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PHYTOCHEMICAL STUDIES ON THE LEAF EXTRACTS OF *STRELITZIA REGINAE*

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ABSTRACT

The plant *Strelitzia reginae* is a widely cultivated ornamental plant which is a native of South Africa. The flower of this plant is known as Bird-of-paradise or crane flower. It is a monocot plant belonging to the order- zingiberales, family – Strelitziaceae, and genus - Strelitzia. The phytochemical study on the leaf extract of *Strelitzia reginae* is done for the first time. Fresh leaves were collected, shade dried, and a crude extract was prepared using soxhlet extraction method. The extractive value of each solvent was calculated and preliminary biochemical studies on the leaf extracts of *Strelitzia reginae* were conducted. The solvents used for extraction namely petroleum ether, chloroform, methanol, and distilled water is chosen based on their increasing polarity. The extractive value is highest with methanol. Phytochemical studies revealed the presence of primary metabolites such as carbohydrates, proteins, oils, and fats as well as secondary metabolites such as phenols, flavonoids, steroids, saponins, alkaloids, tannins, and glycosides.

Keywords: Phytochemical analysis, Soxhlet extraction, biochemical tests, *Strelitzia reginae* leaf, Primary metabolites, Secondary metabolites

INTRODUCTION

Phytochemicals are biomolecules present in plants. Biomolecules are chemical compounds found in living organisms. Some of these compounds are bioactive and have therapeutic value. They are present in minute quantities in plants, animals, fungi, and bacteria. Synthesizing bioactive compounds helps in fighting various diseases such as heart disease, cancer, mental health problems, etc. Examples of bioactive compounds include lycopene, resveratrol, lignan, tannin, indoles, vincristine, vinblastine, etc.

Preliminary studies on the leaf extracts of *Strelitzia reginae* reveal the presence of a few primary and secondary metabolites. Further specific tests for the separation of bioactive compounds are in progress. Primary metabolites help in growth and they have nutritive value for the plants. Secondary metabolites help the plant in self-defence and have been harvested by man for their therapeutic value.

Strelitzia reginae is a popular ornamental plant native to South Africa. The flowers of the plant are commonly called bird-of-paradise flower or crane flower. The plant is a monocot belonging to the Order- Zingiberales, Family – Strelitziaceae, and Genus- Strelitzia. The name *Strelitzia* is in honour of Queen Charlotte, wife of George III, from the house of Mecklenburg-Strelitz. The word *reginae* is derived from Latin which means 'of the queen'.

There are five species of plants in the genus Strelitzia. They are: *Strelitzia alba, Strelitzia juncea, Strelitzia nicolai, Strelitzia caudate, and Strelitzia reginae*. It is a common ornamental plant in Southern California and has been chosen as the Official Flower of the City of Los Angeles.

The plant is quite interesting because recorded data indicates the presence of animal protein bilirubin in the aril and sepals of the flowers. The roots of *Strelitzia reginae* are used in South African traditional medicine to treat diseases caused by bacterial pathogens, particularly urinary tract infections (UTIs) and sexually transmitted infections (STIs). A Literature survey suggests that no research has been carried out on the leaves of *Strelitzia reginae*. Therefore, the present research is aimed at studying the phytochemicals available in this plant, particularly the leaves. The present paper presents a preliminary analysis of the phytochemicals present in the leaf extract.

MATERIALS AND METHODS

Fivekilograms (5 kg) of mature leaves of *Strelitzia reginae* were collected from the garden of the Raman Research Institute, Bangalore, Karnataka, in February 2020.

Soxhlet extractor available in Biopharmaceutical and Nanobiotechnology Laboratory, Department of Biotechnology, Gulbarga University, Kalaburagi.

Petroleum ether (0.117), chloroform (0.259), methanol (0.762), and distilled water (1.0) are used as solvents.

Methods

Identification of the leaf

The average length of *Strelitzia reginae* leaves is around 122 cm. The plant is identified and authenticated by Dr.Sanjeet Kumar, CEO, Ambika Prasad Research Foundation, Bhubaneswar, Odisha.

Preparation of the leaves for the extraction process

The leaves were first washed thoroughly with tap water, later with distilled water and shade dried for twenty days. The dried leaves were powdered using a mixer and 496

Figure 1: *Strelitzia reginae* plant at RRI campus, 2020

grams of dried leaves powder was obtained.

