Ethnobotanical survey of Jaundice curing Plants and Preparation of Extract of *Microcos paniculata* L. and *Justicia vasica* Nees for their Phytochemical Analysis, Antioxidant activity, and Antifungal Properties

A Dissertation for
BOT: 651

Credits: 16

Submitted in partial fulfilment of Master of Science in Botany

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Date: April 2024

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I hereby declare that the data presented in this Dissertation report entitled **"Ethnobotanical survey of Jaundice curing Plants and Preparation of Extract of *Microcos paniculata* L. and *Justicia vasica* Nees for their Phytochemical Analysis, Antioxidant activity, and Antifungal Properties"** is based on the results of investigations carried out by me in the **Botany Discipline** at the SBSB, under the Supervision of **Prof. S. Krishnan, M. Phil., Ph.D.** and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not responsible for the correctness of observations/ experimental or the other findings given in the dissertation.

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ACKNOWLEDGEMENTS

This endeavour of mine would not have accomplished without the Profound help and co-operation of many people, I take this opportunity to remember all of them with sincere gratitude.

I take this opportunity to express my deep sense of gratitude to my guide Dr. S. Krishnan, Professor, Botany Discipline, Goa University, for his guidance, encouragement and motivation rendered to me for the successful completion of this dissertation work.

My special thanks to Research Scholar, Ms. Vaishali Gaonkar, for her constant help and inspiration throughout my work.

I am thankful to non-teaching staff members of the Botany Discipline for their help.

My heartfelt thanks to my Friend Saiksha who stood by me throughout my work. I would also like to thank Rida, Preeti, Samrudhi, Simran, Harshada, Geeta, Saisha, Rupesh for their co-operation while working in the laboratory.

Lastly, I am grateful to my Parents, Sister, Brother for their moral support and encouragement in the successful completion of my work.

Saachi Paresh Rawal

PREFACE

The dissertation work is conducted on the ethnobotanical survey of medicinal plants for curing jaundice from the Amona village of Quepem Taluka. The whole survey is measured through the questionnaire, the data is further analysed and interpreted and the results were obtained.

In this work, I have taken two plants that are *Justicia vasica* and *Microcos paniculata* and have done the analysis on their phytochemical, antioxidant and antifungal activity and compared on the presence of their constituents and other activities. Thus, the report will reveal the details of the work that I have done in my dissertation.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

PLATES

Plate No.	Description
Plate 1	*Microcos paniculata*. a) Habit ,b) Bark, c) Adaxial leaf surface, d) Adaxial leaf surface.
Plate 2	*Justicia vasica*, a) Habit. b) Flower. c)Adaxial leaf surface, d) Adaxial leaf surface.
Plate 3	Pictures of: a) Washed fresh leaves, b) Dry fresh leaves powder, c) Maceration process of leaves extract. d) Filtration after 3 days of maceration process
Plate 4	Preliminary phytochemical analysis of *Microcos paniculata, of a*) Methanolic extract, b) Chloroform extract, c) water extract.
Plate 5	Preliminary phytochemical analysis of *Microcos paniculata, of a*) Methanolic extract, b) Chloroform extract, c) water extract.
Plate 6	Determination of antioxidant activity a) ascorbic standard , b) methanolic extract *of Microcos paniculata*, c) chloroform extract of *Microcos paniculata*
Plate 7	Determination of antioxidant activity: c) water extract, d) methanolic extract *of Justicia vasica*, e) chloroform extract of *Justicia vasica*.
Plate 8	Antifungal activity: a) *Aspergillus* subculture streaking) Spore suspension, c) Serial dilution of 100µl of *Justicia* extract a) *Aspergillus niger* subculture streaking) Spore suspension, c) Serial dilution of 100µl of *Justicia* extract
Plate 9	Antifungal activity: a) Positive control, b) Negative control
Plate 10	Antifungal activity: a) *Justicia vasica* zone of inhibition at 300µl of methanol extract, b) *Justicia vasica* zone of inhibition at 200µl of methanolic extract.

ABBREVIATIONS

Entity	Abbreviation
Centimetre	Cm
Hrs	Hours
gms	Grams
mg	Mililitre
ml	Miligram
min	Minutes
ul	microlitre
PDA	Potato Dextrose Agar
%	Percentage

ABSTRACT

The main aim of this study was to do an Ethnobotanical survey on jaundice curing plants in the Quepem Taluka and to evaluate the phytochemical screening, antioxidant, and antifungal properties of different crude extract from the leaves of *Microcos paniculata* and *Justicia vasica*. Different solvents including methanol, chloroform and distilled water were used to prepare the crude extract from the fresh leaves of the sample. The antioxidant and antifungal activities of *Microcos paniculata* and *Justicia vasica* of different crude extracts from the fresh leaves were determined by the DPPH method and agar well diffusion method. The phytochemical constituents of both the leaves were determined by the qualitative and quantitative tests such as Mayer's test, barfoed's test, Bontrager's test, ninhydrin test, ferric chloride test, benedict's test. *Microcos paniculata* and *Justicia vasica* leaves extract revealed the presence of alkaloids, carbohydrates, flavonoids and terpenoids. The total flavonoid content in *Justicia vasica* leaves extract showed good antioxidant activity according to the IC50 values. For the antifungal activity test *Aspergillus* sp strain was used. The methanolic extract of *Justicia vasica* showed more zone of inhibition against the *Aspergillus* sp as compared to *Microcos paniculata*.

CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION

The medicinal plants are of great importance among various communities in many developing countries. Plants are rich resources of ingredients that can be used in drug development. The whole people over the world prefers herbal medicines than conventional medicines. Herbal medicines are been improved in developing countries as an alternative solution to health problems and the costs of pharmaceutical products. Plants play a significant role in the development of human cultures, and they are known as an important source of nutrition and have therapeutic value.

The Indian traditional medicine is based on various systems such as Ayurveda, Siddha, Unani, etc., which provide primary healthcare, throughout the world, the traditional knowledge system has gained big importance with regards to conservation, sustainable development, and search for the utilization patterns of plant resources. Ayurveda is a complete scientific medicinal system that has originated in India. It's the most ancient and the name itself suggest "info of life" it contains the knowledge on the natural cures.

India has wide biodiversity, which is a rich source of medicinal plants distributed within the country's different geographical and ecological parts. India is known to have many medicinal plants, where its extracts, decoctions and pastes are been used by the tribal's and they have a good knowledge on healthcare for the treatment of cuts, burns, diseases, fever, snake bites and wounds. Basically, the wild plants are known to me weeds or useless and unwanted plants but ayurveda stated that "no plant of this world is useless" (Evans,2008)

1.1.1 Ethnobotany

Ethnobotany basically deals with the study of medicinal plants used by the people as medicines. The word is derived from the terms “ethno” the study of people and “botany for the study of plants. It is the direct relationship between a plants and man. The use of plants as traditional medicine has been documented and this plant-based medicine system have played an important role and the world rely on the traditional medicines for their health care.

Traditional medicine system includes the knowledge, skills and practices based on the theories, beliefs, and experiences of the people over the world. The indigenous communities have their own traditional medicine system with different medicinal plants and various traditional therapies for curing diseases. Many wild and cultivated plants are used by them for the treatment of many ailments (Ramakrishna,2011)

The knowledge of ethnobotany is unique (Maiyo,2020). The tribal people are the main depository of traditional knowledge regarding the various application of plants. The locals and the traditional practitioners, healers have passed on the information of the medicinal plants from generations to generation. Ethnobotany is not only the study of medicinal plants used by the people but it also provides details about the culture specific to communities. In this modern era, ethnobotany has shed light on the many important useful plants that are been unidentified by the people in their own localities.

Ethnobotany helps in understanding traditional knowledge and developing criteria to protect biodiversity and promoting the sustainable management of plants. It plays an important role in preserving the indigenous knowledge, it also helps us to understand the historical and the present relationship between the people and plants hence providing insights into the use of plants for medicinal purposes and the prevention and the

treatment of diseases and aids in the conservation of the important plant species, this medicinal plant helps us to treat diseases such as diarrhea, dysentery, malaria, jaundice, stomach ache, and cough.

Jaundice is a liver disorder which occurs because of the release of a substance called Bilirubin in the blood, jaundice is also known as icterus that is a yellowish pigmentation of the skin. Bilirubin is a kind of a yellow chemical in the blood, it carries the oxygen in the red blood cells. As the red blood cells break down the body builds new blood cells to replace them, when the bilirubin builds up in our body the skin colour changes to yellow. When there is increased level of bilirubin in our body fluid it causes hyperbilirubinemia. Jaundice is liver disease which is occurs from the liver malfunctioning. It makes the person vulnerable to be affected by the hepatitis virus. Among the other ailments, jaundice is one of the most common ailments affecting the people of the world.

There are four types of main jaundice, that are grouped through where the bilirubin gets collected in the body.

A) **Prehepatic jaundice** is where the bilirubin builds up before blood enters the liver, which means break down of red blood cells and creating more bilirubin than the liver can process.

B) **Hepatic** is when the liver cannot process bilirubin well, it's called hepatic jaundice.

C) **Post hepatic** jaundice is when the bilirubin builds up after passing through the liver and the body cannot clean it.

D) **Obstructive jaundice** this is a stage when the bile is not able to drain into the intestines due to the blockage, pancreatic duct, narrow bile. Compared to the other jaundice, this type has the high death rate.

The well-known symptoms of jaundice are fever, stomach pain, skin itching, weight loss, confusion, dark urine, chills, clay-coloured stools, constant vomiting, feeling of irritated and abnormal drowsiness (Elsevier;2021).

Jaundice in adults usually occurs at the middle age and it is treated based on the type, if it's a acute viral hepatitis the jaundice will go away on its own as the liver heals, if its other type than the doctors suggest surgery to open. In the new-borns it is treated by the Phototherapy where it uses white or blue light that breaks down the bilirubin in the body. Even after all the tremendous advances made, no significant and safe hepatoprotective agents is available in modern therapeutics.

In the inadequacy of reliable liver protective drugs in allopathic medical practices, herbs play an important role in the management of various liver disorders. About 80% of people depend on the traditional medicines for their health care, as mentioned by the world health organization and there are certain economic benefits of ancient medicines and their use for the treatment of diseases faced by people of the world. Various plants and formulations have been asserted to have hepatoprotective activity (Joseph, 2016).

