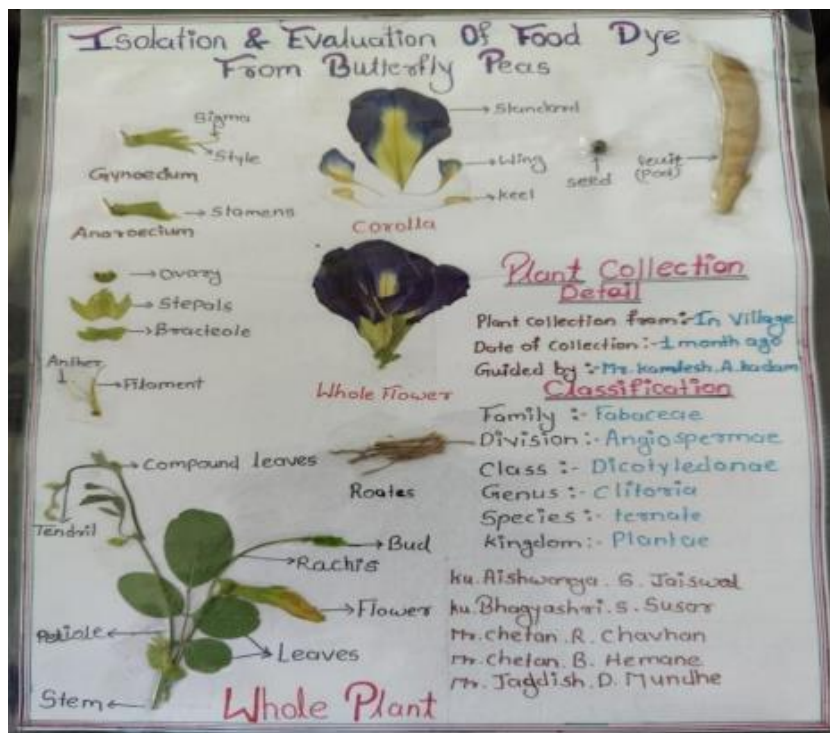


Fig 2 Herbarium sheets

Kingdom: Plantae

Division: Angiospermae

Class: Mangnoliopsida, Magnoliophyta

Order: Fabales

Genus: Fabales

Species: Clitoriaternatea L

Family: Fabaceae

Locality: In village (ajispur)

Habit: Climber

Collected by: Miss. Aishwarya S. jaiswal

Identified by: Prof. A.T. More

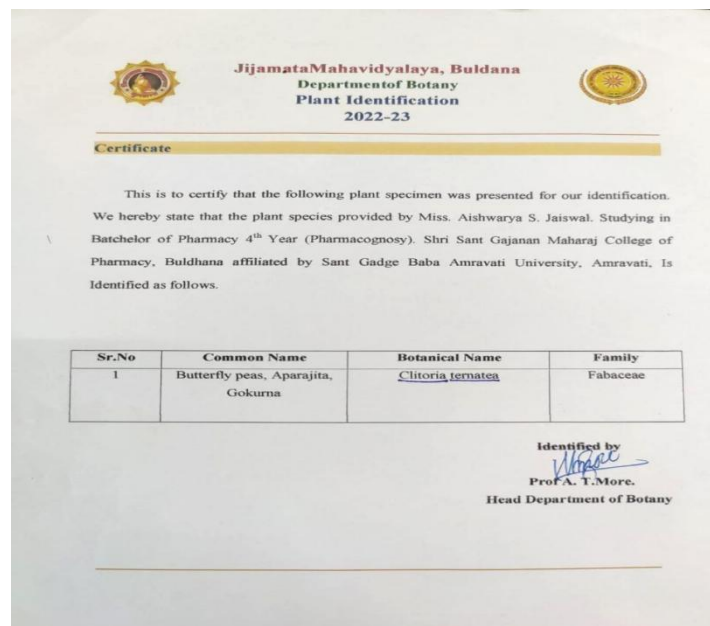


Fig 3 Authentication issued by the jijamata mahavidyalaya Buldana

MACROSCOPY OF LEAF:

10 -15 mature leaves were taken for microscopic study. Size measure means often readings

Length: 3-5 cm

Width: 1.5 - 3.5 cm

Organoleptic characters: odourless, sour taste,

Extra features:

Surface- Gabrous

Texture- Thin and leathery

Dried- Crump led



MICROSCOPIC OF LEAF:

PREPARATION OF LEAF FOR MICROSCOPY:

Fresh leaf of plant was collected and thin transverse section of middle part of the leaf was taken. This section was observed under the microscope and reported. This section was then stained with saffron red dye hydrochloric acid, concentrated H₂SO₄.



Fig 4 T.S. of Leaf butterfly pea's (*pholroglucinol:HCL*)

Macroscopy Of Flower:

Length: 4cm

Width: 3cm

Organoleptic characters:

Odour: pungent taste

Sweet: sweet

Shape: funnel

MICROSCOPIC STUDY OF FLOWER:

Fresh flower of the plant was collected and thin transverse section of T.S of flower was taken. This section was observed under the microscope and reported. This section was then stained with saffronred dye hydrochloric acid, concentrated H_2SO_4 .

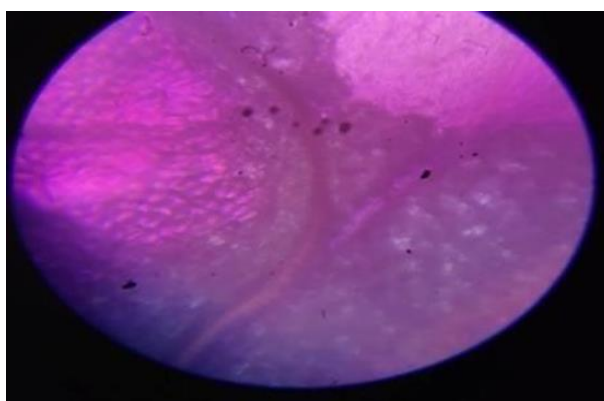


Fig 5 T. S of flower butterfly peas's (phloroglucinol:HCL)

STANDARDIZATION OF PLANT MATERIAL:**Determination of extractive values:**

This method determines the number of active constituents in each amount of medicinal plant material, when extracted with solvents. The determination of water-soluble extractive or alcohol soluble extractive is used as means of evaluating drugs the constituents of which are not readily estimated by the other means.

Determination of water-soluble extractive value:

Method: 5gm of air-dried drug was macerated with 100ml of 0.01% v/v chloroform: water of the specified strength in a closed flask for 24 hrs, it with reference to air dried drug was frequently shaken during the first 6 hrs and allowed to stand for 18 hrs. there after it was filtered rapidly without loss of water, dried in red flat-bottomed shallowed disc, dried at 105 degrees Celsius and weighed. The percentage of water-soluble extractive value was calculated with the reference to the air-dried drug.

Table 2 Determination of water-soluble extractive value

Sr.no.	Reading	I(gm)
1	Wt. of porcelain dish	50.360
2	Drug(gm)	5
3	Solvent (ml)	100
4	Wt. of dish +residue	50.526
5	Wt. of residue	0.166
6	Extractive value (% w/w)	13.5

B. Determination of fat-soluble extractive value:

Method: 5gm of air-dried drug was macerated with 100ml of ethanol of the 95% v/v strength in a closed flask for 24 hrs.; it was frequently Shaked during the first 6 hrs. an allowed to stand for 18 hrs. Thereafter it was filtered rapidly without loss of water, dried in tared flat-bottomed shallow disc, dried at 105degree Celsius, and weighed. The percentage pf ethanol soluble extractive value was calculated with reference to the air-dried drug.