Soxhlet extraction:

Soxhlet extractor is laboratory equipment invented in 1879 by the German agricultural chemist, Franz von Soxhlet. The Soxhlet extraction process is known as a hot continuous extraction process. The main advantage of this method is that it ensures maximum extraction with minimum quantity of solvent[6]. 92 grams of the powdered leaf of *Strelitzia reginae* (with grades between moderately coarse and coarse) was taken into the main chamber of the Soxhlet extractor. Based on the polarity index of the solvents -petroleum ether (0.117), chloroform (0.259), methanol (0.762), and distilled water (1.0) - were taken in the same order and the extraction process was carried out. Around 500 ml of each solvent was used for 92 grams of the plant material.

1.84 grams of dried extract was obtained with petroleum ether, 1.16 grams of dried extract was obtained with chloroform, 9.05 grams of dried extract was obtained with methanol and 4.96 grams of dried extract was obtained with distilled water. All these extracts were dried and preserved in aseptic containers and kept in the refrigerator for future use.

Extractive values are used for extraction and evaluation of crude drugs. Extractive values by different solvents are used to assess quality and purity and to detect adulteration.

The extractive value of the various solvents are calculated using the formula

*Extractive value = (Weight of the dried extract / Weight of the plant material) * 100*

Plant material: *Strelitzia reginae* leaf coarse powder

Figure 2: *Strelitzia reginae* leaves (5 kg)

Figure 3: Processed dry leaves of *Strelitzia reginae*

Figure 4: Size of Strelitzia reginae leaf (122 cm)

Table 1: Extraction conditions with different solvents

Solvent	Temperature	Duration of extraction	Ratio of material to solvent
Petroleum ether	60 degree centigrade	10 hours	1: 7
Chloroform	60 degree centigrade	12 hours	1: 7
Methanol	65 degree centigrade	12 hours	1: 7
Distilled water	100 degree centigrade	1 hour	1: 7

Table 2: Extractive value of various polar and non-polar solvents

Name of the solvent	Weight of the dried extract	Weight of the plant material	Extractive value
Petroleum ether	1.18 grams	92 grams	1.282
Chloroform	1.16 grams	92 grams	1.260
Methanol	9.05 grams	92 grams	9.836
Distilled water	4.96 grams	92 grams	5.391

Figure 5: Leaf extracts of *Strelitzia reginae* using solvents

Figure 6: Carbohydrate test results for *Strelitzia reginae* leaf extracts

Phytochemical analysis of *Strelitzia reginae* leaf extract

Phytochemical screening was done to test the various primary and secondary metabolites in the leaf extracts of *Strelitzia reginae* using petroleum ether, chloroform, methanol, and distilled water as solvents. The phytochemicals were detected by colour tests following the standard protocol as described by Kokate *et al*, (1995). The stock was prepared by dissolving 20 milligrams of the *Strelitzia reginae* leaf extract in 60 ml of respective solvents and