***Microcos paniculata* L.**

Classification

Kingdom: Plantae

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: *Microcos* L.

Species: *Microcos paniculata* L.

It is a flowering shrub native to China and south-east Asia including India. It belongs to family Malvacea. It is an Erect bushy shrub, commonly known in Konkani as Asali, it is a tall semi-deciduous tree, but sometimes it is shrubby. Its leaves are 10-15 cm long, flowers are small, yellow. The fruits are obovoid and globose, the taste of it is mildly sour. It is claimed to have medicinal values as well. It is also included in Indian Ayurveda. In traditional medicine the plant is believed to help cure jaundice, digestive system. It is also used for other health problems including colds, diarrhea, heat stroke. This plant is traditionally used in wound healing, fever and as an insecticide. It is used traditionally as the herbal medicines due to its effective power in curing various health related problems. It is also been used as a tea. It has good biological activities and chemical constituents present which are been revealed by the phytochemical tests. There is the presence of flavonoids, carbohydrates, and alkaloids in it. The decoction of the roots has been used to cure jaundice and cough. People from the hills use its leaves along with turmeric to treat jaundice. A drink of the dried or fresh leaves is given as vermifuge to Childrens. It has also beneficial and preventive effects for heart disease. Based on the

varies research, it is proved that the M. paniculata contains many active components which have pharmacological values.

***Justicia vasica* Nees**

Classification

Kingdom: Plantae

Class: Magnoliopsida

Order: Scrophulariales

Family: Acanthaceae

Genus: *Justicia* L.

Species: *Justicia vasica* Nees

Justicia vasica is a shrub that is widespread throughout the tropical regions of Southeast Asia (Chakrabarty., 2001). It is a perennial, evergreen, and highly branched shrub of 1.0 m to 2.5 mm height with a bitter taste. It has opposite ascending branches with white, pink or purple flowers (Patel and Venkata-Krishna., 1984). Its inflorescences are in spikes or panicles cymes and the species slightly has solitary, terminal, or axillary flowers. They can be easily recognised by their bilabial corolla that has a posterior lip which is a two lobed, and with an anterior lip which is three lobed, two stamens, a capsule with four seeds (Patel and Venkata-Krishna- Bhatt, 1984). *Justicia vasica* is a well-known plant drug in Ayurvedic medicines (Claeson et al., 2000). It is used for the treatment of various diseases and disorders, particularly for the respiratory tract ailments like bronchitis, asthma, tuberculosis, Jaundice, cold and cough (Sharma et al., 1992). Its main action is expectorant and antispasmodic (bronchodilator) (Karthikeyan et al., 2009). In Goa, *Justicia vasica* locally known as **Adulsa** and is been used as a remedy for

treating jaundice, cold, whooping cough and chronic bronchitis and asthma, as expectorant which promotes the discharge of the mucus present in the respiratory tract, and as pharmaceutical drug which relieves the muscle spasms and, as Helminthic.

1.1.2 Phytochemistry

Plants has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from plants, many based on their use in traditional medicine (Cragg & Newman, 2013). Phytochemicals are naturally occurring in plants, leaves and other vegetative parts and roots. These phytochemicals have a role in defence mechanism of plants and in protection of plants from various diseases (Sabovljevic 2008). Plant and plant-based products are the natural sources of different phytochemicals such as phenols. flavonoids, alkaloids, glycosides, lignins, and tannins. In fact, plants produce a varied range of bioactive molecules, making them a rich source of dissimilar types of medicines.

The term "phytochemicals" comes from the Greek word for the plant and refers to non-nutritive elements of a plant-based diet with significant antimutagenic and anticarcinogenic characteristics. Phytochemicals play various roles in the treatment and prevention of cancer and other diseases (Bathaie et al., 2015). Phytochemicals are bioactive substances derived from plants. Since the plant that produces them may not have much need for them, they are regarded as secondary.

1.1.2.1 Alkaloids

Alkaloids are metabolic by-products derived from amino acids and are one of the plants' most essential and significant components. Alkaloids have been removed from various

plant parts using a variety of solvents, including ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and aqueous (water). With the help of these solvents, phytochemical components can be extracted from the leaves, roots, stem bark, and fruits of medicinal plants. Therapeutically, alkaloids are particularly well known as anti-inflammatory agents, cardioprotective. Michael (2018). Alkaloids make up to 20% known secondary metabolites.

1.1.2.2 Flavonoids

Plants contain flavonoids, they are the members of a class of natural compounds that have recently been the subject of considerable scientific and therapeutic interest. Flavonoids are hydroxylated phenolic substances known to be synthesised by plants in response to microbial infection (Dixon 1983). Flavonoids and the secondary phenolic metabolites are responsible for the varies pharmacological activities (Mahomoodaily et al., 2005). They constitute the largest class of the phenolic compounds with more of three thousand structures. They have two aromatic rings and are known to be synthesised by the plants in response to microbial infection, and they are antimicrobial substances against a wide array of microorganisms.

1.1.2.3 Terpenoids

Terpenoids are the most common natural products and are tiny molecules that plants produce. Terpenoids exhibit remarkable pharmacological properties, including antibacterial, antiviral, antimalarial, anti-inflammatory, and anticancer properties (Boroushaki et al.,2016).

1.1.2.4 Glycosides

Glycosides are usually compounds of plant origin, they are made up of sugars combined with a phenol and a complex molecule. The commonly reported benefits of glycosides are the antioxidants and anti-inflammatory activities which has application in prevention and management of disease (Lin and harnly,2007)

1.1.2.5 Carbohydrates

The plants store carbohydrates in long polysaccharides chains called starch. These large polysaccharides contain various chemical bonds and are therefore store a lot of chemical energy. (Koch,1996, 2004)

1.1.3 Antioxidants Activity

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Matsui, 2011). The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots, and bark (Mathew and Abraham, 2006). The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles (Scartezzini et al., 2000).

1,1 -Diphenyl- 2-picryl-hydrazyl radical scavenging (DPPH) assay, DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the nonradical form DPPH-H (Blois, 1958). This transformation results in a colour change from purple to

yellow, which is measured spectrophotometrically. The disappearance of the purple colour is monitored at 517 nm.

The scavenging reaction between DPPH and an antioxidant can be written as,

(DPPH) + (H-A) → DPH-H + (A)

Here, the antioxidant reacted with the DPPH and reduced it to DPPH-H, and thus the absorbance decreased with an increase in the concentration of the extract. This indicated the scavenging potential of the antioxidant compound in the extracts in terms of hydrogen donating ability (Kedare et al., 2011).

The percentage of inhibition was calculated by using the formula:

% Inhibition = (A blank- A sample)/ A blank; X 100

A blank is the absorbance of the negative control, and A sample is the absorbance of the sample/ standard (Ahmed et al., 2013; Brand-Williams et al., 1995).

1.1.4.Antifungal Activity

The antifungal activity of leaf extracts of the methanolic, chloroform and water extracts of *Justicia vasica* and *Microcos paniculata* was tested against the fungal (Aspergillus niger) strain and was compared with the positive control (Fluconazole) and negative control (methanol) using well diffusion method. PDA plates were prepared, and the test organisms were spread plate onto the respective PDA plates using the glass spreader. The wells were filled with 200, 300, 500 mg/ul plant extracts using a micropipette and incubated for 48 hours at room temperature. After incubation, the diameter of the inhibitory zones formed around each well was noted down.

1.2 Objectives of the present investigation

1. To gather the ethnobotanical knowledge of jaundice curing plants from the people of Amona village of Quepem Taluka.
2. To analyse the phytochemical profile of the chosen medicinal species by the qualitative and quantitative phytochemical analysis.
3. The screening and comparing antioxidant activity of the selected plant parts using the DPPH method.
4. The evaluation of antifungal activities of the selected medicinal species against the *Aspergillus niger*.

1.3 Hypotheses

Plants are a source of large amount of bioactive compounds which are been used for curing various diseases and play an important role in healing. The phytochemical analysis will reveal the presence of the chemical compounds present in the plant and its antioxidant and antifungal activities that give them the potential to act as a source of useful drugs and to improve the health status of the consumers.

1.4 Scope

Bringing in light the importance of Ethnobotany knowledge about the medicinal plants that are been used to cure jaundice and the preservation of this important medicinal plants for the future and understanding the scientific data on the qualitative and quantitative analysis of the secondary metabolites and their antioxidant profile from the selected plants which makes them a good source of drug.

CHAPTER 2: REVIEW OF LITERATURE

2. Review of Literature

The relevant literature on the present study has been briefly reviewed to understand the different parameters of the study done on the mentioned objectives.

Ethnobotany

Siddalinga *et al.* (2012), worked on the traditional herbal medicines for jaundice in Bellary district, Karnataka, India. A survey was conducted to collect information of the medicinal plants used in curing jaundice. The information was collected through oral and personal interviews. Total 24 species of folk drug plants were documented during the time of survey that are been used as a remedy for the jaundice treatment. The study indicates that the locals rely medicinal plants for their treatment.

Mohammad *et al. (*2014), examined the ethno-medicinal plants used to cure jaundice by the traditional healers of Mashhad, Iran. An ethnobotanical survey of medicinal plants was conducted used by the traditional healers for the treatment of Jaundice. A total 37 plants were documented belonging to different families such as Fabaceae, Malvaceae, Asteraceae and Polygonaceae. The use of decoction is the most preferred method. In all of the cases the treatment involved oral dose of the extracts for 2 to 3 times a day.

Deb *et al*. (2016), worked on the ethno-medicinal plants used for the herbal medications of jaundice by the indigenous community of Tripura, India. A total of 50 ethno-medicinal plants were documented that were used for the treatment of jaundice. The necessary specimens were collected and cross checked with the existing literature. This community seems to hold the knowledge of all the herbal remedies for small to chronic

diseases. This community has led to the use of various plants for medicine and food. And this indigenous knowledge has been passed from generation to generations.

Pohekar *et al.* (2018), studied on the ethno-medicinal plants used to cure jaundice by traditional healers Kamtee Tehsil, MS, India. The Ethnobotanical survey was conducted in the Kamtee Tehsil, India the people still depend on own systems of herbal medicine. The standard questionnaire was prepared and the information was and documented. The information of plants used for jaundice of folklore origin was obtained during the survey. The leaves were highly utilized followed by the roots, seeds, and flowers.

