PHYTOCHEMICAL ANALYSIS AND MEDICINAL PROPERTIES OF *STRELITZIA REGINAE* LEAF EXTRACT

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Abstract:

Our project involves the collection of leaves of *Strelitzia reginae* and to access its physiochemical and medicinal properties. Leaves sample were collected and extract was prepared by using polar and non polar solvent. Qualitative analysis of primary metabolites and secondary metabolites were carried out and result were tabulated. *Staphylococcus aureus, Klebsiella pneumoniae* were taken from accessing antimicrobial activity of the extract. The extract were taken in different concentration and zone of inhibition value and measured. More Over, minimum inhibitory activity was determined for gram positive bacteria and Gram negative bacteria.

Keywords *-- Strelitzia reginae,* polar and Non polar solvent*, Staphylococcus aureus, Klebsiella pneumoniae* and Minimum inhibitory concentration.

I. INTRODUCTION

Plants are fascinating and unique living organisms. They are playing a very important role in supplying oxygen to all life on earth. Plants are vital for the health and well- being of life on our planet. Human beings have explored plants as a food source initially and subsequently for their healing properties for thousands of years. Out of a quarter million species of higher plants that are documented ,about 30% are reported to have at least some medicinal value (Joy et al., 1998).

History of Medicinal Plants

An advantage of using plant-based compounds is the long history of their usage over several generations ,acceptance in folk medicine and the ability for its tolerance among patients. Scientific validation of the long-used plant medicine communities , irrespective of the ability of is mandatory for their acceptance as viable drugs for commercial use. So far, the pharmacological properties of around 80,000 plant species out of 2,50,000 known plant species are studied (Joyetal., 1998).

Thus, plant-based medicines are being commercially harnessed after passing rigorous scientific processes in treating diseases like Cancer, Malaria, Alzheimer's disease, Parkinson's disease, Chronic Obstructive Pulmonary Disease, Anti tyrosinaemia and as pain killers.

Plant Based Medicine & Global Dependency

Indigenous Traditional Medicine can be defined as the collective knowledge and practices of traditional medicine in local the traditional medicine practitioners to explain the rationale behind. Traditional

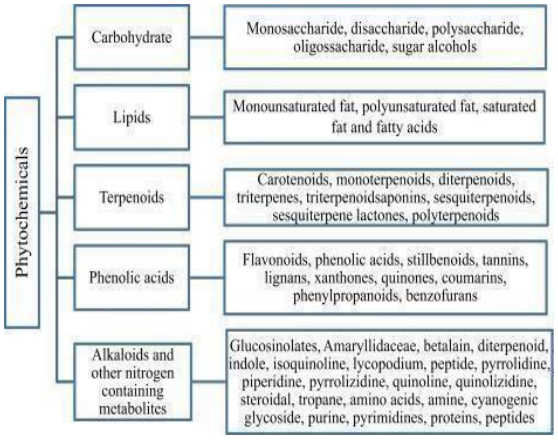
medicine is used to diagnose, prevent, and eliminate ailments of either physical, mental ,or social nature. The usage of herbal medicine practice is passed on from one generation to the next as practical experience or observation. The knowledge transfer happens either verbally or is documented in writing (Che et al., 2017).

Necessity of standardisation in medicinal plants research

Despite the whopping dependency of the world population on traditional medicine, some serious challenges need to be addressed. The data currently available on traditional medicine regarding its safety and effectiveness is not really meeting the rigorous standard to support its usage on a global scale. Lack of appropriate healthcare policies and dearth of adequate, acceptable and rigorous methodology to evaluate traditional medicine have hindered the generation of high quality research data(Wilson,2000).

Phytochemicals and their uses

The term 'phytochemical' refers to a wide variety of compounds that occur naturallyin plants. The term 'bioactive' refers to the compounds that are capable of interacting with living tissues and resulting to cause a wide range of plausible effects.



Phytochemicals are classified as primary and secondary metabolites based on their metabolic function (Krishnaiah et al., 2007).

