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Pharmacognostic Standardization and Preliminary Phytochemical Screening of Extract of *Strelitzia Reginae* Flowers

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Abstract:
This research work was proposed to study the pharmacognostic standardization parameters and phytochemicals present in the flower extract of *Strelitzia reginae* flowers. Based on this study, it can be concluded that the flowers are not just an epitome of elegant beauty but are of therapeutic importance also. The various pharmacognostic and phytochemical standards helpful in ensuring the purity, safety and efficacy of the flowers were determined. The research dealt with the comprehensive study of pharmacognostical parameters which were carried out by study of various parameters like organoleptic, powdered microscopy characteristics, physico-chemical and phytochemical studies. The flowers were washed, air dried, then the aqueous and alcoholic extracts of powdered flower material were prepared. Phytochemical Screening was done for the phytochemical profiling of the aqueous and alcoholic extracts. Physico-chemical studies included the various parameters of ash values (total ash value, acid-insoluble ash value, and water soluble ash value), moisture content, fluorescence analysis which were done for the identification, authentication and standardization of the flowers. The data generated from this study will help to elaborate the phytoconstituents present in the extract and the pharmacognostic standardization parameters necessary for its authentication. It would be easy to establish the botanical identity of the plant and its standardization parameters.

Keywords: Pharmacognostic Standardization, Phytochemical Screening, Physico-chemical standardization parameters, Botanical identity, Ash values.

1. Introduction:

The study was performed to elaborate the pharmacognostic and phytochemical evaluation of the flowers of *Strelitzia reginae*. The flower and its extract were studied.

Pharmacognostic Standardization [(1)]

All the crude drugs that are being used as sources of drugs for the formulation of medicaments need to be standardized as per the specifications of World Health Organization .As in today's world the side effects of allopathic drugs is too high and can cause serious damage to various organs of the human system, so man has turned towards mother nature to cure himself.

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Herbal Drugs are playing a crucial role in today's health care scenario. Herbal drugs have been used since times immemorial to alleviate certain health conditions and as nutraceuticals. The main advantage with the herbal drugs is that they are natural in origin and cause lesser side effects than the allopathic drugs. A major drawback is that the standardization parameters of certain crude drugs are not available. The main aim of this project is to explore the nature (mother earth) and to set some standards for the drugs that have not been developed till now.

2. Materials And Methods:

2.1. *Procurement of the flower and its authentication:*

The flower was procured in the month of September from a local florist (Ashok Florist) at Bhopal in M.P. It was identified and authenticated in the Department of Botany, Safia College, and Bhopal (M.P.) by the botanist **Dr Zia-Ul-Hassan** and a voucher specimen of the plant was deposited at the herbarium for future reference.

2.2. Preparation of Plant Extract:

Plant was shade dried and powdered using an electric mixer-grinder and sieved through sieve number 22.Then, the coarsely powdered plant material was macerated with solvents in increasing order of polarity i.e. ethanol, water. After 24 hours, the extracts were filtered through a Whatmann filter paper and concentrated in a rotary vacuum evaporator followed by removal of the residual moisture in a dessicator.

2.3. Macroscopic and microscopic analysis: *(2.3.1)*

Macroscopic characters of the plant were analyzed by WHO Quality Control Methods of herbal remedies. Powder microscopy was performed according to the method of **Khandelwal.** The photomicrographs of different magnifications were taken with Olympus C x 2 1i trinocular microscope under illumination of halogen lamp.

2.4. Physicochemical Parameters: *(2.4.1, 2.4.2, 2.4.3)*

Physicochemical parameters such as ash value; moisture content and extractive values were determined according to the procedures mentioned in WHO Guidelines for herbal materials as follows*:*

2.4.1: Determination of loss on drying:

10 gms of drug was weighed and placed in a tared evaporating dish. It was air dried at 105 °C for 5 hours and weighed. The drying and weighing was continued at 1 hour intervals until the difference between 2 successive weighings corresponded to not more than 0. 25%. A constant weight was supposed to have reached when 2 successive weighings after drying for 30 minutes and cooling for 30 minutes in a dessicator, showed a difference of not more than 0.01gm.

2.4.2. Determination of Ash values of Strelitzia reginae flowers:

2.4.2. a) Determination of total ash value of Strelitzia reginae flowers:

Weighed accurately about 3 gm of the powdered drug in a tared silica crucible. Incinerated the powdered drug material by gradually increasing the heat until free from carbon at a

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temperature not exceeding 450°C and cooled it, then kept it in a dessicator. The process was repeated to obtain a constant weight. Weighed the ash and calculated the percentage of total ash with reference to the air dried sample.