Vandana *et al*. (2019), worked on the plants used for the treatment of icterus in the central India. The study states that the jaundice is not a disease but it's a sign or symptom of liver disorder that occurs when the bilirubin level increases that mixes in the blood due to improper metabolism and excretion. This study also describes total 55 medicinal plants that are been used by the rural and the tribal people of the different area for curing the jaundice.

Disha *et al*. (2021), studied on the ethnobotanical plants traditionally used for the treatment of jaundice in Himachal Pradesh in Western Himalaya. This study describes the different ethnobotanical plants that are been used for curing jaundice by the tribal people and the rural people of the Himachal Pradesh. The study has also revealed 87 ethno-medicinal plants which are used for curing jaundice in Himachal Pradesh. Some of

the plant extracts have been explored for their pharmacological and phytochemical activities and have also proved its potential in the treatment of jaundice.

Phytochemistry

Saha *et al*. (2010), evaluated the phytochemical analysis of the leaves of *Microcos paniculata.* The Soxhlated extraction and the standard methods were used for phytochemical analysis. *Microcos paniculata* revealed the presence of phytochemicals such as glycosides, tannins, phytosterols, and flavonoids, alkaloids from the petroleum and chloroform extracts of *Microcos paniculata* leaves.

Narendra *et al*. (2012), worked on the phytochemical studies and the presence of various compounds such as flavonoids, terpenoids & alkaloids were mentioned. The extracts of *Microcos paniculata* have a wide range of pharmacological activities like hepatoprotective, antioxidant, anticancer, and diuretic.

Deepak *et al.* (2012), worked on the detection and confirmation of alkaloids in the leaves of *Justicia adhatoda*. It was performed and confirmed by the Phyto analysis, there were total six different quinazoline alkaloids found in the leaves of *Justicia adhatoda*. The alkaloids were vasicoline, vasicolinone, vasicine, vasi-cinone, adhatodine and anisotine.

Akhtar *et al*. (2017), studied the phytochemical screening of *Microcos paniculata* collected from Kerala region and its antioxidant and antimicrobial potentials, The Soxhlated extraction and the standard methods were used for phytochemical analysis. *Microcos paniculata* has shown various pharmacological activity.

Suvendu *et al*. (2017), The study was planned to evaluate the phytochemical analysis and Pharmacological activities with methanolic extract of *Microcos paniculata* stem, barks and the leaves of *Microcos paniculata*. The maceration method was used for extractions and phytochemical analysis.

Malathi *et al.* (2018), studied the preliminary phytochemical analysis of *Justicia adhatoda*_leaves extract using different solvents, this paper gives an amount of phytochemical studies. Five solvents were used namely methanol, ethanol, acetone, chloroform, and diethyl ether to obtain the extracts from powdered plant leaves. The result showed the presence of alkaloids, flavonoids, glycosides, tannins, steroids and phenols. The presence of all this phytoconstituents suggest that *Justicia adhatoda* leaf could serve as a source of useful drug.

Antioxidant Activity

Yau *et al*. (2007), carried out the study on the total phenolic content (TPC) and antioxidant activity of *Microcos paniculata* fresh and dried plant materials were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. The study results indicate that *Microcos paniculata may* provide a source of plant compounds with the antioxidant activity.

Hua *et al*. (2010), investigated the antioxidant Properties from the leaves of *Microcos paniculata* using such as 1,1-diphenyl-2-picrylhydrazyl (DPPH). The compounds were identified by spectroscopic methods. The further identification of the chemical constituents will be helpful to understand the traditional medicines. The result from this study helps us understand the antioxidant capacity profiles of these plant.

Nizar *et al.* (2013), focused on the screening of natural antioxidant from selected medicinal plants. The medicinal plants contain high levels of natural antioxidant and they exhibited strong antioxidant activity. The total phenolic content, flavonoids, anthocyanins, and tannins were measured and the antioxidant capacities were evaluated using DPPH and the Trolox equivalent antioxidant capacity assays. The result from this study helps us understand the antioxidant capacity profiles of these medicinal plants and also investigate the new sources of natural antioxidants.

Chanu *et al. (*2014), investigated on the antioxidant activity and the phytochemical analysis of *Justicia adhatoda*. The leaves of this plant are used as a main source of drug, used for the treatment of wide variety of diseases and disorders. The antioxidant activity was carried out by the DPPH method and the phytochemical studies showed the presence of alkaloids, flavonoids and terpenoids from the leaf extracts prepared using different solvents. The alkaloid presents in the adhatoda leaves contributed a vast variety of pharmacological property.

Arvinder *et al. (*2015), worked on the evaluation of antioxidant and antimutagenic potential of *Justicia adhatoda*_leaves extract. In this study the ethanolic extract of *Justicia adhatoda* leaves was prepared by the successive extraction procedure in the increasing polarity order. They aim to determine the antioxidant potential of the different fractions of ethanolic extract of the leaves. The antioxidant activity of the ethanolic extract of *Justicia adhatoda*_leaves was determined by the DPPH method.

Armijos *et al,* (2017), worked on the antioxidant properties of medicinal plants used in the Southern Ecuador. In this study 64 organic extracts from 34 species belonging to 23 botanical families were obtained with different solvents. The antioxidant activity was determined by the two methods, the DPPH and the ABTS. The total phenolic content was determined by Folin's-Ciocalteau colorimetric technique. The results obtained from this study suggest good antioxidant activity described for these species could play an important role in the medicinal properties claimed for the plants under study and could be useful in the pharmaceutical industries.

Ibrar *et al.* (2018), focused on the pharmacological activities of *Justicia adhatoda* including antifungal, antibacterial and antioxidant by DPPH method. The methanolic extract showed good antifungal activity against Aspergillus Niger. The DPPH activities were proportional to the concentration of the fractions, as the concentration increased, the percent scavenging activity also increased.

Nipunika *et al.* (2022), examined the distribution of bioactive compounds and the antioxidant capacity of different parts of *Justicia adhatoda*. *J. adhatoda* have been used for the treatment of various ailments. The study was conducted to quantify the total antioxidant capacity, the total phenolic content, and the total flavonoid content of different parts. The results revealed that all the tested parts of the plant contained marked amount of TAC, TPC and TFC. Hence could be used effectively in the pharmaceutical industries.

Ravali *et al.* (2023), worked on the alkaloids from *Justicia adhatoda*. and its antioxidant property. *Justicia adhatoda* has been used as an ayurvedic medicine in India over a long period of time. It is used in the treatment of various ailments. The alkaloids from adhatoda are found to have many pharmacological properties. The vasicine alkaloid that can be potentially used in the treatment of various disorders. The antioxidant activity of vasicine is measured in this paper by the method DPPH. The results indicate that the inhibition concentration IC50 of vasicine is 187 ug/ml.

Antifungal Activity

Aziz *et al*. (2010), worked on the antibacterial and phytochemical screening of the leaves of *Microcos paniculata*. The phytochemical studies revealed the presence of various

compounds such as flavonoids, terpenoids & alkaloids. The antibacterial activity was carried out using microbial strains.

Sumathi *et al.* (2010), examined the antimicrobial activity of some traditional medicinal plants using the leaves and fruits. They used pure isolates of *E. coli* strains, *salmonella Para typhi A, S. typhi and S. aureus strains.* The results of the present investigation showed minimum inhibitory concentration of some of the medicinal plants fruit extract against Escherichia coli and S. aureus with values being 50 and 200 ug/ml respectively.

Rashmi *et al.* (2012), worked on the antimicrobial activity of methanolic leaf extracts of *Justicia adhatoda.* The activity was evaluated by determination of the diameter of zone of inhibition against the bacteria and fungi. The minimum inhibitory concentrations were determined against all the pathogens.

Sharma *et al. (*2016), worked to uncertain the presence of antifungal activity of leaf extracts of *Justicia adhatoda* and the phytochemical constituents present in the leaves and bark. The phytochemical studies revealed the presence of alkaloids, flavonoids and terpenoids in it and that were active against the bacteria and fungi.

Ibrar *et al.* (2018), focused on the pharmacological activities of *Justicia adhatoda* including antifungal, antibacterial and antioxidant by DPPH method. The methanolic extract showed good antifungal activity against *Aspergillus niger*. The DPPH activities were proportional to the concentration of the fractions, as the concentration increased, the percent scavenging activity also increased.

Muh *et al. (*2023), investigated the therapeutic potential of the medicinal plant *Justicia adhatoda* and its antibacterial and antifungal activities. The methanolic crude extract was prepared at the concentration of 100, 500 and 1000 ug/ml. the crude extracts were evaluated for the antifungal activity against the *aspergillus niger* and fusarium oxysporum indicating different zones of inhibition. The highest antifungal activity was recorded in *Justicia adhatoda* methanolic extract.

CHAPTER 3: MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Ethnobotanical Data Collection

Ethnobotanical data was collected from July 16th to August 13th 2023 from the local people of Amona village of Quepem taluka. The standard data collection method has been followed to document indigenous knowledge of the local community of Quepem taluka on the uses of medicinal plants for curing jaundice. The data was collected from basic oral interview and the information was noted.

3.2 Study Area and Plant Collection

The selected species (*Microcos paniculata* and *Justicia vasica*) were collected from the Quepem Goa. Mature and healthy leaves were collected and appropriately cleaned with distilled water and dried for 10 days under shade at room temperature until dry.

3.3 Preliminary qualitative phytochemical analysis

3.3.1. Preparation of plant extract

Methanol, chloroform, and distilled water were used for maceration method. 10g of dried powder sample was mixed with respective solvents and kept at room temperature for 3 days. The extract was stirred after every 4 to 5 hours. The solution was filtered using Whatman filter paper Grade A.

The extracts were evaporated using rotary evaporator. The crude extracts were stored in glass vials and kept in freezer for future use. The extract obtained with different solvents were used for the preliminary qualitative and quantitative estimation, which was carried out according to the methods described by Raman (2006).

QUESTIONAIRE

- **Sr.no:** _____
- **Date:** _____
- **Age:** _____
- **Sex:** **M/F**
- **Name of interviewer:** _____
- **Language:** _____
- **Occupation:** _____
- **Name of the plant:** _____
- **Plant description:** _____
- **Plant parts used:(Roots/stem/leaves/bark/seeds/flowers/whole plants)**
- **Mode of consuming:(Juice/decoction/paste/poultice)**
- **Preparation:** _____
- **Dosage:** _____
- **Duration of treatment:** _____
- **Important note:** _____

3.3.2. Test for Alkaloids

Take 50mg of extract and stir it with 1Ml of dilute hydrochloric acid and filter it. The filtrate is used for the following test. Precipitate will reveal the presence of Alkaloids.

a). Mayer's test

Add one drop of Mayer's reagent to 2ml of filtrate. A white or creamy precipitate reveals the presence of Alkaloids.