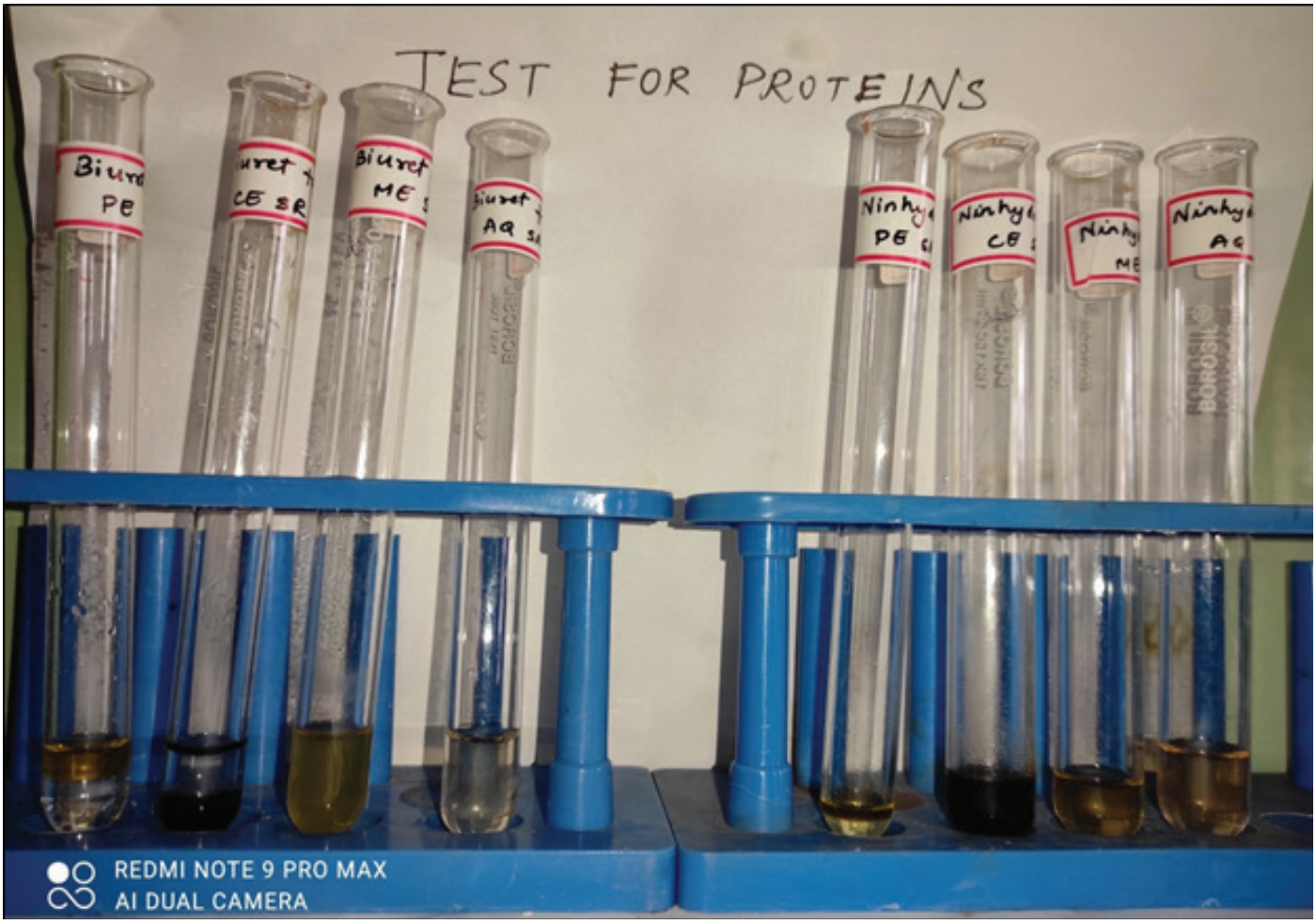

Figure 7: Protein test results on leaf extracts of *Strelitzia reginae*

Table 3: Test results for primary metabolites (+ indicates presence and – indicates absence)

Sl. No.	TEST	PE	CE	ME	AQ
1	**Test for carbohydrates**				
	• Mollisch's test	+	+	+	+
	• Fehling's test	+	-	+	+
	• Benedict's test	+	+	+	+
	• Anthrone test	+	+	+	+
2	**Test for proteins**				
	• Biuret test	-	-	-	-
	• Ninhydrin test	+	+	-	-
3	**Test for oils and fats**				
	• Spot test	+	+	+	+
	• Saponification test	+	+	+	+

the solution was filtered using Whatman filter paper No.1. The filtrate obtained was used for phytochemical tests.

Qualitative analysis of primary metabolites

Primary metabolites are compounds directly involved in the metabolic pathways of an organism which are necessary for growth, development, and reproduction. The primary metabolites usually occur in a high quantity and they can be extracted easily through simple extraction procedures. They are found in most cells throughout the body and are also termed as central metabolites. Primary metabolites do not have pharmacological activities against other factors. Primary metabolites are categorised into two groups-

- Primary essential metabolites such as carbohydrates, proteins, vitamins, enzymes, lipids, etc.
- Primary metabolic end products such as ethanol, lactic acid, certain amino acids, etc.[7]

Listed below are the various tests conducted to detect primary metabolites.