Inflammation Studies and Medicinal Plants

Inflammation is a part of the body's defense mechanism. Rather it is the process by which the immune system recognizes and removes harmful and foreign stimuli and begins the healing process. Inflammation is of two types viz., acute inflammation and chronic inflammation (Pahwa et al., 2021).

Acute Inflammation

Acute inflammation is induced due to tissue damage caused by trauma, microbial infection, or toxic substances

Plant Profiling

Strelitzia reginae is a species of beautiful flowering plant which is native to South Africa. It is commonly called as 'bird of paradise' or 'crane flower' and it typically grows in temperate regions. It is a common evergreen perennial flowering plant, widely cultivated in the parks for its ornamental flower. The open flower resembles the head and beak of a colourful exotic bird (Gurung & Kumar, 2020).





Strelitzia is named in the honour of Queen Charlotte who was also an amateur botanist. She belonged to Mecklenburg-Strelitz royal family. The epithet *reginae*

means 'of the queen' in Latin (da Silva Vieira et al., 2020).
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Taxonomic Classification

The taxonomic tree of S.reginae is as given below:

Kingdom : Plantae
Phylum : Spermatophyta
Subphylum : Angiospermae
Class : Mononcotyledonae
Order : Zingiberales
Family : Strelitziaceae
Genus : Strelitzia
Species : S.reginae
Binomial nomenclature: S.reginae

Objectives of the Present work

Considerable scientific work and documentation has accumulated on *Strelitzia nicolai* while the scientific work on *Strelitzia reginae* is limited. The plant is interesting because, according to recorded data, the animal protein bilirubin is present in the aril and sepal part of the flowers (Pirone et al., 2010). Also, there is no comprehensive scientific literature available on the anticancer properties of the leaf extracts of *Strelitzia reginae*.

- The objectives of the present study include: Collection, identification, authentication and processing of the leaves of *Strelitzia reginae*.
- Extraction of the ingredients from the leaves of *Strelitzia reginae* using nonpolar and polar solvents.
- Preliminary screening of phytochemicals from the leaf extracts of *Strelitzia reginae.*
- Evaluation of the leaf extracts for antimicrobial properties.
- Evaluation of the leaf extracts for anti-inflammatory properties.

II. MATERIALS AND METHODS

COLLECTION, IDENTIFICATION, AUTHENTICATION & PROCESSING OF PLANT MATERIAL

Collection of the plant material

The mature leaves of *Strelitzia reginae* were collected.

Identification and Authentication of the plant material

The collected plant material was pressed and dried, an herbarium was prepared and submitted for identification.

Preparation of the leaves for the extraction process

The petioles and leaf blades were separated and were washed thoroughly with tap water, later with distilled water and shade dried for twenty days. The dried leaves were coarsely powdered in a mixer grinder and 496 grams of dried leaves of *Strelitzia reginae* was obtained.

Extraction of Active compounds from Plant Material by Soxhlet Method

The Soxhlet extraction process is known as a hot continuous extraction process. By this method, maximum extraction can be obtained with minimum quantity of solvent. About 92 grams of the coarsely powdered leaves of *Strelitzia reginae* were put in the main chamber of Soxhlet extractor. The solvents were taken in the increasing order of their polarity index (petroleum ether - 0.117, chloroform - 0.259, methanol - 0.762, and distilled water - 1.0) and the extraction process was carried out. Around 500 ml of each solvent was used for about 92 grams of the plant material per solvent.

Phytochemical Screening of the Leaf Extracts

Phytochemical screening was conducted in order to detect the presence of various primary and secondary metabolites in the leaf extracts of *Strelitzia reginae*. For this purpose, petroleum ether, methanol, chloroform, and distilled water were used as solvents. The phytochemicals were detected by colour tests following the standard protocol as described by Kokate & Gokhale (2014). The solutions were prepared by dissolving 20 mg of the *Strelitzia reginae* leaf extract in 60 ml of respective solvents. Subsequently, the solutions were 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 high quantities. Simple extraction procedures can be used to extract them quite easily. They are also called 'central metabolites' because they are present in most of the cells in the body. Primary metabolites are classified into:

1. Primary essential metabolites. E.g., carbohydrates, proteins, vitamins, enzymes, lipids.
2. Primary metabolic end products. E.g., lactic acid, certain amino acids, ethanol.