2.4.2. b) Determination of acid-insoluble ash of Strelitzia reginae flowers:

Boiled the total ash obtained as above for 5 minutes with 25 ml of 2 M hydrochloric acid ,filtered and cooled the insoluble matter on an ashless filter paper, washed the filter paper with hot water, ignited for 15 minutes at a temperature not exceeding 450° C in a tared silica crucible, cooled and kept it in a dessicator. Weighed the residue and calculated % acid-insoluble ash of the powdered drug with reference to the air dried drug.

2.4.2. c) Determination of water soluble ash of Strelitzia reginae flowers:

Boiled the total ash obtained as above for 5 minutes with 25 ml of water, filtered and cooled the insoluble matter on an ashless filter paper, washed the filter paper with hot water, ignited for about 15 minutes at a temperature not exceeding 450° C in a tared silica crucible , cooled and kept it in a dessicator. Weighed the residue and calculated % water soluble ash of the powdered drug with reference to the air dried drug.

2.4.3. Determination of extractive values of Strelitzia reginae flowers:

2.4.3. a. Determination of alcohol-soluble extractive value of Strelitzia reginae flowers:

Macerated about 5 gram accurately weighed coarsely powdered air-dried drug with 100 ml of alcohol (90%) in a stoppered flask for 24 hours, shaking frequently during the first 6 hours and allowed it to stand for 18 hours, filtered it rapidly through filter paper taking precaution against excessive loss of alcohol, evaporated 25 ml of alcoholic extract to dryness in a tared flat bottomed shallow dish. Dried at 105° C and weighed. Kept it in a dessicator. Calculated % w/w of alcohol (90% v/v) **soluble** extractive value with reference to the air-dried drug.

2.4.3. b. Determination of water soluble extractive value of Strelitzia reginae flowers:

Macerated about 5 gram accurately weighed, coarsely powdered air-dried drug with 100 ml of water in a stoppered flask for 24 hours, shaking frequently during the first 6 hours and allowed it to stand for 18 Hours, filtered it through a filter paper, evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dried the extract at 105° C and weighed. Kept it in a dessicator. Calculated % w/w of water soluble extractive value with reference to the air-dried drug (***Strelitzia reginae*** **flowers)**.

2.5. Qualitative Preliminary Phytochemical screening of crude drug. *(2.5.1. , 2.5.2.)*

The plant material was subjected to preliminary phytochemical screening for the detection of various constituents .The first step was to extract the air dried powdered plant material (***Strelitzia reginae*** **flowers)**.

Chemical tests were performed on the aqueous and alcoholic extracts so obtained for the phytochemical screening.

Chemical tests were performed on the ash for the detection of elements present**.**

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Table I .: Procedure for qualitative preliminary phytochemical screening of extract of *Strelitzia reginae* flowers:

S No	Experiment	Observation	Inference
	i)Test for Carbohydrates		
1	**a) Molish's Test**		
	To 2-3 ml aqueous extract; added a few drops of α-naphthol solution in alcohol; shaken and added concentrated H_2SO_4 along the sides of the test tube.	If violet ring was formed at the junction of two liquids	**+ve**
	b)Test for Reducing Sugars:		
	i) Fehling's test:		
	1 ml Fehling's A and 1ml Fehling's B solution were mixed and boiled for 1 minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 minutes.	If yellow then brick red precipitate was formed	**+ve**
	ii) Benedict's Test :		
	To the crude drug extract; added equal volume of Benedict's reagent and heated for 1-2 minutes in a boiling water bath and cooled.	If red precipitate was formed	**+ve**
	c) Test for Pentose Sugars:		
	i) Mixed equal volume of test solution and hydrochloric acid .It was heated and then added a crystal of phloroglucinol.	If red colour was formed	**+ve**
	d) Test for Non-Reducing Sugars: If test solution does not respond to fehling's test and benedict's test, then iodine test was performed: Mixed 3 ml **of** test solution and added a few drops of dilute iodine solution .	If blue colour appeared ;disappeared on boiling and reappeared on cooling	**+ve**
	e) Test for Gums:		
	Hydrolysed test solution using dilute hydrochloric acid, then performed fehling's test or benedict's Test.	If red colour was formed	**+ve**
	f) Test for Mucilage:		
	i) To powdered drug material, added ruthenium red solution. ii)To powdered drug material, added water or aqueous potassium hydroxide	If red colour was formed. If drug swelled	**+ve** **+ve**
	a) Test for Proteins:		
2	**Biuret Test:**		
	To 3ml of test solution, added 4% sodium hydroxide solution + a few drops of 1% copper sulphate solution.	If violet or pink colour was observed	**+ve**
	ii) Million's test: Mixed 3ml test solution with 5 ml millon's reagent .	If white precipitate was formed which turned red on heating	**+ve**