3.3.3 Detection of carbohydrates

The extract (100mg) is dissolved in 5ml of water and filtered. The filtrate is subjected to the following tests.

a). Molish's test

To 2ml of filtrate, two drops of alcoholic solution of a-naphthol are added, the mixture is shaken well and 1ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

3.3.4 Detection of Glycosides

a). Borntragers's test

To 2ml of filtered hydrolysate, 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

3.3.5 Detection of saponins

The extract (50mg) is diluted with distilled water and made up to 20ml. The suspension is shaken in a graduated cylinder for 15min. A 2cm layer of foam indicates the presence of saponins.

3.3.6 Detection of proteins

a). Ninhydrin test

2 drops of ninhydrin solution are added to 2ml of aqueous filtrate. A characteristic purple colour indicates the presence of proteins.

3.3.7 Detection of phenolic compounds and tannins

a). Ferric chloride test

The extract (50mg) is dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

3.3.8 Detection of fats and oils

Add 4 to 5 drops of 0.5N alcoholic potassium hydroxide solution is added to a small quantity of extract and a drop of phenolphthalein. This mixture is heated in a water bath for 2 hours. The soap formation reveals the presence of saponins.

3.3.9 Detection of Flavonoids

a.) NaOH test

Add 1ml of extract then add few drops of dil NaOH, the intense yellow color change indicates the presence of the Flavonoids.

3.3.10 Detection of Terpenoids

Add 2 ml of extract in the test tube, then add in 2 ml of chloroform and add 3 ml of sulphuric acid. The reddish yellow colouration will indicate the presence of the Terpenoids.

3.4 Quantitative Phytochemical Analysis

3.4.1 Total of Flavonoids Estimation

The Total Flavonoid content was determined by ALCL3 colorimetric method according to Chang *et al*., 2002

Take the 0.5ml of plant extract of serial dilutions of 200, 400, 600, 800 and 1000ul. Then add 1.5ml of methanol and 0.1 ml of 10% ALCL3. To this add 0.1ml of 1 M potassium acetate and 2.8ml of distilled water and keep this mixture at room temperature for 30 minutes. Take the absorbance of reaction mixture at 415 nm.

Calibration curve was prepared using quercetin in methanol and the results expressed as amount of flavonoid content. 10 mg of quercetin 10 ml of 80% Methanol and diluted to 200,400, 600, 800 and 1000 ul. Then further they were treated same like extracts. Blank was prepared by substituting distilled water in place of 10% ALCL3. The test was performed in triplicates.

Concentration of total flavonoid content was calculated as mg quercetin equivalent to plant extract using quercetin calibration curve.

3.4.2 Total of Tannins Estimation

The Total Tannic content is determined according to Amorium *et al.,* 2009.

Take 1 ml of the plant extract, to that add 0.5 ml of Folin's reagent. To this add 7.5 ml demineralised water and add 1 ml of 35% sodium carbonate and shake well. Incubate the solution for 30 minutes at room temperature and take the absorbance at 725nm.

The calibration curve was determined using the Tannic acid standard. The stock solution of the tannic acid was prepared by dissolving 10 mg of tannic acid in 10 ml of distilled water.

The concentration of total tannic acid equivalent of extract using tannic acid calibration curve.

3.4.3 Total of Phenols Estimation

The total Phenolic content was determined by Ciocalteau's method according to Dewanto *et al*., 2002.

Gallic acid was used as standard for determining calibration curve. The stock solution of gallic acid was prepared by mixing 10 mg of gallic acid in 10 ml of distilled water.

Calibration curve plotted by 100µl aliquots of 20, 40, 60, 80 and 100 µl of gallic acid solution. Make final volume as 500 µl with distilled water. Add 150 µl of 10% Folin's reagent and vortexed. Keep mixture at room temperature for 10 minutes. Add 500 µl of 7.5% Sodium carbonate and incubate the mixture in dark for 1hour. Take absorbance at 650 and 760 nm. The blank was prepared by adding 100ul of distilled water. Concentration of total phenolic content was calculated as mg gallic acid by using gallic acid calibration curve.

3.5 Antioxidant studies

The antioxidant studies in selected plant species for leaves were carried out using the 1,1 - diphenyl-2-picrylhydrazyl **(DPPH**) method.

A. Preparation of DPPH: Stock solution was prepared by dissolving 24mg of **DPPH** in 100mL of ethanol in the dark and stored in an Amber-coloured bottle. The working solution was prepared by adding 10mL of Stock solution to 45mL of ethanol.

B. Preparation of L-ascorbic acid solution: 10mg of ascorbic acid was dissolved in 10mL of distilled water. Serial dilution was performed to prepare solution with different concentration (12.5 g/mL- 200g/mL).

C. Preparation of Test solution: 10mg of methanolic extract of leaf was dissolved in 10mL of ethanol, and then serial dilution was performed to prepare the required concentrations (12.5g/mL- 200g/mL).

D. Preparation of control: 3mL DPPH was used as a negative control.

3.6 Anti- Fungal Studies

Anti- fungal studies were done on 2 species of *Microcos paniculata* and *Justicia adhatoda*

3.6.1 Sterilization of glassware's

Sterilization of Equipment and Media: Dry Heat Sterilization- All the glass wares previously washed and they were kept in oven for complete drying. Petri-dishes, pipettes, test tubes were also washed and were wrapped separately in the paper and sterilized by autoclaving at 15lbs for 1 hour.

3.6.2 Preparation of media

PDA were accurately weighed separately as per given proportion. The ingredients were then dissolved in 250 ml distilled water. The media was digested in micro-oven by gentle heating and constant stirring and autoclaved at 15lbs pressure for 1 hour and cooled at room temperature.

3.6.3 Spreading of fungal Suspension

The **PDA** was poured on to sterile Petri plates and allowed for solidification. The plates with media were seeded with the respective fungal suspension using micropipette and with the help of sterile glass spreader the suspension was spread on the plates uniformly under aseptic conditions. The plates were dried for 1 hour.

Using subculture strains, antifungal activity was carried out by following method:
a) Agar Well Diffusion Method

Preparation of Wells and Addition of extract in Wells

Wells were made with the help of sterile cork borer having a size of 7 mm diameter at four corners. The extract with different concentrations (100,200,300ul) individually was added in respective wells using micropipettes of each Petri dishes. Methanol was also poured in the second well as a positive control well. Later the plates were kept for incubation in an incubator at 37º C for 24 hr. After incubation period, antifungal activity was determined by measuring the zone of inhibition around each well and expressed in mm (Jayprakash., 2013).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Ethnobotanical survey data

Table 1. List of documented medicinal plant species, family, local names, botanical names, parts used.

Sr.no	Botanical name	Family	Local name	Parts used	Mode of consumption
1.	*Abrus precatorius* L.	Fabaceae	Gunji	Roots	Juice
2.	*Allamanda catharitca* L.	Apocynaceae	korno	Roots	Juice
3.	*Azadirachta indica* A. Juss	Meliaceae	Kodulim	Leaves	Decoction
4.	*Aegle marmelos* (L.) Correa	Rutaceae	Bel	Leaves	Juice/decoction
5.	*Andrographis paniculata* (Burm.f.) Wall. Ex Nees	Acanthaceae	Kirayte	Leaves	Juice
6.	*Aloe vera*	Liliaceae	Katekavar	Leaves	Paste/juice
7.	*Asparagus racemosus* (Willd.)	Liliaceae	Shatavari	Roots	Juice
8.	*Boerhravia diffusa* L.	Nyctaginaceae	Punarnava	Whole plant	Decoction
9.	*Bridelia retusa* (L.) Spreng.	Euphorbiaceae	Fatarphal	Leaves/fruit	Juice/decoction
10.	*Carica papaya* L.	Caricaceae	Popaii	Seeds	Decoction/ directly consumed
11.	*Chrysanthemum indicum* L.	Asteraceae	Shevanti	Flower	Decoction
12.	*Cuscuta reflexa* Roxb.	Cuscutaceae	Benulo	Whole plant	Juice/decoction
13.	*Cassia fistula* L.	Fabaceae	Bayo	Roots/bark	Decoction

14.	*Celastrus paniculatus* Willd.	Celastraceae	Kanglin	Roots	Juice/decoction
15.	*Centella asiatica* (L.) Urb	Apiaceae	Brahmi	Leaves	Decoction
16.	*Calotropis procera* W.T. Aiton	Asclepiadaceae	Rui	Leaves	Juice
17.	*Curcuma longa* L.	Zingerberaceae	Halad	Rhizome	Paste
18.	*Justicia vasica* Nees	Acanthaceae	Adulsa	Leaves	Decoction/poultice
19.	*Leucas aspera* (Willd.) Link	Lamiaceae	Thumo	Leaves	Juice
20.	*Momordica charantia* L.	Cucurbitaceae	karela	Leaves	Juice/ paste
21.	*Microcos paniculata* L.	Malvaceae	Asale	Leaves/bark	Decoction/juice/ paste
22.	*Moringa oleifera*	Moringaceae	Maska shango	Leaves/flowers	Decoction/ juice
23.	*Ocimum sanctum* L.	Lamiaceae	Tulsi	Leaves	Juice
24.	*Phyllanthus embilica* L.	Euphorbiaceae	Amla	Fruit	Juice/ paste
25.	*Phyllanthus niruri* L.	Euphorbiaceae	Ran avalo	Leaves	Decoction
26.	*Raphanus sativus* L.	Brassicaeae	Mulo	Root	Juice/ poultice
27.	*Saccharum officinarum* L.	Poaceae	Uas	Stem	Juice
28.	*Syzgium cumii* (L.) Skeels	Myrtaceae	Jamla	Seeds/bark	Decoction
29.	*Tinospora cordifolia* (Willd.)	Menispermaceae	Giloy	Leaves	Decoction/juice
30.	*Tamarindus indica* L.	Fabaceae	Chincha	Leaves	Decoction/juice

4.1.1 Ethnobotanical survey data analysis

In the present survey all together 30 plant species were documented from the study area, which were used by the people for the treatment of jaundice. The mode of preparation and administration depends on the plant used. But the data collected shows that most of the remedies are been taken orally. The medicines prescribed by the people are either made up of single plant or made from the mixtures. This medicine remedies are consumed in the form of juice, decoction, seeds and paste. It is also studied that the all parts of the plant are been used for the medicinal purpose. In accordance to the survey most of the medicinal plants belong to the following families Apocynaceae, Acanthaceae, Euphorbiaceae, Fabaceae, lamiaceae etc. the result of the present study provides evidence that the medicinal plants play an important role in the healthcare of the people.