The different tests conducted to detect primary metabolites in *S. reginae* leaf extracts are listed below.

Test for carbohydrates

Molisch test:

About 2 ml of the *Strelitzia reginae* leaf extract is mixed with 1-2 drops of Molisch reagent. 2 ml of concentrated H2So4 is added carefully along the sides of the test tube. The test tube is allowed to stand for 2 minutes. Presence of carbohydrates is indicated if a reddish violet ring is formed at the interface between the layers of concentrated sulphuric acid and leaf extract of *Strelitzia reginae*.

Fehling's test: A small quantity of Fehling's reagent (1-2 ml) is added to 2 ml of the leaf extract of *Strelitzia reginae*. The mixture is boiled carefully for about 3 min in a water bath. Carbohydrates are confirmed to be present if a reddish brown precipitate is formed.

Benedict's test: A small quantity of Benedict's reagent (1-2 ml) is added to 2 ml of the leaf extract of *Strelitzia reginae*. The mixture is kept for about 3 min in a hot water bath. Carbohydrates are confirmed to be present if a reddish precipitate is formed.

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. Carbohydrates are confirmed to be present if blue or green colour appears in the solution.

Test for proteins

Biuret test: A small quantity of Biuret reagent (1-2 ml) is added to 2 ml of the leaf extract of *Strelitzia reginae*. A purple colour appears indicating the presence of proteins.

Ninhydrin test: 2 ml of the leaf extract of *Strelitzia reginae* is boiled in 0.1% ninhydrin solution for 1-2 minutes. If a blue colour appears, it indicates the presence of amino acids in the leaf extract.

Test for oil and fats

Spot test: A small amount of *Strelitzia reginae* leaf extract (2 ml) ispressed on butter paper. If oil stains are found on the paper, it suggests that oils are present. **Saponification test:** A small quantity of 0.5 N alcoholic potassium hydroxide (2 ml) is added to 2 ml of the leaf extract of *Strelitzia reginae*. A small quantity of the indicator phenolphthalein is now added. Solution of these mixture is heated for 30 min on a water bath. If soap is formed in the form of a white precipitate, it confirms that fatty acids are present in the leaf extract.

Qualitative analysis of secondary metabolites

Organic compounds that are produced by plants which help them to get a distinct selective advantage are termed as plant secondary metabolites. In plants, these secondary metabolites are produced by the plant cells through metabolic pathways, which are in turn, derived from primary metabolic pathways.

The major secondary metabolites in plants include phenols, steroids, alkaloids, flavonoids, saponins, tannins, and glycosides. Secondary metabolites are used as medicines, flavouring agents, for making pigments, and for preparing drugs

Test for Phenols

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

Ellagic test: 2 ml of *Strelitzia reginae* leaf extract is 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 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 1 ml 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 is added to ml of the leaf extract of *Strelitzia reginae*. A blackish green colour is produced which suggests the presence of flavonoids.

Lead acetate test: 2 ml 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

Salkowski test:

Around 2 ml of *Strelitzia reginae* leaf extract is taken in a glass test tube. 4-5 drops of chloroform and 1 ml of concentrated H2SO4 are added and shaken well. Appearance of red colour suggests the presence of steroids.

Liebermann-Burchard test:

Around 2 ml of *Strelitzia reginae* leaf extract is taken in a glass test tube. A few drops of chloroform, 1-2 ml of acetic anhydride and 1 ml of concentrated H2SO4 are added to it. Appearance of a wine red ring at the interface between the two layers suggests the presence of steroids.