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	b) Test for Proteins containing Sulphur :		
	Precipitation test:		
	i) To test solution; added 5% w/v copper sulphate solution ii) To test solution; added alcohol	If white precipitate was formed	**+ve**
	Test for Alkaloids:		
3	Stirred a small portion of the solvent free alcoholic and water extracts separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was then tested with hager's reagent.	If yellow precipitate was formed	**+ve**
	Test for Fixed oils and Fats:	If oil stains were	
4	Pressed a small quantity of petroleum ether and benzene extracts separately between 2 filter papers.	present on the paper.	**+ve**
	Test for Saponins:	If 1 centimetre layer of	**+ve**
5	Diluted 1ml of alcoholic and aqueous extracts separately with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes.	foam was formed	
6	**Test for Volatile oils:**		
	Taken about 50 gm of powdered material in a volatile oil estimation apparatus and subjected it to hydro-distillation	If volatile oil layer was formed over water layer; noted the volume of volatile oil	**+ve**

3 . Results :

Macroscopic characters of the flowers were analyzed by WHO Quality Control methods of herbal remedies.

Table II: Macroscopic features of fresh flower sepals:

S No	Macroscopic Features of Fresh Flower Sepals	Observations
1	Colour	Orange
2	Colour(At Base)	White (Inverted V Shape, cream colored of a length 2.5cm)
3	Shape	Lanceolate
4	Surface	Smooth and glossy with a waxy coat. Longitudinally striated and pitted all over.
5	Length of sepal	15.9 cm
6	Breadth of sepal	2.7 cm
7	Extrafeature	Tapering at apex and base
8	Odour	Aromatic
9	Margin	Simple, Entire

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Table III : Macroscopic Features of fresh flower outer most sepal (unopened in bud form)

S No	Macroscopic Features of Fresh Flower outermost Sepal (unopened)	Observations
1	Colour	Green
2	Colour (At Base)	Red
	(At Middle)	Dark Green
	(At Tip)	Light Green
3	Shape	Lanceolate
4	Surface (Outer)	Dotted and Pitted Waxy with Longitudinal Striations
5	Surface (Inner)	Smooth with interconnection between veins .Parallel Venation.
6	Length of sepal	25 cm
7	Breadth of sepal	7cm
8	Extrafeature	One flap covering the other and containing rest of the sepals
9	Odour	None
10	Margin	Simple; Entire
11	Apex	Acute
12	Breadth of apex	7 mm
13	1 Longitudinal striation line thickness	1 mm
14	Spacing between 2 striating lines	2 mm
15	Thickness of Sepal	3mm
16	Fractured Surface	Rough with projecting Fibres

Table IV: Macroscopic Features of Fresh Flower Petals

S No	Macroscopic features of fresh flower petal	Observations
1	Colour	Purple
2	Colour(At Base)	White
3	Shape	Cordate
4	Surface	Rough; dotted
5	Length of sepal	8.5 cm
6	Breadth of sepal	2.2cm

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7	Extrafeatures	Fine striations are present on the surface
8	Odour	Specific ; characteristic
9	Margin	Simple

Image Number 3.1: Image of *Strelitzia reginae* flower

3.2. Microscopic analysis:

Powder microscopy was performed according to the method of Khandelwal.

Table V : Powder Microscopy of dried flowers of *Strelitzia reginae*

S No	Microscopic features	Observations
1.	Ovary	Present in flowers as a cluster ; **Shape of Ovary**: Oval or rounded. Present in clusters of 12-15.
2.	Crystals	Calcium Oxalate Crystals. **Characteristic Features**: They are present as isolated individual crystals. **Shape:** Prismatic; Rhomboidal; cubical, sometimes in groups of two.
3.	Epidermis	It is present in 10-15 layers arranged as a wall. They are thin walled ;living cells forming the outermost layer of leaf blade. **Shape:** Rectangular cells joined end to end; concisely arranged in rows.