Table 2. Names of the selected plant species and their location.

Sr.no	Botanical Names	Local Names	Place of collection
1.	*Justicia vasica* Nees	Adulsa	Quepem
2.	*Microcos paniculata* L.	Asali	Quepem

4.2 Phytochemical Analysis

4.2.2 Qualitative Phytochemical Analysis

The phytochemical analysis of *Justicia adhatoda* and *Microcos paniculata* was carried out to understand the different secondary metabolites present in both leaves. The crude

extracts were prepared using the solvents such as methanol, chloroform, and distilled water. From leaves of each plant and the phytochemical was carried out.

Aziz *et al*., 2010, worked on the phytochemical screening of the leaves of *Microcos paniculata.* The study revealed the presence of various compounds such as flavonoids, terpenoids & alkaloids, whereas in this present study the results revealed the presence of alkaloids, carbohydrates, terpenoids and flavonoids were present. The alkaloids were found to be present in both methanol and chloroform solvent and absent in water. It showed very intense colouration in methanol solvent. The terpenoid was found present in Distilled water extract of plant. Flavonoids showed its presence in the methanol solvent. The carbohydrate showed its presence in both that is the methanol solvent and distilled water. However, the glycosides were absent in all the 3 solvents. (Table no.3)

Malathi *et al.,* 2018, studied the preliminary phytochemical analysis of *Justicia vasica* leaves extract using different solvents, The result showed the presence of alkaloids, flavonoids, carbohydrate and phenols. Whereas in this present study *Justicia vasica* leaves showed the presence of the alkaloids, flavonoids, carbohydrates and terpenoids. Alkaloids were found to be present in only methanol solvent and absent in the rest 2 solvents that is water and chloroform. While the Terpenoid showed its presence only in the water extract and absent in the other 2 solvents. The carbohydrate showed presence in the chloroform extract. The flavonoid showed absence in all the 3 solvents. (Table no.4)

Table no.3 Phytochemical analysis of *Microcos paniculata* (Leaves)

Sr. no.	Phytochemical Test	Methanol	Chloroform	Distilled water
1	**Detection of Alkaloids**			
a.	Mayer's test	+	+	-
2	**Detection of Carbohydrates**			
a.	Benedict's test	+	-	+
3	**Detection of Glycosides**			
a.	Borntrager's test	-	-	-
4	**Detection of saponins**	-	-	-
5	**Detection of Proteins**			
a.	Ninhydrin test	+	-	-
6	**Detection of Fats and oil**			
a.	saponification	-	-	-
7	**Detection of Phenolics**			
a.	Ferric chloride test	-	-	-
8	**Detection of Flavonoids**			
a.	NaOH test	+	-	-
9	**Detection of Terpenoids**	-	+	+

(+: Presence and -: Absence)

Table no.4 Phytochemical analysis of *Justicia vasica* (Leaves)

Sr. no.	Phytochemical Test	Methanol	Chloroform	Distilled water
1	**Detection of Alkaloids**			
a.	Mayer's test	+	-	-
2	**Detection of Carbohydrates**			
a.	Benedict's test	-	+	-
3	**Detection of Glycosides**			
a.	Borntrager's test	-	-	-
4	**Detection of saponins**	-	-	-
5	**Detection of Proteins**			
a.	Ninhydrin test	-	-	-
6	**Detection of Fats and oil**			
a.	saponification	-	-	-
7	**Detection of Phenolics**			
a.	Ferric chloride test	-	-	-
8	**Detection of Flavonoids**			
a.	NaOH test	-	-	-
9	**Detection of Terpenoids**	-	-	+

(+: Presence and -: Absence)

4.3 Quantitative Phytochemical Analysis

4.3.1 Determination of Total Flavonoid

The Total Flavonoid content of methanol extract of *Microcos paniculata* was found to be 0.391mg of quercetin/g of plant extract and that of *Justicia vasica* was 1.175mg. The chloroform extract of *Microcos paniculata* revealed 0.647mg quercetin/g of plant extract while *Justicia vasica* was 0.105mg quercetin. Water extract of *Microcos paniculata* revealed 0.027mg quercetin of plant extract and 0.099mg quercetin of *Justicia vasica* as in Table no. 5,6,and 7. The flavonoid content was found to be high in *Justicia vasica.*

4.3.2 Determination of Total Tannin

The Total Tannin content of methanol extract of *Microcos paniculata* was found to be 0.0481 mg of /g of plant extract and that of *Justicia vasica* was 0.0418 mg. The chloroform extract of *Microcos paniculata* revealed 0.0191mg /g of plant extract while *Justicia vasica* was 0.0616 mg .Water extract of *Microcos paniculata* revealed 0.0154mg of plant extract and 0.0164mg of *Justicia vasica* as in Table no. 8,9,and 10. The tannin content was found to be high in *Justicia vasica.*

4.3.3 Determination of Total Phenol

The Total Phenol content of methanol extract of *Microcos paniculata* was found to be 202.76mg of /g of plant extract and that of *Justicia vasica* was 184.52mg. The chloroform extract of *Microcos paniculata* revealed 219.82mg /g of plant extract while *Justicia vasica* was 185.70mg.Water extract of *Microcos paniculata* revealed 143.35mg of plant extract and 215.70mg of *Justicia vasica* as in Table no 11,12 and 13. The Phenol content was found to be high in *Microcos paniculata.*

Table no 5: Preliminary quantitative phytochemical analysis of Flavonoid standard

Quercetin concentration (µg/ml)	Absorbance at 415 nm			Average	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
200	0.217	0.207	0.207	0.210	0.005774
400	0.276	0.253	0.261	0.263	0.011676
600	0.325	0.316	0.361	0.334	208.2384
800	0.416	0.486	0.445	0.449	0.035171
1000	0.525	0.527	0.532	0.528	0.003606

Table no 6: Preliminary quantitative phytochemical analysis of Flavonoid content of *Microcos paniculata*

Extracts (µg/ml)	Absorbance at 415 nm			Average	Total Flavonoid content (mg q/plant dry extract)	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3			
Methanol	0.252	0.247	0.246	0.248	0.391	0.003215
Chloroform	0.386	0.358	0.386	0.376	0.647	0.016166
Water	0.041	0.038	0.039	0.066	0.027	0.001528

Table no 7: Preliminary quantitative phytochemical analysis of Flavonoid content of *Justicia vasica*

Extracts (µg/ml)	Absorbance at 415 nm			Average	Total Flavonoid content (mg q/plant dry extract)	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3			
Methanol	0.621	0.0.648	0.653	0.640	1.175	0.017214
Chloroform	0.756	0.752	0.762	0.756	0.105	0.005033
Water	0.100	0.102	0.100	0.102	0.099	0.002517

Table no 8: Preliminary quantitative phytochemical analysis of Tannin standard

Tannic concentration (µg/ml)	Absorbance at 415 nm			Average	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
200	0.236	0.229	0.239	0.232	0.003512
400	0.317	0.392	0.383	0.364	0.040951
600	0.399	0.427	0.440	0.422	0.020952
800	0.570	0.562	0.584	0.572	0.011136
1000	0.509	0.526	0.589	0.541	0.042147

Table no 9: Preliminary quantitative phytochemical analysis of Tannin Content of *Justicia vasica*

Extracts (µg/ml)	Absorbance at 415 nm			Average	Total Tannin content (mg q/plant dry extract)	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3			
Methanol	0.118	0.109	0.108	0.111	0.0418	0.010693
Chloroform	0.007	0.006	0.009	0.007	0.0616	0.001528
Water	0.420	0.434	0.441	0431	0.0164	0.005508

Table no 10: Preliminary quantitative phytochemical analysis of Tannin content of *Microcos paniculata*

Extracts (µg/ml)	Absorbance at 415 nm			Average	Total Tannin content (mg q/plant dry extract)	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3			
Methanol	0.380	0.378	0.372	0.376	0.0481	0.004163
Chloroform	0.100	0.102	0.100	0.100	0.0191	0.000577
Water	0.116	0.118	0.112	0.115	0.0154	0.003055

Table no 11 : Preliminary quantitative phytochemical analysis of Phenols standard

Gallic concentration (ug/ml)	Absorbance at 415 nm			Average	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3		
Blank	0	0	0	0	0
200	0.591	0.602	0.608	0.600	0.008622
400	0.578	0.585	0.585	0.582	0.004041
600	0.685	0.509	0.678	0.657	0.099654
800	0.706	0.771	0.766	0.747	0.036171
1000	0.782	0.801	0.807	0.796	0.013051

Table no 12 : Preliminary quantitative phytochemical analysis of Phenols content of *Microcos paniculata*

Extracts (ug/ml)	Absorbance at 760 nm			Average	Total phenols content (mg q/plant dry extract)	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3			
Methanol	0.238	0.243	0.245	0.242	202.76	0.003606
chloroform	0.213	0.215	0.211	0.213	219.82	0.002000
water	0.361	0.325	0.345	0.343	143.35	0.018037

Table no 13: Preliminary quantitative phytochemical analysis of Phenols of *Justicia vasica*

Extracts (ug/ml)	Absorbance at 415 nm			Average	Total Flavonoid content (mg q/plant dry extract)	Deviation
	Absorbance 1	Absorbance 2	Absorbance 3			
Methanol	0.284	0.286	0.288	0.273	184.52	0.002
Chloroform	0.276	0.286	0.269	0.271	185.70	0.004359
Water	0.220	0.222	0.219	0.220	215.70	0.001528

4.4 Antioxidant Analysis

The antioxidant studies in the 2 selected medicinal plants, *Microcos paniculata* and *Justicia vasica* was carried out using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. This method measures the decrease in the absorption of the DPPH solution after adding an antioxidant at 517nm. The Ascorbic acid (10mg/ml distilled water) was used as positive control.