Test for carbohydrates

Molisch test: Around 2 ml of the *Strelitzia reginae* leaf extract is mixed with one or two drops of Molisch reagent and then 2 ml of concentrated sulphuric acid is added along the sides of the test tube[16]. The test tube is allowed to stand for 2 minutes. Formation of a reddish violet ring at the interfacebetween the layers of *Strelitzia reginae* leaf extract and concentrated sulphuric acid indicates that the

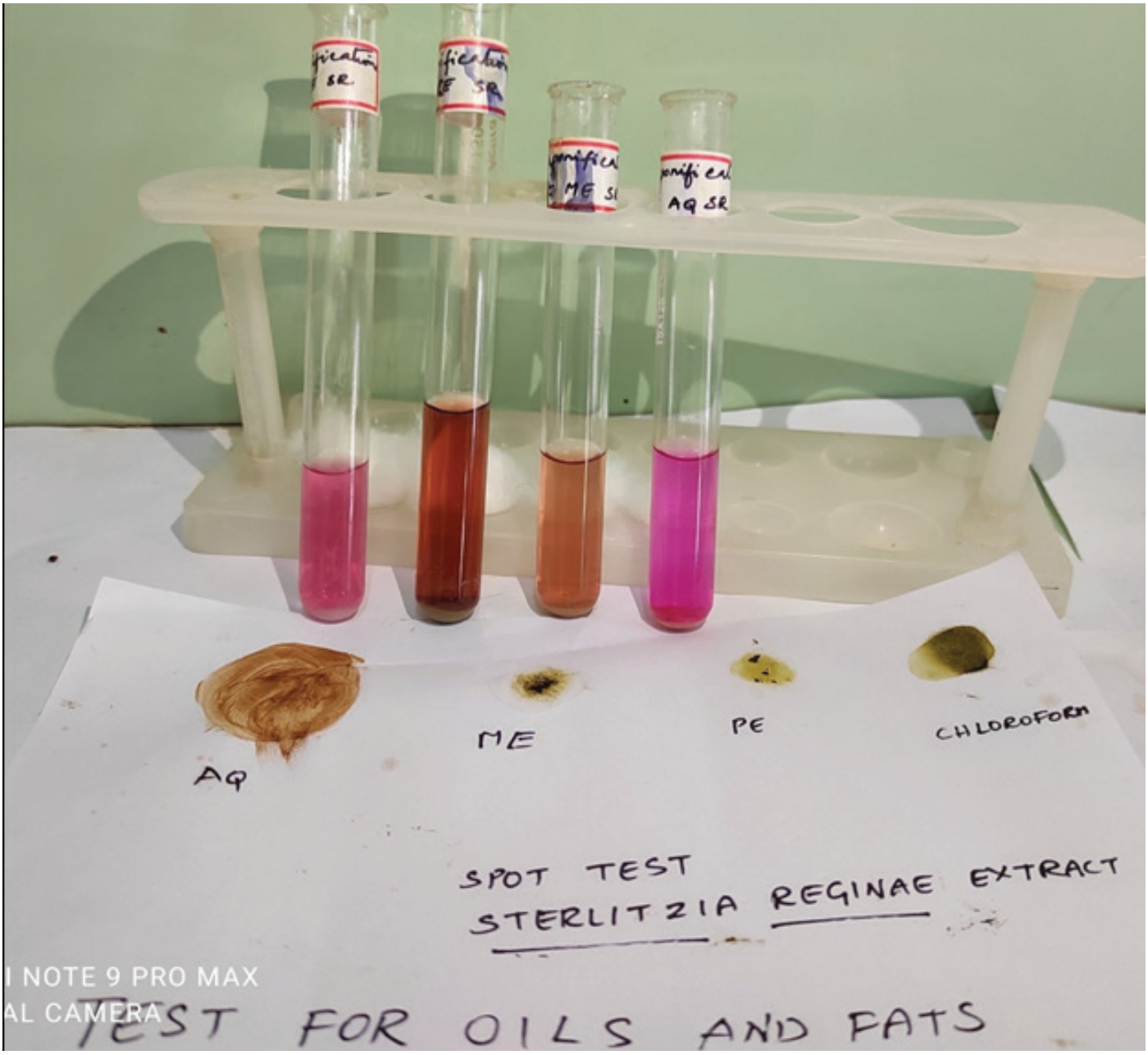

Figure 8: Oil andFat test results on leaf extracts of *Strelitzia reginae*

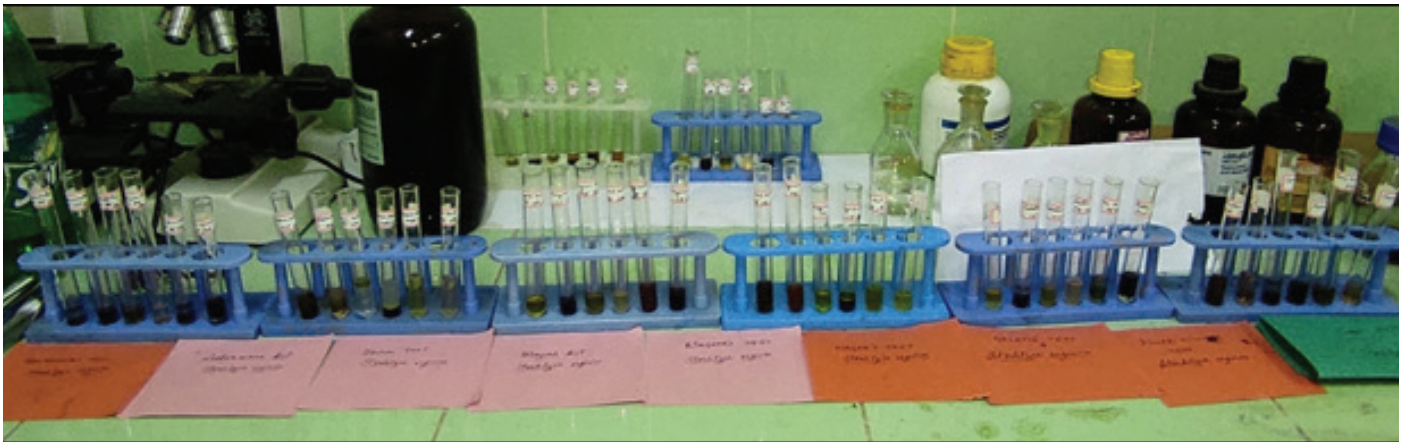

Figure 9: Secondary metabolite test results for *Strelitzia reginae* leaf extracts

carbohydratesare present in the *Strelitzia reginae* leaf.

Fehling's test: Around 2 ml of the *Strelitzia reginae* leaf extract is mixed with 2 ml Fehling's reagent. The mixture is boiled in a water bath for 3 minutes. A reddish brown precipitate is formed which suggests the presence of carbohydrates.

Benedict'stest: Around 2 ml of the *Strelitzia reginae* leaf extract is mixed with Benedict's reagent. The mixture

Table 4: Test results for secondary metabolites

Sl. No.	TEST	PE	CE	ME	AQ