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4.	Fibers:	Cylindrical ;Thin ;hair like; curved in shape; sickle shaped; Thick in centre and tapering at the ends. They are lignified.
5.	Phloem	They are non lignified fibres and perform the function of conduction of food in the flowers.
6.	Xylem Vessels	They are spiral; twisted like a ribbon and swirling through the vessel providing it the necessary support.
7.	Xylem Tubes	It performs the function of conduction of water through the flowers. They are lignified.

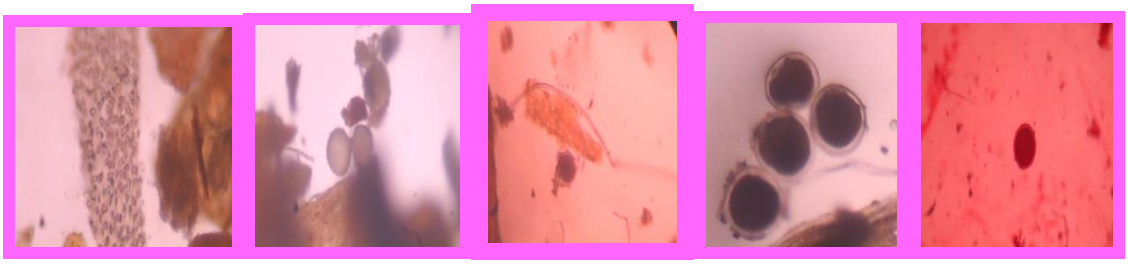

Image 3.1. Images of Ovary

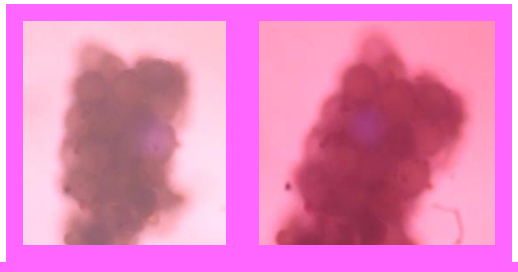

Image 3.2.: Images of Cluster of Ovaries

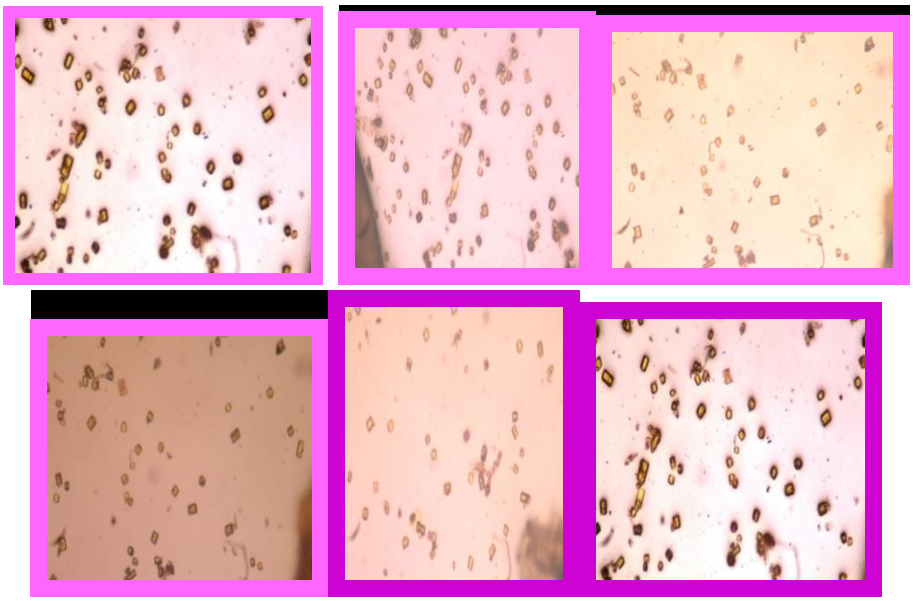

Image 3.3.: Image of Calcium Oxalate

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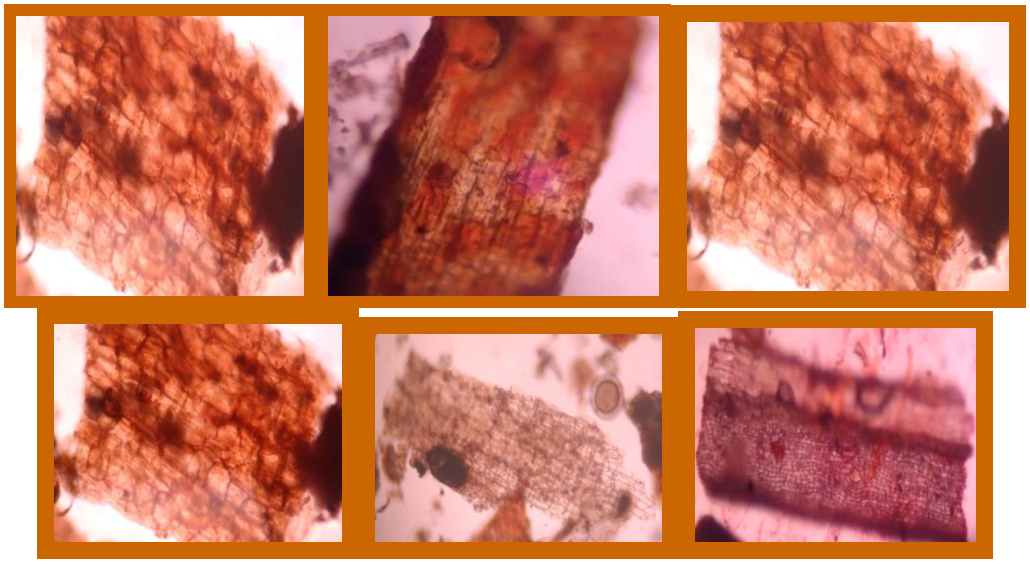

Image 3.4: Images of Epidermis

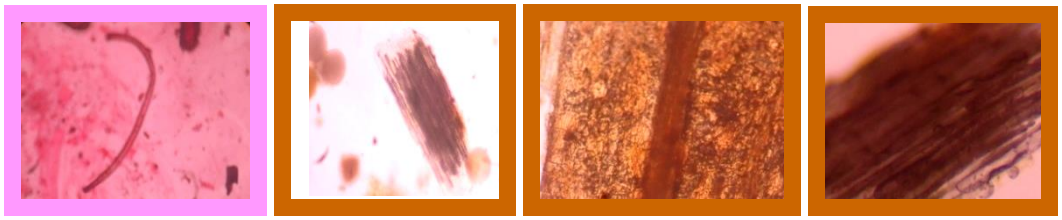

Image 3.5.: Images of Phloem Fibre

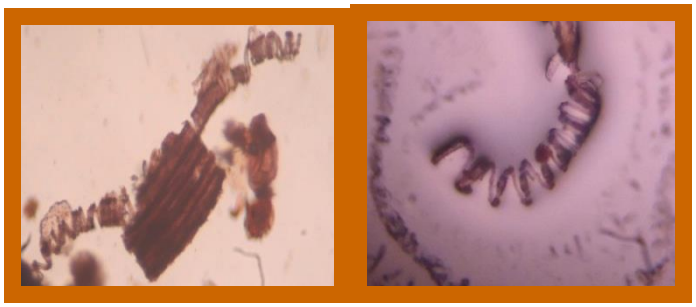

Image 3.6: Image of Xylem Vessels

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3.3. Phytochemical screening

The aqueous and alcoholic (ethanolic extract) extracts were prepared and the *in-vitro* chemical tests were performed.

Table VI: Extractive values of *Strelitzia reginae* flowers (alcohol soluble and water soluble)

S No	Weight of air-dried drug taken(gms)	Volume of solvent used for extraction (ml)	Time of extraction (Hrs)	Weight of extract obtained(gms)	Colour of extract obtained	Consistency of extract obtained	Extractive Values (%)
1	**500gm**	**2l alcohol**	**72 Hours**	**45gm**	**Reddish Brown**	**Dried Mass**	**9%w/w**
2.	**400gm**	**2l water**	**72 Hours**	**126.74gm**	**Reddish brown**	**Dried Mass**	**32%w/w**

Table VII : Physicochemical standardization parameters of flowers of *Strelitzia reginae:*

S. No.	Parameters studied	Values Observed
A	**Total Ash Value**	5.80%
B	**Acid insoluble Ash Value**	0.89%
C	**Water soluble Ash Value**	3.20%
	Ether soluble extractive	0.20%
	Loss on drying	7.54%
	Moisture Content	7.20%

Table VIII: *In-vitro* chemical tests performed on aqueous and alcoholic extracts of *Strelitzia reginae* flowers.