Here, the antioxidant reacted with the DPPH and reduced it to DPPH-h, and thus the absorbance decreased with an increase in the concentration of the extracts. This indicated the scavenging potential of the antioxidant compound in the extracts in terms of hydrogen donating ability. The IC50 value represents the concentration at which a substance exerts half of its maximal inhibitory effect. Thus, IC50 value is used to represent the effectiveness of an antagonist in inhibiting a specific biochemical process.

The result showed that all the two plants exhibited antioxidant activity. The highest free radical scavenging activity was shown by the methanol extract of *Justicia vasica* with an IC50 value of 33.37µg /ml. while the methanol extract *Microcos paniculata* exhibited the antioxidant activity with an IC50 value of 188.86µg /ml. while the chloroform extract of *Justicia vasica* exhibited antioxidant activity with an IC50 value of 324.36 µg/ml. while the chloroform extract *Microcos paniculata* exhibited the antioxidant activity with an IC50 value of 295.89µg /ml. The water extract of *Justicia vasica* with an IC50 value of 235.88µg /ml. while the water extract *Microcos paniculata* exhibited the antioxidant activity with an IC50 value of 216.02µg /ml .The L-ascorbic acid was used as a standard

to compare the radial scavenging activity of the extract, and its IC50 value was 4.206µg/ml.

Table no 14. DDPH free radial scavenging assay : % scavenging activity of DPPH by ascorbic acid and methanolic, chloroform and water extract of *Justicia vasica.*

Sr. No	Concentration (ug/ml)	L-Ascorbic acid	M.E	C.E	W.E
1	12.5	25±0.01769	17.4±0.01357	1.0±0.00493	3.8±0.00602
2	25	75.8±0.39402	24.1±0.00057	2.85±0.00251	4.1±0.000577
3	50	85±0.07133	30.8±0.00057	17.8±0.02844	13.3±0.00493
4	100	87.7±0.02405	32.8±0.00057	26.7±0.00057	25.7±0.00529
5	200	94.8±0.05602	36.6±0.00458	27.6±0.004041	40.9±0.00550

Table no 15. DDPH free radial scavenging assay : % scavenging activity of DPPH by ascorbic acid and methanolic, chloroform and water extract of *Microcos paniculata*.

Sr. No	Concentration (ug/ml)	L-Ascorbic acid	M.E	C.E	W.E
1	12.5	25±0.01769	2.4±0.02055	3±0.014799	2.84±0.00251
2	25	75.8±0.39402	3.0±0.00850	12.3±0.01417	6±0.014742
3	50	85±0.07133	11±0.00115	17.7±0.00513	10.7±0.00709
4	100	87.7±0.02405	37.9±0.00173	22.2±0.00251	20.5±0.00152
5	200	94.8±0.05602	48.3±0.00152	34.6±0.01135	46.2±0.00057

Table no 16. IC50 values of Methanol, Chloroform and Water extract of *Microcos paniculata* and *Justicia vasica*

Sr. No	Sample	Type of extract	IC50
1	**L-Ascorbic acid**		**4.206**
2	***Microcos paniculata***	**Methanol extract**	**188.86**
3		**Chloroform extract**	**295.89**
4		**Water extract**	**216.02**
5		**Methanol extract**	**33.37**

6	***Justicia vasica***	**Chloroform extract**	**324.36**
7		**Water extract**	**235.88**

4.5 Antifungal Analysis

The antifungal activity of the crude leaves of *Microcos paniculata* and *Justicia vasica* were studied using different concentrations (100, 200, 300 µl). The leaf extract of *Microcos paniculata* and *Justicia vasica* was extracted using different solvents (methanol, chloroform and water). Their antifungal activities were studied on *Aspergillus niger*. The results showed that methanol extract of *Justicia vasica* showed higher antifungal activity at 300 µl concentration, inhibition area ranging from (0-4mm) as compared to other two concentrations. While the *Microcos paniculata* showed relatively smaller zone of inhibition area ranging from (0-3mm) at 300 µl. It is seen that the water extract of both the plants displayed, no or less zone of inhibitory effects against the fungi tested. It is also noted that chloroform extracts of both the plant species did not show any zone of inhibition against the fungi. Therefore, the inhibition zones were measured and presented in tables(17, 18)

From the above results, it is seen that the among the extracts, the methanol extract of *Justicia vasica* showed high inhibitory effect at 200 µl. and 300 µl. The methanol extract was more potential against *Aspergillus niger*. The water solvent extract of *Justicia vasica* exhibited low activity against the fungi. It was observed that there were no inhibitory zones in the chloroform extract of both plants. It was noted that the positive control Fluconazole showed excellent zone of inhibition against the fungi. It was even observed that, the 300 µl concentration showed good

inhibitory effect when compared to the other plant that is *Microcos paniculata* against the strain of fungi viz. *Aspergillus niger.*

The present study revealed that the *Justicia vasica* showed high antifungal effect against the fungi *Aspergillus niger* than the *Microcos paniculata.* This may be due to the presence of terpenoids, flavonoids, saponins, phenolics, alkaloids and bioactive phytochemicals found in the leaves. Some of these compounds such as tannins, phenolics, saponins were found to be absent in the other plant that is *Microcos paniculata.*

Table no 17. Antifungal Activity of *Microcos paniculata* (Leaves)

Fungus	Concentration (µl)	Water	Methanol	Chloroform
Zone of inhibition in (mm)				
Aspergillus niger	**100µl**	**-**	**-**	**-**
	200µl	**-**	**-**	**-**
	300µl	**-**	**1**	**-**
	+ve Control	**4**	**4**	**4**
	-ve Control	**-**	**-**	**-**

Table no 18. Antifungal Activity of *Justicia vasica* (Leaves)

Fungus	Concentration (µl)	Water	Methanol	Chloroform
Zone of inhibition in (mm)				
Aspergillus niger	**100µl**	**-**	**-**	**-**
	200µl	**-**	**2**	**-**
	300µl	**-**	**3**	**-**
	+ve Control	**4**	**4**	**4**
	-ve Control	**-**	**-**	**-**

4. Conclusions

The present study was undertaken with the aim of ethnobotanical survey, producing an inventory of the varies medicinal plants used by the people in curing jaundice. The investigation indicates that the local inhabitants still rely on the medicinal plants for treatment. Total 30 medicinal plants were documented during the survey in Amona village of Quepem taluka. The leaves were highly utilized followed by the roots, seeds, and flowers.

The study on qualitative phytochemical analysis of selected two species revealed the presence of secondary metabolites in the leaves. The phytochemical tests done using maceration method revealed the presence of alkaloids, carbohydrates, flavonoids, saponins and terpenoids. Mayer's test was carried out to detect alkaloids. It gave positive results in methanol and chloroform extracts and showed negative result in both the plants.

Carbohydrates, flavonoids were intensely present in both the plant species. Whereas the glucosides were absent in all the plant species. The detection of proteins was performed by the Ninhydrin test, which showed absence in both plants. Terpenoid was intensely present in the water extract of both the plants. Overall methanol being the polar solvent, gave better results for the phytochemical analysis.

The quantitative phytochemical screening resulted in high amount of flavonoid content followed by the phenolic content and tannic content. The *Justicia vasica* showed presence of more Flavonoid and tannin content than the *Microcos paniculata.* The total phenolic content was found to be similar in both the plants. Overall, the quantitative analysis suggests that leaves of *Justicia vasica* contains more amount of phytoconstituents than the *Microcos paniculata.*

The antioxidant studies were carried out for the leaves with methanolic, chloroform and water extract of both the plant species using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Both the plants exhibited antioxidant activity. L ascorbic acid was used as a standard to compare the radical scavenging activity of the extract, and its IC_{50} value was 4.206µg /ml. The highest free radical scavenging activity was exhibited by *Justicia vasica* with an IC_{50} value 33.37µg /ml, while the *Microcos paniculata* exhibited the lowest antioxidant activity with an IC_{50} value 188.86µg /ml.

In the antifungal studies, agar well diffusion method was used and the same was adopted for the evaluation of antifungal activity of two species that is *Microcos paniculata* and *Justicia vasica* using their leaves. Further the three concentrations of extract were prepared (100µl, 200 µl, 300 µl) to evaluate the zone of inhibition of both the plants. The 300 µl concentration showed more zone of inhibition as compared to the other two concentrations. Overall, result indicated that the leaves of *Justicia vasica* shows zone of inhibition. The antifungal activity of *Justicia vasica* at 300 µl concentration was slightly equivalent to that of the Positive control.

REFERENCES

Alzoreky, N. S., & Nakahara, K. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int. J. Food Microbiol*., 80, 223-230.

Ates, D. A., & Turgay, Ö. (2003). Antimicrobial activities of various medicinal and commercial plant extracts. *Turkish Journal of Biology*, 27(3), 157-162.

Akinyemi, K. O., Oluwa, O. K., & Omomigbehin, E. O. (2006). Antimicrobial activity of crude extracts of three medicinal plants used in South-West Nigerian folk medicine on some food borne bacterial pathogens. *Afr. J. Trad. Compl. Altern. Med*. 3 (4) pp. 13-22.

Ahmad, M., Saeed, F., & Jahan, N. (2013). Evaluation of insecticidal and antioxidant activity of selected medicinal plants. Journal of Pharmacognosy and Phytochemistry, 2(3), 153-158.

Aziz, M. A., Akter, M. I., & Islam, M. R. (2016). phytochemical screening, toxicity, larvicidal & antidiabetic activity of aqueous extract of Microcos paniculata leaves. Pharmacology online, 2, 50-7.

Abbas, M. W., Shamshad, T., Ashraf, M. A., & Javaid, R. (2016). Jaundice: a basic review. Int J Res Med Sci, 4(5), 1313-9.

Ahmed, H. M., Ramadhani, A. M., Erwa, I. Y., Ishag, O. A. O., & Saeed, M. B. (2020). Phytochemical screening, chemical composition, and antimicrobial activity of microcos paniculata bark. Int. Res. J. Pure Appl. Chem, 21, 36-43.

Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. Bulletin of the National Research Centre, 46(1), 1-22.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol., 28, 25-30.

Deshpande, A.R., S.P. Mohd, Rothe Musaddiq and A.M. Banole. (2005). Screening of some plant extracts for antibacterial activity. Asian Jr. of Microbial. Biotech, 4, 755-758.

Goresky, C. (Ed.). (2012). Jaundice (Vol. 2). Springer Science & Business Media.