1	**Test for phenols**				
	Phenol test	-	-	-	-
	Ellagic test	-	+	-	-
2	**Test for flavonoids**				
	Flavonoid test	-	-	+	+
	Shinoda test	-	-	+	+
	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
3	**Test for oils and fats**				
	Spot test	+	-	-	-
	Saponification test	+	+	-	-
4	**Test for Saponins**				
	Foam test	-	+	+	+
5	**Test for alkaloids**				
	Mayer's test	-	-	-	+
	Wagner's test	+	-	+	+
	Hager's test	+	-	-	-
6	**Test for tannins**				
	Ferric chloride test	-	-	+	+
	Gelatin test	-	-	+	+
7	**Test for glycosides**				
	Keller-Kiliani test	+	-	+	+
	Concentrated H_2SO_4 test	-	-	-	-

is then kept in a hot water bath for 3 minutes. A reddish precipitate is formed which suggests the presence of carbohydrates.

Anthrone test: Around 2 ml of the *Strelitzia reginae* leaf extract is mixed with 1-2 ml of water and 1-2 ml of anthrone reagent. The solution is shaken well and the contents are gently warmed. A blue or green colour appears indicating the presence of carbohydrate.

Test for proteins

Biuret test: Around 2 ml of the *Strelitzia reginae* leaf extract is mixed with 2 ml of biuret reagent. A purple colour appears indicating the presence of proteins.