S.No.	**Experiment**	**Result**	
		Ethanolic Extract	**Aqueous Extract**
1. Alkaloids			
1.1	Wagner's reagent test	-ve	+ve
1.2	Hager's reagent test	-ve	+ve

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2. Carbohydrates			
2.1	Molish's test	-ve	+ve
2.2	Barfoed's test	-ve	+ve
3. Test for Reducing Sugar's			
3.1	Fehling's test	-ve	-ve
3.2	Benedict's test	-ve	-ve
4. Flavonoids			
3.1	Alkaline reagent test	+ve	+ve
3.2	Lead acetate test	+ve	+ve
5. Glycoside			
4.1	Legal's test	-ve	-ve
4.2	Keller- Killiani test	-ve	-ve
6. Tannin and Phenolic compounds			
6.1	Ferric chloride test	+ve	+ve
6.2	Lead Acetate test	+ve	+ve
6.3	Gelatin test	+ve	+ve
7. Saponin			
7.1	Foam Test	+ve	+ve
8. Test for Proteins and amino acids			
8.1	Ninhydrin test	-ve	-ve
8.2	Biuret test	-ve	-ve
9. Test for Triterpenoids and Steroids			
9.1	Salkowski Test	+ve	+ve
9.2	Liebermann and Burchard's test	+ve	+ve
10. Tests for Fats and Oil			
10.1	Solubility Test	-ve	-ve

Table IX : Fluorescence Analysis of the powdered drug of *Strelitzia reginae* flowers with various solvents:

S. No.	Solvents	Short UV	Long UV	Visible
	Normal Coarse Powder	Green	Grey	Orange-Brown
	50% H_2SO_4	Dark Green	Dark Grey-Black	Brown
	50% HNO_3	Dark Green	Dark Brown	Golden Orange
	5% NaOH	Dark Green	Dark Grey	Brown
	1N Methanolic NaOH	Green	Dark Grey	Brown

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	5% KOH	Dark Green	Grey	Brown
	Methanol	Green	Grey	Yellowish Brown
	Conc. HCl	Dark Green	Brown	Orange-Brown
	Conc. H_2SO_4	Dark Green	Black	Brown
.	Ammonia	Dark Green	Grey	Brown
.	Conc. HNO_3	Green	Grey	Yellowish Brown