Test for saponins

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

Test for Alkaloids

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

Wagner's test: Around 2 ml of the leaf extract of *Strelitzia reginae* 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 2 ml 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 2 ml 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 2 ml of *Strelitzia reginae* leaf extract is taken in a glass test tube. 2 ml of 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 2 ml of *Strelitzia reginae* leaf extract is taken in a glass test tube to which 1 ml of glacial acetic acid and 4-5 drops of ferric chloride is added and add 2 ml of concentrated H2SO4 is 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 2 ml of *Strelitzia reginae* leaf extract is taken in a glass test tube to which 1 ml of concentrated sulphuric acid is added. After about 2 min, if there is a formation of red colour, it suggests the presence of glycosides.

Antimicrobial Activity of *Strelitzia reginae* leaf extracts

Antimicrobial activity of *S. reginae* leaf extracts against *S. aureus* and k.pneumoniae

This experiment involves determining the antimicrobial activity of *Strelitzia reginae* leaf extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae* by well diffusion method.

Determination of Antimicrobial activity

1. *Staphylococcus aureus* and *Klebsiella pneumonia*e are inoculated on Soybean Casein Digested agar plates (90 mm).
2. Test compounds including sample (20µl, 10µl) and standard ciprofloxacin (20µl) for *Staphylococcus aureus* and *Klebsiella pneumonia*e are added to the 5mm well of each agar plate.
3. The treated plates with *Staphylococcus aureus* and *Klebsiella pneumonia*e are incubated in the chamber at 37°C for 24-48hrs. The treated plates were observed for the appearance of zone of inhibition just around the wells.

Antimicrobial activity of CE and ME of *S. reginae* leaves against Gram positive and Gram-negative bacteria

This experiment involves the determination of the antimicrobial activity by well diffusion method against six Gram-positive and six Gramnegative bacteria using the chloroform leaf extract and methanol leaf extract of *Strelitzia reginae*.

Determination of Antimicrobial activity

- *S. aureus, M. tuberculosis, S. mutans, S. epidermis, E. faecalis, L. acidophilus, K. pneumoniae, P. aeruginosa, P. mirabilis, S. typhimurium, E. aerogenes,* and *E. coli* were inoculated on Soybean Casein Digested agar plates (90 mm).
- Test compounds: Sample (20, 10µl), Standard Ciprofloxacin (20µl), and control (20µl) were added to the 5mm well on each agar plate.
- The treated plates with *S. aureus, M. tuberculosis, S. mutans, S. epidermis, E. faecalis, L. acidophilus, K. pneumoniae, P. aeruginosa, P. mirabilis, S. typhimurium, E. aerogenes,* and *E. coli* were incubated in an incubator for 24hrs at 37°C. After incubation, the treated plates were observed for the zone of inhibition just around the wells.

Determination of MIC of *S. reginae* CE against Gram-positive and Gram-negative bacteria

This experiment involved the determination of Minimum Inhibitory Concentration (MIC) of *Strelitzia reginae* leaf extracts against three Gram positive an five Gram- negative bacteria using chloroform as the solvent.

III. RESULT AND DISCUSSION

Standardization of Extraction and Determination of Extractive Value

In the present study successively the solvents from nonpolar to polar are used for Soxhlet extraction. Considering the parameter such as type of solvent, polarity of the solvent, extraction temperature, solvent sample ratio is taken into account before processing the plant material for the standardization.

Qualitative phytochemical screening

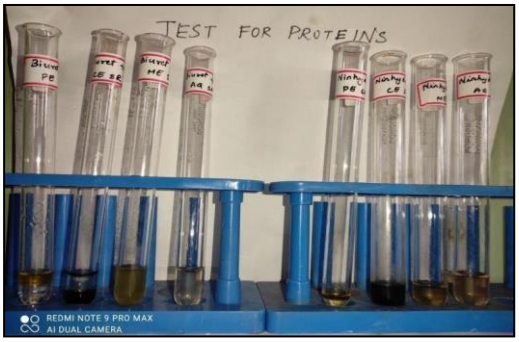
Four successive extracts namely petroleum ether extract, chloroform extract, methanol extract and aqueous extract of *Strelitzia reginae* leaves are used for qualitative analysis of primary and secondary metabolites.