Ninhydrin test: Around 2 ml of the *Strelitzia reginae* leaf extract is boiled in 0.1% ninhydrin solution for 1-2 minutes. Appearance of blue colour indicates that the amino acids are present in the leaf extract.

Test for oil and fats:

Spot test: A small quantity of *Strelitzia reginae* leaf extract is pressed on butter paper. Appearance of oil stains on the paper suggests the presence of oils.

Saponification test: Around 2 ml of the *Strelitzia reginae* leaf extract is mixed with 2 ml of 0.5N alcoholic potassium hydroxide. A drop of phenolphthalein is added and heated on a water bath for 30 minutes. Formation of soap in the form of a white precipitate confirms the presence of fatty acids.

Qualitative analysis of secondary metabolites:

Secondary metabolites are secondary products or organic compounds produced by bacteria, fungi, or plants, that help the host organism get a selective advantage. In plants, secondary metabolites are produced by the plant cell through metabolic pathways derived from the primary metabolic pathways[8]. Phenols, flavonoids, steroids, alkaloids, saponins, tannins, and glycosides are the major secondary metabolites in plants. Humans have understood the role of secondary metabolites as medicines, flavouring agents, for making pigments, and recreational drugs. Thus, there is a constant search for secondary metabolites by various researchers.

Test for Phenols

Phenol Test: 1ml of ferric chloride solution and 1ml of *Strelitzia reginae* leaf extract are taken in a glass test tube. Appearance of intense colour suggests the presence of phenols.

Ellagic test: 2ml of *Strelitzia reginae* leaf extractis treated with a few drops of 15% acetic acid and a few drops of 5% sodium nitrate solution. The appearance of muddy or brown precipitate suggests the presence of phenols.

Test for Flavonoids

Flavonoid test: Around 2 ml of the *Strelitzia reginae* leaf extract is taken in a glass test tube to which a 5-6 drops of sulphuric acid are added along with magnesium turnings. Development of pink or magenta colour suggests the presence of flavonoids.

Shinoda test: Around 2 ml of the *Strelitzia reginae* leaf extract is taken in a glass test tube. A few magnesium turnings are added along with 1ml of concentrated hydrochloric acid. Appearance of magenta colour in

the test tube after few minutes suggests the presence of flavonoids.

Ferric chloride test: A few drops of ferric chloride solution are added to 2ml of the *Strelitzia reginae* leaf extract. A blackish green colour is produced which suggests the presence of flavonoids.

Lead acetate test: 2ml of *Strelitzia reginae* leaf extract is taken in a glass test tube. A few drops of lead acetate solution (10%) are added and shaken well.Formation of a yellow precipitate suggests the presence of flavonoids.

Test for steroids

Salkowaski test: Around 2ml of *Strelitzia reginae* leaf extract is taken in a glass test tube. 4-5 drops of chloroform and 1ml of concentrated sulphuric acid are added and shaken well. Appearance of red colour suggests the presence of steroids.

Liebermann-Burchard test: Around 2ml of *Strelitzia reginae* leaf extractis taken in a glass test tube. A few drops of chloroform, a few drops of acetic anhydride and 1 ml of concentrated sulphuric acid are added to it. Appearance of a wine red ring at the junction of the two layers suggests the presence of steroids.

Test for saponins

Foam test: Around 2ml of *Strelitzia reginae* leaf extract is taken in a glass test tube. 2ml of distilled water is added and shaken vigourously. Formation of a persistent foam that lasts for more than a minute suggests the presence of saponins.

Test for Alkaloids:

Mayer's test: Around 2ml of *Strelitzia reginae* leaf extract is taken in a glass test tube. 1 ml of liquid ammonia, a few drops of chloroform and 1ml of dilute hydrochloric acid is added. Formation of a white precipitate suggests the presence of alkaloids.

Wagner's test: Around 2ml of *Strelitzia reginae* leaf extract is taken in a glass test tube. 2 ml of Wagner's reagent is added to it. Formation of reddish brown colour suggests the presence of alkaloids.

Hager's test: Around 2ml of *Strelitzia reginae* leaf extract is taken in a glass test tube.1 ml of picric acid is added to the same. Appearance of a yellow precipitate suggests the presence of alkaloids.