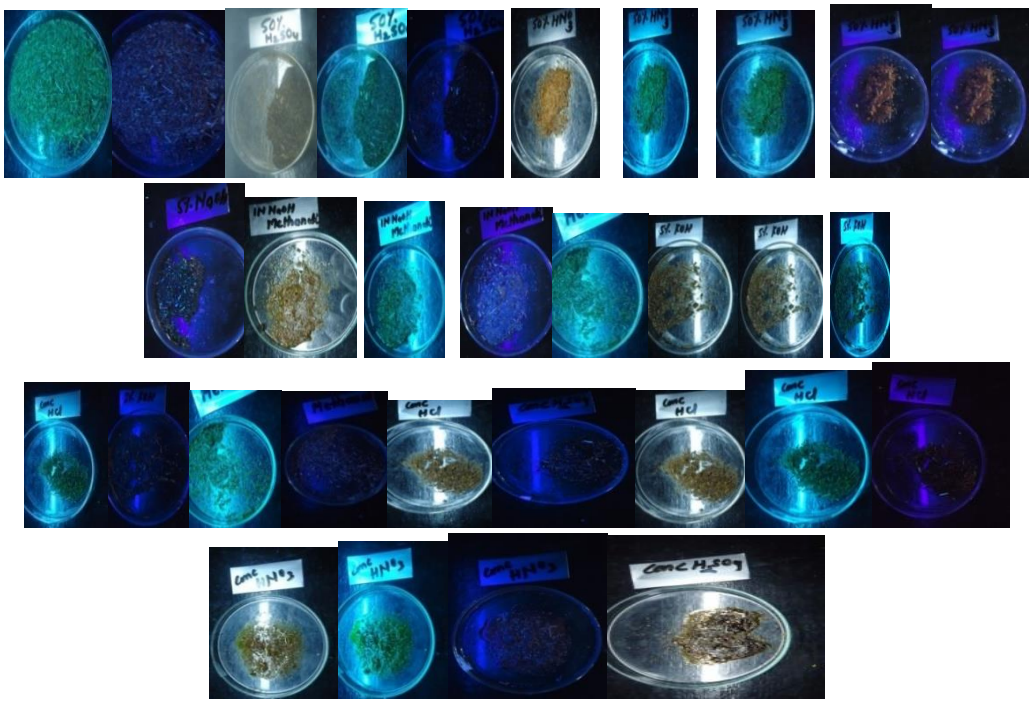

Image 3.7.: Images of fluorescence of powdered drug with various reagents

4. Discussion

The pharmacognostic standardization parameters were studied and have been revealed. The flowers of ***Strelitzia reginae*** were chosen and research was done for the study of their diagnostic features. The authentication and pharmacognostic profiling of the flower was performed .The study revealed that the flowers are not just an epitome of beauty.

The flowers were selected and were authenticated by Dr Zia-ul-Hassan at the Department of Botany, Safia College, Hamidia Road ,Bhopal.

A voucher specimen of the sample was submitted for referencing in future. The plant was authenticated and named "***Strelitzia reginae*** flower" or **"Birds of Paradise"** .The nomenclature of the flower was done according to the shape of the flower which resembles the beak of a bird. It is also named as the "***Crane Flower"***. The shape of the flower is similar to a bird in flight as exemplified by broad beautiful orange sepals with beautiful purple cordate petals, enclosed with in a thick green perianth possessing a reddish tinge at its periphery. The androecium is white, long with cordate anthers having, creamish pollen

grains, the ovary being positioned at the base of the flower, possessing the ovules which are further converted to seeds with an orange seed coat and black aril.

The flower is an exotic variety due to the vividity in the perianth, sepals and petals. The sunbirds are attracted towards the flower which helps in the pollination of the flowers. They transfer pollen grains from one flower to another while sucking the nectar of the flowers.

The flowers were collected from a Local Florist Shop (Ashok Florist) at 10 Number Market, Bhopal. They were washed, dried in shade, powdered and passed through sieve number 22, stored in a plastic bag for further studies.

The powder microscopy and fluorescence analysis were performed with a series of reagents to identify the presence of unsaturated compounds which may act as the chromophoric groups. The powder microscopy revealed the presence of ovary present in clusters of 12-15.The next characteristic feature was the presence of calcium oxalate crystals which were prismatic; rhomboidal and cubical in structure. The fibres were cylindrical, thin and hair like, curved in shape ,sometimes sickle shaped, thick in center and tapering towards the ends, lignified in nature. The phloem fibres are non lignified and perform the function of conduction of food in the flowers. The xylem vessels are spiral; twisted like a ribbon and swirling through the vessel providing it the necessary support. The xylem tubes are lignified.

The shade dried flowers were defatted with Petroleum Ether and then extracted with alcohol and water, with the help of maceration. These solvents were chosen as the solubility of the main phytoconstituent in these solvents is high. The alcoholic and aqueous extracts obtained were reddish brown in colour and had a % yield of 9% w/w for aqueous extract and 31.685% w/w for alcoholic extract

The Pharmacognostic standardization of the flowers was performed and reported. The physico-chemical standardization parameters included a comprehensive study of loss on drying, moisture content, total ash value, acid-insoluble ash value, water soluble ash value, ether soluble extractive value. The physico-chemical standardization parameters studied were found out to be as moisture content 7.20%, loss on drying was 7.54%, total ash value was 5.80%, acid insoluble ash was 0.89%, water soluble ash was 3.20% , ether soluble extractive was 0.20%.

The qualitative preliminary phytochemical screening was performed on the aqueous and alcoholic extracts to determine the various metabolites present in the extract. The phytoconstituents present in aqueous extract were revealed to be alkaloids, carbohydrates, flavonoids, tannins and phenolic compounds, saponins, triterpenoids and steroids. The phytoconstituents present in alcoholic extract were found out to be flavonoids, tannins and phenolic compounds, saponins, triterpenoids and steroids.

The fluorescence was observed in ultra-violet light on treatment with the following reagents : 50% H_2SO_4, 50% HNO_3, 5% NaOH, 1 N Methanolic NaOH, 5% KOH, Methanol, Concentrated HCl, Concentrated H_2SO_4 ,Ammonia ,Concentrated HNO_3 .

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