The stocks of four extracts of *Strelitzia reginae* leaves namely petroleum there extract, chloroform extract, methanol extract and aqueous extract were tested for the presence or absence of carbohydrates in preliminary phytochemical.

Test results for primary metabolites



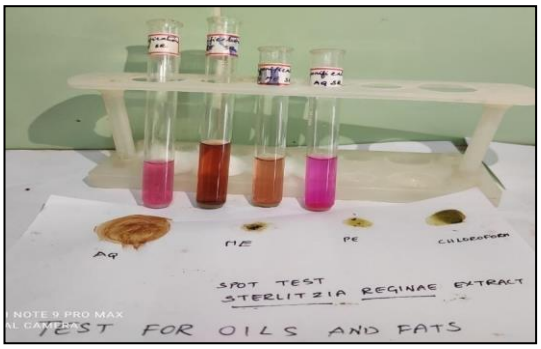
The tests conducted for detection of carbohydrates in the *Strelitzia reginae* leaf extracts. Molisch test produced a reddish purple ring at the junction between concentrated sulphuric acid and leaf extracts of *Strelitzia reginae* confirming the presence of carbohydrates. Absence of the ring denotes the absence of carbohydrates. Fehling's test confirms the presence of carbohydrates by the formation of reddish brown precipitate. Benedict's test denotes the presence of carbohydrates when a reddish precipitate is formed. Anthrone test indicates the presence of carbohydrates when a blue or green colour.



indicates the tests conducted for the detection of proteins**.** The stocks off our extracts of *Strelitziareginae* leaves namely petroleum ether extract, chloroform extract, methanol extract and aqueous extract were tested for the presence or absence of proteins in preliminary phytochemical tests.

Biuret test confirms the presence of proteins if a purple colour appears in the contents of the test tube. Ninhydrin test indicates the presence of proteins if a blue colour appears. Biuret test showed negative results for all the four extracts.

Ninhydrin test was positive for PE and CE, while it was negative for ME and AE. The presence of proteins was confirmed in the PE and CE of *Strelitziareginae* leaf extracts through Ninhydrin test.



shows the tests conducted for the detection of oils and fats. Spot test and saponification tests are done to test the presence of oils and fats in the sample. Appearance of oil stains suggest the presence of oils.

The white precipitate indicating the formation of soap is also seen in the test for fats by all the four extracts.

Carbohydrates: All the four extracts of *S.reginae* leaf namely PE, CE, ME and AE showed positive results in all the four tests conducted to test the presence of carbohydrates namely Molisch's test, Fehling's test, Benedict'S stand Anthrone test. Hence, the presence of carbohydrate in the sample is confirmed.

Proteins: PE and CE extracts of the leaf extracts of *S.reginae* showed positive result for ninhydrin test. Thus presence of proteins is confirmed in these extracts. While all the four extracts have shown negative result for Biuret test.

Oils and Fats: All the four extracts PE, CE, ME and AE of the *S.reginae* leaf extract show positive result for both the spot test and saponification tests confirming the presence of fats and oils in the sample. test(appearance of wine red ring).

Test results for secondary metabolites

Various tests were conducted to detect the presence of secondary metabolites such as phenols, flavonoids, steroids, saponins, alkaloids, tannins and glycosides. A detailed explanation of the test results areas given below:

Phenols: The test for phenols was negative in all the four extracts because no intense colour was noticed, CE has shown positive result for Ellagic test with the appearance of reddish brown muddy precipitate.

Flavonoids: Flavonoids were present in ME and AE extracts as confirmed by positive results in Flavonoid test (development of pink or magenta colour), Shinoda test (appearance of magenta colour), Ferric chloride test (appearance of blackish green colour) and Lead acetate test (formation of yellow precipitate) where as flavonoids tests are negative in PE and CE.