Test for tannins

Gelatin test: Around 2ml of *Strelitzia reginae* leaf extract is treated with 1 ml of 1% gelatin solution containing 10% sodium chloride. Appearance of a white precipitate suggests the presence of tannins.

Ferric chloride test: Around 2ml of *Strelitzia reginae* leaf extract is taken in a glass test tube. 2mlof 1% ferric chloride solution is added to the test tube. Appearance of blue green or brown green colour suggests the presence of tannins.

Test for glycosides

Keller-Kiliani test: Around 2ml of *Strelitzia reginae* leaf extract is taken in a glass test tube to which 1ml of glacial acetic acid and 4-5 drops of ferric chloride are added. 2ml of concentrated sulphuric acid is added to the test tube along the sides. Appearance of a reddish brown ring at the junction of the two layers suggests the presence of glycosides.

Concentrated sulphuric acid test: Around 2ml of *Strelitzia reginae* leaf extract is taken in a glass test tube to which 1ml of concentrated sulphuric acid is added. The solution is allowed to stand for 2 minutes. Formation of red colour suggests the presence of glycosides.

RESULTS AND DISCUSSION

Test results showing the presence or absence of primary and secondary metabolites.

The tests were conducted using the 60 ml stock solution prepared with the respective mother solvents.

Two of the primary metabolites viz., oils and fats, and carbohydrates were found in all the solvent extracts namely those using petroleum ether, chloroform, methanol, and distilled water. However, proteins were found only in the chloroform extract.

Further isolation and characterisation of each of the primary metabolites is necessary for a greater understanding of the nutritional and medicinal value of *Strelitzia reginae* leaves. However, these test results presented above constitute the first ever such attempt at preliminary phytochemical isolation and phytochemical testing of *Strelitzia reginae* leaves.

The secondary metabolites, namely, flavonoids, saponins, alkaloids, tannins, and glycosides were present in the methanolic and aqueous extracts. Chloroform extract has shown positive result for saponin. Petroleum ether extract indicated a positive result for alkaloids.

Only chloroform extract indicated a positive result for phenols.

Petroleum ether extract and chloroform extract indicated a positive result for steroids.

This study constitutes an important first step towards a greater understanding and further study of the various secondary metabolites present in the *Strelitzia reginae* leaves.

CONCLUSION

As mentioned in the introduction, the present investigation is the first phytochemical study conducted on the leaves of *Strelitzia reginae* leaf extracts. Based on a literature study the presence of saponins in the leaf extracts of *Strelitzia reginae* indicates probable anti-fungal (Porsche *et al;* 2018), anti-cancer (Wang *et al;* 2019), insecticidal (Dolma *et al*; 2017), molluscicidal (Hostettmann *et al;* 1982) activity. The presence of tannins in the leaf extracts of *Strelitzia reginae* indicates probable antiviral (Galabov *et al;* 2019), antibacterial (Sung *et al*; 2012), anti-cancer (Sukagami *et al;* 2000) and antioxidant (Sung *et al;* 2012)

activity. The presence of steroids in the leaf extracts of *Strelitzia reginae* indicates probable anti-inflammatory (Moura *et al;* 2018), immune suppressant (Liu *et al;* 2016), drug potential for pulmonary diseases (Hasan *et al;* 2020) and maintenance of male and female sexual characters (Tatem *et al;* 2019).

Further tests in this direction needs to be carried out. It is reported that the decoctions prepared from crushed *Strelitzia reginae* roots were considered particularly useful for easing the symptoms of sexually transmitted diseases (including inflamed glands) in cultures from the Kwazulu-Natal region of South Africa. There are reports of the presence of bilirubin (animal protein) in the arils of *Strelitzia reginae* and *Strelitzianicolai*. Further studies can be carried out to test the presence of bilirubin in other plant parts too, including the leaf. This paper is an account of the preliminary phytochemical analysis of the leaf extracts of *Strelitzia reginae* which has shown the presence of both primary and secondary metabolites.

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DISCLOSURE STATEMENT

All the authors declare that there is no conflict of interest.

ABBREVIATIONS

PE- Petroleum ether extract, **CE**- Chloroform extract, **ME**- Methanolic extract, **AQ**- Aqueous extract

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