Harborne J. B. (1973). Phytochemicals Methods - A guide to Modern Techniques of Plant analysis. 2'd edition. Chapman and Hall Publishing Company Ltd., London. 275-295.

Harkare, B. R., Suryawanshi, J. S., Kadam, S. S., Osmani, R. A., and Bhosale, R. R. (2013). Phytochemical analysis and antibacterial activity of methanolic seed extract of microcos paniculata. IJPBS, 3(2), 373-378.

http://www.worldfloraonline.org/taxon/wfo-0000451652.

http://www.worldfloraonline.org/taxon/wfo-0000354128

Jiang, Y. Q. (2019). Microcos paniculata: a review on its botany, traditional uses, phytochemistry and pharmacology. Chinese journal of natural medicines, 17(8), 561-574.

Kala, C.P., Dhyani, P.P. and Sajwan, B.S. 2006. Developing the medicinal plant sector in north India: challenges and opportunities. J. ethnobiology and ethnomedicine.

Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food chemistry, 94(4), 550-557.

Kedar, S. B., & Singh, R. P. 2011). Genesis and development of DPPH method of antioxidant assay. Journal of food science and technology, 48, 412-422

Lai, P. K., & Roy, J. (2004). Antimicrobial and chemo preventive properties of herbs and spices. Current medicinal chemistry, 11(11), 1451-1460

Lim, Y. Y., & Murtijaya, J. (2007). Antioxidant properties of Microcos paniculata extracts as affected by different drying methods. LWT-Food Science and Technology, 40(9), 1664-1669.

Maridass, M. (2010). Survey of phytochemical diversity of secondary metabolism in selected wild medicinal plants. Ethnobotanical leaflets, 2010(5), 8.

Maity, S., Chatterjee, S., Variyar, P. S., Sharma, A., Adhikari, S., & Mazumder, S. (2013). Evaluation of antioxidant activity and characterization of phenolic constituents of Microcos paniculata root. Journal of agricultural and food chemistry, 61(14), 3443-3450.

Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research, 10(2).

Pattanaik, C., & Sudhakar Reddy, C. (2008). Medicinal plant wealth of local communities in Kuldiha Wildlife Sanctuary, Orissa, India. Journal of herbs, spices & medicinal plants, 14(3-4), 175-184.

Roche, S. P., & Kobos, R. (2004). Jaundice in the adult patient. American family physician, 69(2), 299-304.

Ramandeep, K., Nahid, A., Neelabh, C., & Navneet, K. (2017). Phytochemical screening of Microcos paniculata collected from Kerala region and its antioxidant and antimicrobial potentials. Journal of Pharmaceutical Sciences and Research, 9(8), 1312.

Sherlock, S. (1962). Jaundice. British Medical Journal, 1(5289), 1359.

Stillman, A. E. (1990). Jaundice. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition.

Scartezzini, P., & Speroni, E. (2000). Review on some plants of Indian traditional medicine with antioxidant activity. Journal of ethnopharmacology, 71(1-2), 23-43.

Sumathi, P., & Parvathi, A. (2010). Antimicrobial activity of some traditional medicinal plants. Journal of Medicinal plants research, 4(4), 316-321.

Sharma, H., & Kumar, A. 201). Ethnobotanical studies on medicinal plans of Rajasthan (India): A review. Journal of Medicinal plants research, SCT), 1107- 1112. Sharma, P., Kumar, P., Sharma, R., Gupta, G., & Chaudhary, A. (2017).

Shraim, A. M., Ahmed, T. A., Rahman, M. M., & Hiji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. LWT, 150,.

Vashist, H., & Jindal, A. (2012). Antimicrobial activities of medicinal plants-Review. Int J Res Pharm Biomed Sci, 3(1), 222-230.

Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. Journal of phytology, 3(12)

Figure 1: Map of Quepem Taluka, Goa.

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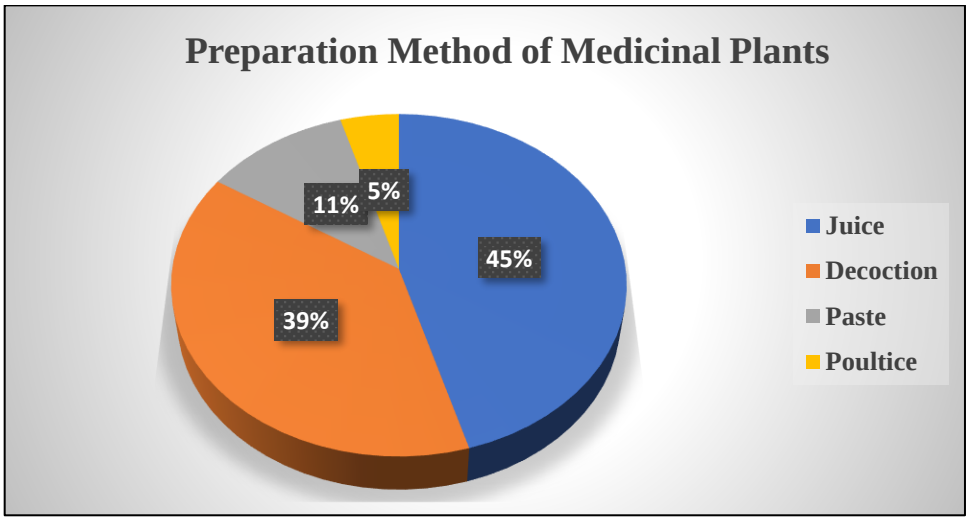

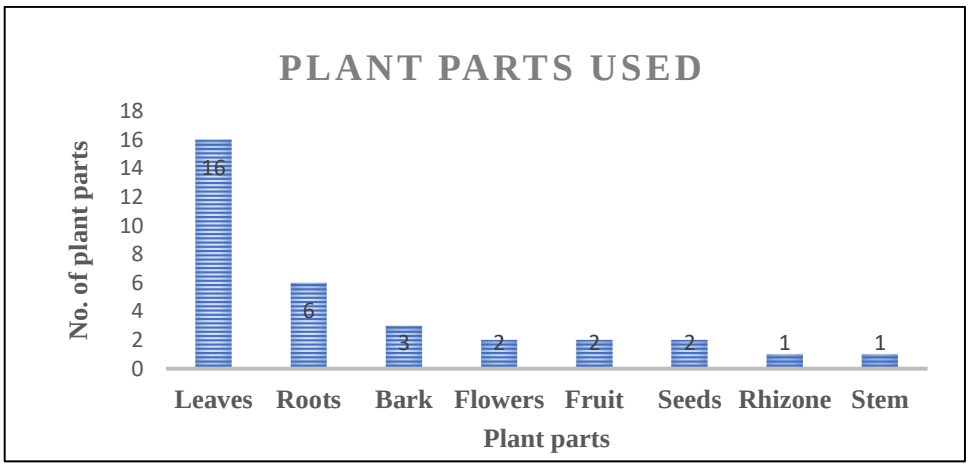

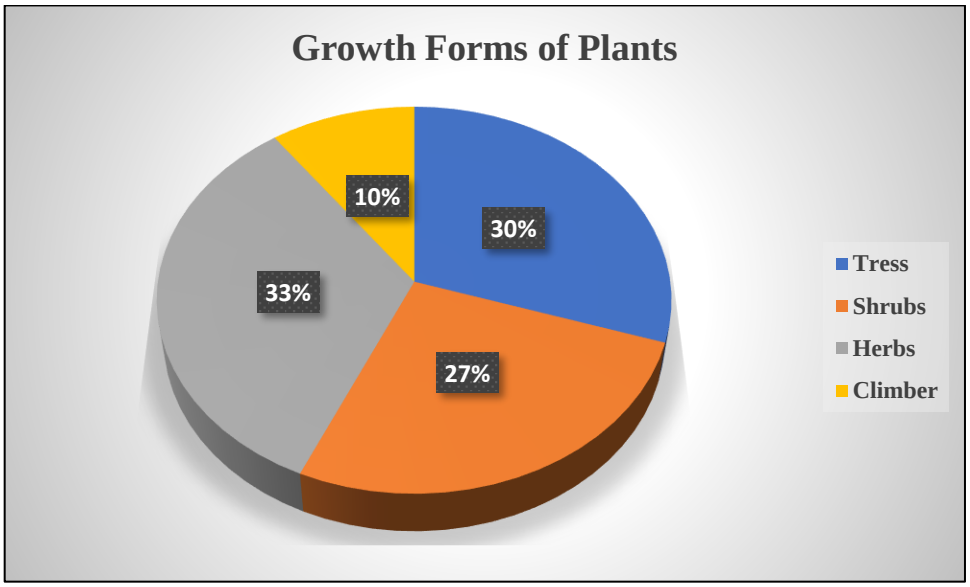

Figure 2. a) Pie chart of preparation method of medicinal plants. b) Graph of plants parts used. c) pie chart of growth forms of plants.