Table 3 Determination of alcohols soluble extractive value

Sr no.	Readings	I(gm)
1	Wt. Of porcelain dish	50.325
2	Drug(gm)	5
3	Solvent (ml)	100
4	Wt. of dish + residue	50.429
5	Wt. of residue	0.104
6	Extractive value (%w/w)	7.9

II. Determination of ash value**Determination of total ash value:**

Method: 5gm of the air-dried crude drug was weighed in a tared silica crucible and incinerate data temperature not exceeding 450 degrees Celsius until free from carbon. After incineration the material was cooled and weighed. The percentage of ash value calculated either reference to the air drug. The average value was recorded.

Table 4 Determination of total ash value

Sr.no.	Reading	I
1	Weight of crucible (g)	18.12
2	Weight of crucible + air dried material (g)	23.12
3	Weight of crucible + ash (g)	19.13
4	total ash	1.1
5	Percentage of total ash	22

Determination of acid insoluble ash value

Method: the total ash was boiled with 25 ml of 2M hydrochloric acid for 5 minutes. The insoluble matter was collected in ashless Whatman filter paper. The collected insoluble matter was washed with hot water, ignited, and cooled in desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug. The average value was recorded.

Table 5 Determination of the acid insoluble ash value

Sr.no.	Reading	I
1	Weight of crucible(g)	18.12
2	Weight of crucible + air dried material(g)	23.12
3	Weight of crucible + ash(g)	19.13
4	Total acid insoluble ash(g)	1.8
5	Percentage of total ash acid insoluble ash(%w/w)	17

Determination of water-soluble ash value

Method: The total ash was boiled with 25ml of water for 5 minutes. The insoluble matter was collected in ashless Whatman filter paper. The collected insoluble matter was washed hot water, ignited, and cooled in desiccators and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug. The average value was recorded.

Table 6 Determination of water-soluble ash value

Sr.no.	Reading	I
1	Weight of crucible(g)	18.12
2	Weight of crucible+air dried material(g)	23.12
3	Weight of crucible+ash(g)	19.13
4	Total water-soluble ash(g)	1.9
5	Percentage of total water-soluble ash (%w/w)	15

PHYTOCHEMICAL INVESTIGATIONS:**Preparation of crude material for extraction:**

Dried flower was processed for size reduction by using Cutter Mill (portable mixer). Crushed material was passed through 60# sieve (coarse powder) for uniform particle size, which gave efficient extraction and yield of extract.

Extraction methodology:

Successive extraction method:

Specification of extraction –

Method: solid-liquid extraction.

Mode- Continuous extraction (Batch process)

Type- Successive extraction. (Increasing order of polarity)

Instrument- Soxhlet extractor. (Hot extraction)

Solvent- ethanol (60-80)

Test	Observation	Results
TEST FOR CARBOHYDRATE		Pass
a) Fehling's Test Fehling's solution A + Fehling's solution B + 1ml sample solution	Brickred ppt observed	
b) Barford's Test: 1ml Barford's reagent + 1ml sample	no red ppt observed	Fail
Hexose Test		
a) Selwinoff's Test: selwinoff's reagent 3ml + 1ml sample	Red ppt	Pass
Test For Starch		
a) Iodine Test: 3ml sample + few drops of dil. Iodine solution	No disappear colour	Fail
b) Tannic Test: tannic acid + sample	No ppt	
TEST FOR PROTEIN		Fail
Biuret Test: 3ml sample + 1ml CuSO ₄ + 2ml NaOH	No colour appear (violet or pink)	
TEST FOR FIXED OIL		Pass
a) Saponification Test: 2ml H ₂ O + 1gm powder of sample	Foam is observed	

<p>TEST FOR OIL AND FATTEST</p> <p>a) Take a glass slide and take a thin section of flower +put in Sudan red III+ after 2 min wash with 50 ml alcohol + add glycerin</p> <p>b) solubility test: 2ml H₂O+1gm sample</p>	<p>Observed under microscope red oil globule are appear (shown in fig.6)</p> <p>Soluble blue color observed</p>	<p>Pass</p>
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Rationale of method:

The method was selected for its efficient and easy separation of phytochemical constituents on the basis of polarity of solvent. The following factors were considered for the selection of extraction methodology.