Steroids: Two tests were done to test the presence of steroids namely the Salkowski test and Liebermann-Burchard test. PE showed positive result in Salkowski test (appearance of red colour).PE and CE both have shown positive results in Liebermann-Burchard test(appearance of wine red ring).

Saponins: Foam test was conducted to detect the presence of saponins. CE, ME and AE all have shown positive results (formation of foam) confirming the presence of saponins while PE did not show foam formation.

Alkaloids: Three tests were conducted to identify the presence of alkaloids namely Mayer's test, Wagner's test and Hager'stest. AE extract has shown positive result for Mayer's test (formation of white precipitate). Similarly PE, ME, and AE extract have shown positive result for Wagner's test (formation of reddish brown colour)and PE showed positive result for Hager's test (appearance of yellow precipitate).

Tannins: Ferric chloride test and gelatin test were conducted to detect the presence of tannins. ME and AE extracts have shown positive test results for ferric chloride test (appearance of blue green or brown green colour) and gelatin test (formation of white precipitate) confirming the presence of tannins whereas PE and CE both showed negative result for ferric chloride test and gelatin test.

Glycosides: Keller-Kilianite stand concentrated H2SO4 test were conducted to test the presence of glycosides. PE, ME and AE extracts all have shown positive result for Keller-Kiliani test (appearance of reddish brown ring). Whereas all the four extracts have shown negative result for concentrated H2SO4 test.

Test for Phenols:

Phenol Test: No intense colour was seen in all the four extracts namely PE, ME, CE and AE extracts of the *Strelitzia reginae* leaves that indicates negative result for phenol test.

Ellagic test: The appearance of muddy or brown precipitate was seen only in the CE suggesting the presence of phenols. The other three extracts namely PE, ME and AE have shown negative results suggesting the absence of phenols.

Test for Flavonoids:

Flavonoid test: Development of pink or magenta colour was seen in ME and AE suggesting the presence of flavonoids. It is represented by + symbol in the Table 4.2.

Shinoda test: Appearance of magenta colour in the test tube was seen in the ME and AE after few minutes suggesting the presence of flavonoids.

Ferric chloride test: A blackish green colour was produced in both the ME and AE extracts which suggests the presence of flavonoids.

Lead acetate test: Formation of a yellow precipitate was seen in the ME and AE extracts, indicates the presence of flavonoids.

Test for steroids:

Salkowski test: Appearance of red colour suggests the presence of steroids. PE showed positive result for Salkowski test indicating the presence of steroid. Where as ME, CE and AE extracts have shown negative result for Salkowski test.

Liebermann-Burchard test: Appearance of a wine red ring at the interface between the two layers suggests the presence of steroids. In the present study the PE and CE have shown positive result for Liebermann Burchard test suggesting the presence of steroid.

Test for saponins:

Foam test: Formation of a persistent foam that lasts for more than a minute suggests the presence of saponins. CE, ME and AE extracts have shown positive results for the foam test suggesting the presence of saponins.

Test for Alkaloids:

Mayer's test: Formation of a white precipitate suggests the presence of alkaloids. AE extract has shown positive result for Mayer's test reveals the presence of alkaloids in the leaf extracts of *Strelitzia reginae*. The other three extract PE, ME and CE have shown negative result, represented by a – symbol in the Table 4.2.

Wagner's test: Formation of reddish brown colour suggests the presence of alkaloids. In current study PE,ME and AE extracts have shown positive result for Wagner's test confirms the presence of alkaloids.

Hager's test: Appearance of a yellow precipitate suggests the presence of alkaloids. PE showed positive result for Hager's test indicating the presence of alkaloid in the sample. The other three extracts have shown negative results for Hager's test.

Test for tannins

Gelatin test: Appearance of a white precipitate suggests the presence of tannins. Both ME and AE extracts have shown white precipitate formation confirming the presence of tannins.