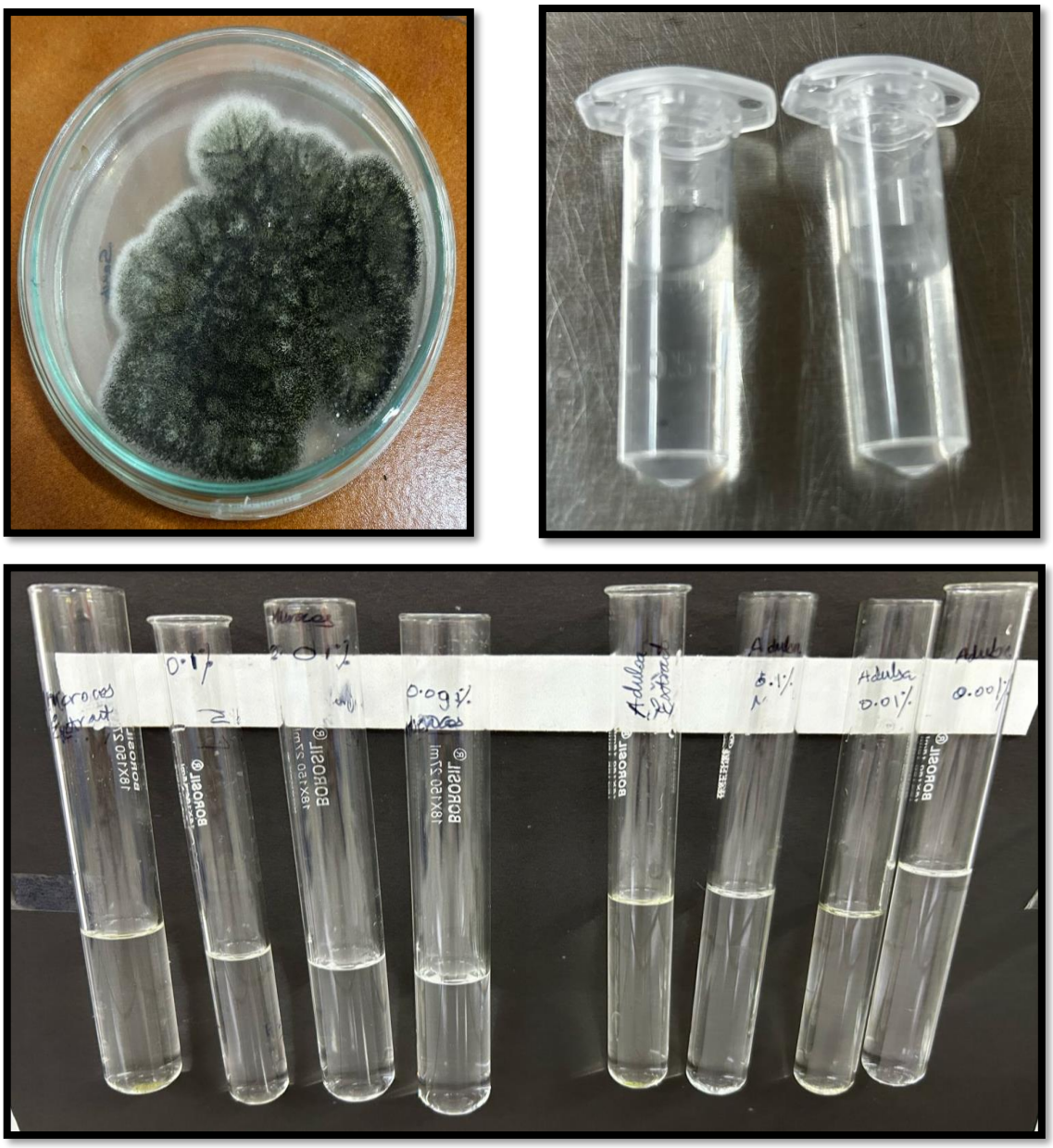

Plate8. a) *Aspergillus sp* subculture streaking, b)Spore suspension, c) Serial dilution of 100µl of *Justicia vasica* extract and *Microcos paniculata*

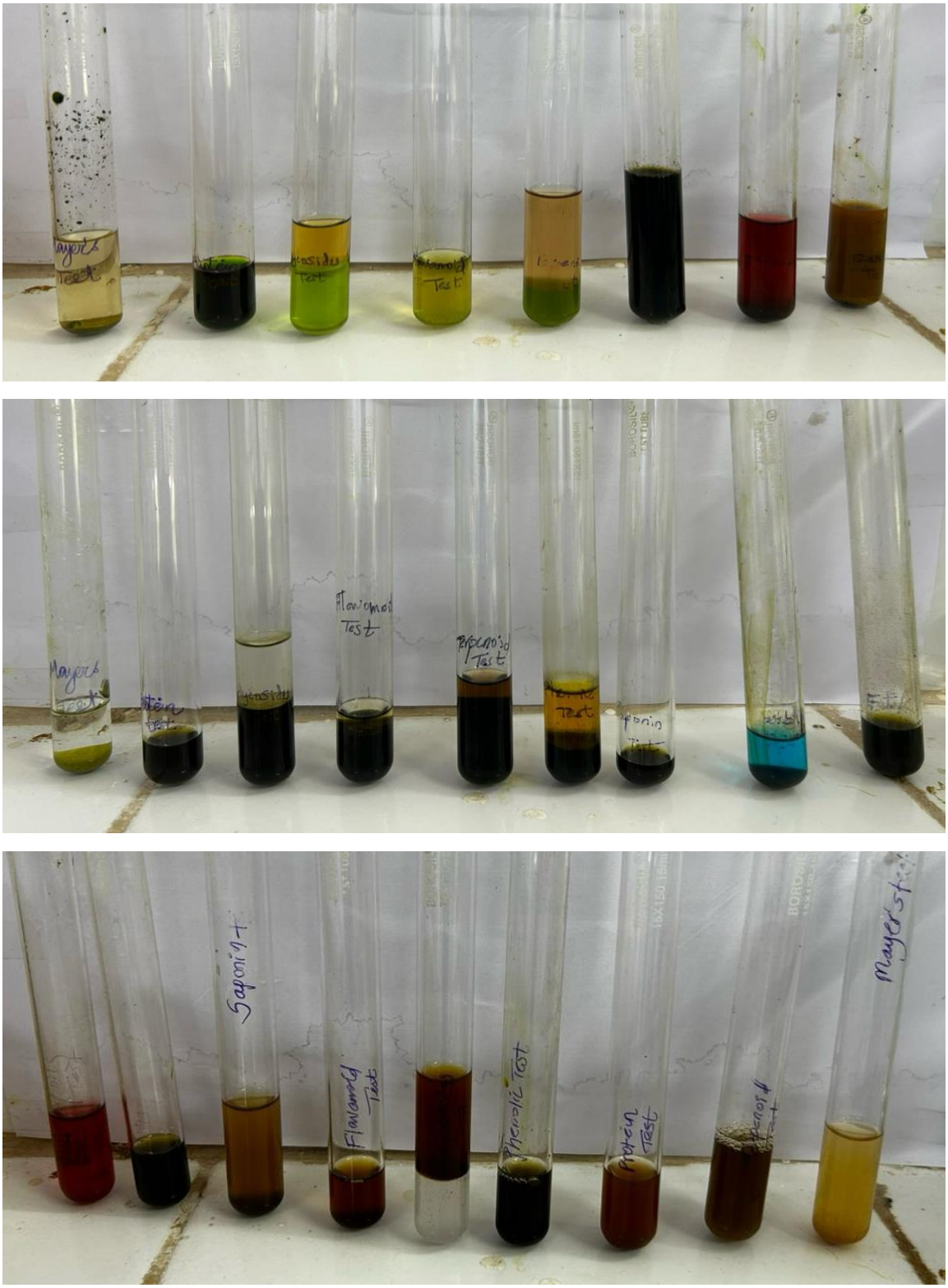

Plate 4. Preliminary phytochemical analysis of *Microcos paniculata, of a*) Methanolic extract, b) Chloroform extract, c) water extract.

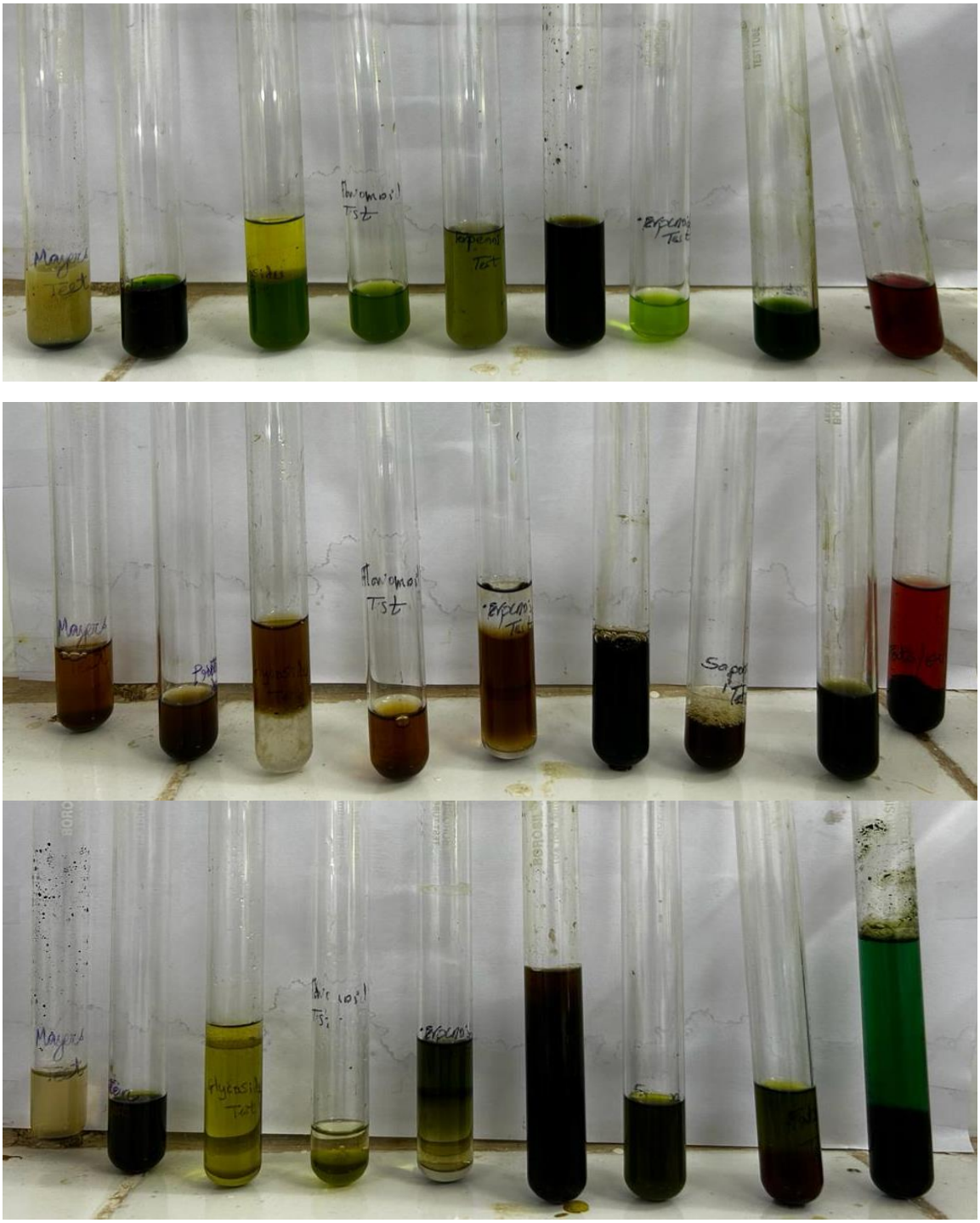

Plate 5. Preliminary phytochemical analysis of *Justicia vasica, of a*) Methanolic extract, b) Chloroform extract, c) water extract.