Temperature:

It is the rate- limiting factor for the extraction method. Constant temperature would increase the efficiency of the extraction by increasing the diffusivity of the solvent and solubility of the solute.

Another factor is extraction efficiency, which covers wide range of factors. It consists of time, economy, and completion of extraction.

Water extraction:

The marc after exhaustive extraction with water and subjected to exhaustive Soxhlet extraction with water. The point of completion of extraction was determined by reaction with iodine vapours.

CHEMICAL TEST:

Table 7 Chemical Tests

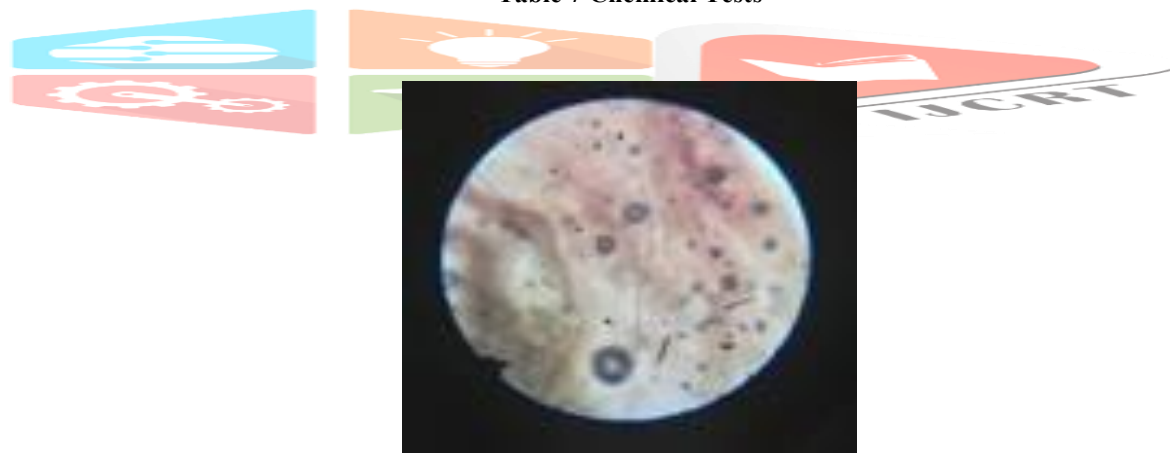


Fig 6 oil and fattest

RESULT AND DISCUSSION:

The present work was carried out on flower of *clitoria ternatea*. Family – Fabaceae and emphasis were given on pharmacogenetic, phytochemical and pharmacological studies on flower of *clitoria ternatea* to find out their usefulness to human being. This plant was collected from Ajispur. Herbarium of the plant specimen was deposited at jijamata mahavidyalaya buldana. The plant was authenticated by Prof A. T. more (head department of botany) dated 27 feb 2023.

Here more emphasis was given on pharmacogenetic, phytochemical and pharmacological studies on flower of *clioria ternatea*. The flower of *clioria ternatea* were studies for its microscopy. Flower and leaf show the presence of stomata, spongy tissue, and characteristics arrangement of vascular bundles.

Standardization of *clioria ternatea* flower was carried out, it includes determination of ash values, extractive values

Phytochemical investigation found following investigation:

Extraction of flower powder (100gm of drug) was done in Soxhlet apparatus according to the increasing order of polarity.

The extracts obtained were characterized by chemical test for rough ideas of main constituents present. Carbohydrates, oil, and fat, flavonoid, color pigments were presents in the ether extract. These findings help for isolation of constituents from each extract.

After collection of different fractions from extract, it was further processed to separate single constituents.

CONCLUSION AND FUTURE PROSPECTS

a) Conclusion:

from all the experiments perform on flower of *clitoria ternates*, it is concluded that the plants show presence of carbohydrate, fat an oil (fixed oil), flavonoids, colour pigment in other part of plant

b) Prospects of research in *clitoria ternatea*

we use the butterfly pea color extract for giving shining texture to the cloth with bleaching

It is also used as the food dye for all type of food products

It has an anti-aging property so butterfly peas also use in cosmetics

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