Ferric chloride test: Appearance of blue green or brown green colour suggests the presence of tannins.ME and AE extracts both showed positive result for ferric chloride test revealing the presence of tannins. Test for glycosides

Keller-Kiliani test: Appearance of a reddish brown ring at the junction of the two layers suggests the presence of glycosides. PE, ME and AE extracts all have shown positive result for KellerKiliani test infers the presence of glycosides in the leaf extracts of *Strelitzia reginae*.

Concentrated sulphuric acid test: Formation of red colour suggests the presence of glycosides. However in the present investigation all the four extracts namely PE, ME, CE and AE have shown negative result for concentrated sulphuric acid test affirming the absence of glycosides. It is represented by a – symbol in the Table 4.2 .

ANTI MICROBIAL ACTIVITY

The petroleum ether, chloroform, methanol and aqueous extracts of *Strelitzia reginae* Leaf was screened for anti-microbial activity through a set of experiments.

In the followings etof experiments PE,ME,CE and AE were test edagains tone Gram positive and one gram-negative bacteria namely, *Staphylococcus aureus* ATCC 6538 (Gram-positive) and *Klebsiella pneumonia*e ATCC 13882 (Gram negative).

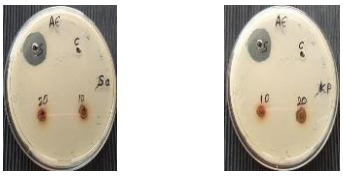
The zone of inhibition is the clear area roundan antibiotic phytochemical in which there is no growth of micro-organisms.

The zone of inhibition at various concentrations of each of the extract was recorded against a standard antibiotic ciprofloxacin and a control.
When AE extract is used sterile water is taken as the control, when CE is used

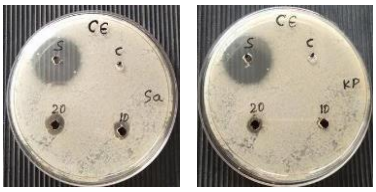
5% chloroform is maintained as the control ,when M E is used

5% methanol is taken as the control and for PE, 5% PE is used as control.

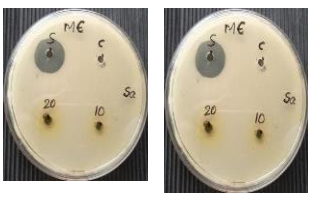
Anti microbial activity of *S.reginae* leaf extracts against *S. aureus* and *K. pneumoniae*



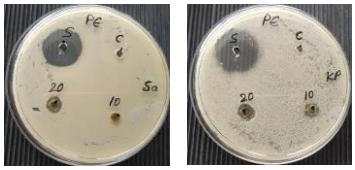
Showing Inhibitory activity of AE against *S.aureus* and *K. pneumoniae* S-Standard (Ciprofloxacin);C-Control (distilled water)



Inhibitory activity of CE against *S.aureus* and *K.pneumoniae* S Standard (Ciprofloxacin); C-Control (5%Chloroform)



Inhibitory activity of ME against *S.aureus* and *K.pneumoniae* S-Standard (Ciprofloxacin);C-Control(5%Methanol)



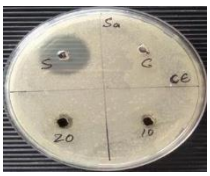
Inhibitory activity of PE against *S.aure* and *K.pneumoniae* S-Standard (Ciprofloxacin);CControl(5%Petroleum Ether)

Test Organisms	Sample	Test Compounds	Conc. per well	Zone of inhibition (mm)	Figure reference number
S. aureus	*AE*	Control (sterile water)	-	-	Figure 4.5
		Ciprofloxacin (Standard)	2µg	20	
		Sample (20µl)	2mg	8	
		Sample (10µl)	1mg	6	
K. pneumoniae		Control (sterile water)	-	-	
		Ciprofloxacin (Standard)	2µg	21	
		Sample (20µl)	2mg	9	
		Sample (10µl)	1mg	7	
S. aureus	*CE*	Control (Chloroform)	-	-	Figure 4.6
		Ciprofloxacin (Standard)	2µg	24	
		Sample (20µl)	2mg	12	
		Sample (10µl)	1mg	10	
K. pneumoniae		Control (Chloroform)	-	-	
		Ciprofloxacin (Standard)	2µg	25	
		Sample (20µl)	2mg	8	
		Sample (10µl)	1mg	5	
S. aureus	*ME*	Control (Methanol)	-	-	Figure 4.7
		Ciprofloxacin (Standard)	2µg	20	
		Sample (20µl)	2mg	-	
		Sample (10µl)	1mg	-	
K. pneumoniae		Control (Methanol)	-	-	
		Ciprofloxacin (Standard)	2µg	23	
		Sample (20µl)	2mg	10	
		Sample (10µl)	1mg	8	
S. aureus	*PE*	Control (Petroleum Ether))	-	-	Figure 4.8
		Ciprofloxacin (Standard)	2µg	22	
		Sample (20µl)	2mg	9	
		Sample (10µl)	1mg	5	
K. pneumoniae		Control (Petroleum Ether)	-	-	
		Ciprofloxacin (Standard)	2µg	24	
		Sample (20µl)	2mg	10	
		Sample (10µl)	1mg	8	

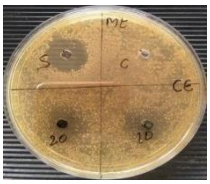
summarises the effect of AE,CE,ME and PE on two bacteria *S.aureus* and *K. pneumoniae*. CE shows good anti-bacterial effect on *S. aureus* exhibiting a zone of inhibition as(10mm)at1mg/well concentration whereas ME and PE have shown good anti-bacterial effect on *K. pneumoniae*.

Antimicrobial activity of CE and ME of *S.reginae* leaves against Gram positive and Gram-negative bacteria

CE showed good activity against gram-positive bacteria *S.aureus* and ME showed good activity against gram-negative bacteria *K.pneumoniae* in the earlier set of experiments.



Inhibitory activity of CEagainst *S.aureus*S-Standard (Ciprofloxacin);C-Control(5%Chloroform)



Inhibitory activity of CE against *M.tuberculosis* S-Standard (Ciprofloxacin); C-Control (5%Chloroform)



Inhibitory activity of ME against *E.coli* S Standard(Ciprofloxacin);C-Control(5% Methanol)

Determination of MIC of *S.reginae* CE against Gram-positive and Gram-negative bacteria

Since CE showed the be stanti-bacterial activity in experiment1 andexperiment2 ,itis considered as potential extract and CE was taken further for Minimum Inhibitory Concentration (MIC) studies.

Minimum Inhibitory Concentration (MIC)was determined against three Gram-positive bacteria and five Gram-negative bacteria using chloro form extract of *Strelitziareginae* leaves are summarized in the below tables and figures.

Minimum inhibitory activity of Std and CE of *S.reginae* leaves against Gram-negative bacteria E. coli

These bacteria are treated at various concentrations ciprofloxacin and CE of *Sreginae* leaves as shown in the table. MIC for *E. coli* is at 0.5 µg for standard drug. For CE of *S reginae* leaves, MIC is at 2.5mg.

IV. CONCLUSION

The present study is the first and original work on the leaf extracts of *Strelitzia reginae*. Around 5kg leaves of *Strelitzia reginae* collected were cleaned in distilled water, shade dried for 20 days, and 496g of coarse powdered plant material was obtained.

The powdered and dried leaves of *Strelitzia reginae* were taken up for extraction and phytochemical studies were done using both polar and nonpolar solvents namely petroleum ether, chloroform, methanol and distilled water. The leaf extracts showed the presence of primary metabolites namely carbohydrates, proteins, oils & fats, and secondary metabolites namely phenols,

flavonoids, saponins, alkaloids, steroids, tannins, and glycosides.

The PE, ME, CE and AE were subjected to antimicrobial studies. All these extracts exhibited antibacterial activity while the CE showed maximum antibacterial activity.

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