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Determination of ANC of *Zhizhiphus Mauritiana* (Ethenolic Extract of Leaves)

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Abstract: *Zhizhiphus mauritiana (Rhamnaceae), commonly known as Indian jujube, is a pharmacologically diverse medicinal plant. A plethora of active phytochemical constituents of thisplant has been revealed so far, namely, berberine, quercetin, kaempferol, sitosterol, stigmasterol, lanosterol, diosgenin, and so forth. Several studies demonstrated the exploration of pharmacological potential of various parts such as fruits, leaves, and stems of the plant as antioxidant, cytotoxic, antimicrobial, anti-diarrhoeal, antidepressant, immunomodulator, and hepatoprotective. This review gives a unique summary including phytochemical screening ,analytical parameters , and significant pharmacological importance of Z. mauritiana. The phytochemical analysis of the plant was carried out . The plant has rich source of flavonoids and ascorbic acid which has significant importance in wound healing property and can act as against ulcers . The acid neutralizing property of the plant was determined by back titration method which was found useful in neutralizing the HCL. In the present study, anti microbial activity of Zhizhiphus mauritiana was tested with ethanol extracts against various medically important bacteria such as Staphylococcus aureus, and pathogenic fungi such as Candida albicans. The invitro anti microbial activity was performed by agar well diffusion method. The ethanol extract from Z. mauritiana leaves exhibited significant antibacterial and antifungal activity. In anti bacterial studies, the results revealed that significant zone of inhibition was observed in ethanol extract of Z. mauritiana leaf against Staphylococcus aureus . In anti fungal studies, ethanol leaf extract showed promising results against Candida albicans..*

Keywords: Zhizhiphus mauritiana, phytochemical screening, acid neutralising capacity, antibacterial , antifungal activity

I. INTRODUCTION

Zhizhiphus mauritiana a plant belonging to the family Rhamnaceae is one of those plants which have been used in many disorders since long time in many parts of India as well as other countries . Traditionally, various parts of the plant is useful in variety of disease conditions like, Roots are useful in vitiated conditions of pitta, fever, wounds, ulcer . Bark is useful in dysentery, diarrhea, gingivitis and boils and ulcers. Leaves are useful in stomatitis, wounds, syphilitic ulcers, asthma, leucorrhoea, typhoid fever, diarrhea and obesity. Paste of leaves is applied on wounds, cuts and boils, etc. Fruits are useful in vitiated condition of pitta, burning sensation, hyperdipsia, constipation, flatulence, leprosy, thirst, anorexia, fatigue, , pruritis, wounds ulcers. Seeds are useful in encephalopathy, ophtalmopathy, cough, and asthma, vitiated condition of pitta, burning sensation, diarrhea, vomiting, leucorrhoea, general debility and insomnia. Looking to the very vast uses of the amazing traditional uses we decided to evaluate antiulcer activity of the leaves .

1.1 Introduction to Plant

Zhizhiphus mauritiana comes from a family of Rhamnaceae and is commonly known as ber in India . This tree can be found native to warm climate region of Southeastern Asia and can withstand unfavorable condition such as salinity, drought and water-logging which making it susceptible to grow in various type of Soil. This hardy tree has drooping branches with stipular spines and usually grew up to 15 m tall with trunk 40 cm or more in diameter . Z. mauritiana leaves has tip rounded or slightly notched base. The leaves have a shiny green color above and whitish color below . Meanwhile, the fruit has variable shape and sizes depending on the varieties. The local fruit variety has either round or oblong shape. Moreover, the unripe fruit has light green peel color with white crisp flesh and become yellowish peel color with soft white flesh upon ripening.

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1.2 Pharmacological Actions

A. Antioxidant Activity

An antioxidant is broadly defined as any substance that delays or inhibits oxidative damage to a target molecule by trapping free radicals . There is increment in the interest and study of antioxidant activity of various leaves among scholars and researchers worldwide although antioxidative compounds in leaves are not fully known. Many age related human diseases are also the result of cellular damage by free radicals and antioxidants could play a crucial role in preventing such diseases.

B. Antimicrobial Activity

In current world, a microbial infection was one of the major threats to human and animal population. Antimicrobial drugs provide the essential basic for treatment of various microbial infections instead of the elevated genetic inconsistency of some microorganisms enhance them to quickly develop antimicrobial resistance; therefore, there has been a continuing study for new potent antimicrobials drugs. Due to drug resistance nature of microorganisms, there is a need to find out new lead molecules from alternative sources like plants and algae. The present study shows that leaves ethanolic extracts have inhibitory effect on bacterial growth. The plant extracts show varying degrees of action adjacent to bacteria Staph aureus . Phytochemical components were extracted from Zhizhiphus leaves to have and inhibition activity against bacteria strains namely alkaloids, saponins, tannins, glycosides, flavonoids and terpinoids.

C. Antifungal Activity

Fungal infections are common throughout much of the natural world. In humans, fungal infections occur when fungus invades our body and the immune system unable to destroy the fungus. Fungal infections are often caused by fungi that are common in the environment. One the most common fungus is known as Candida albicans which normally lives as normal flora in human body, cause no harm and has symbiotic relationship with the host. This study aims to reduce the usage of synthetically derived antifungal by determining the antifungal activities of the methanol leave extract of Z. mauritiana.

II. PLANT PROFILE

Fig 1: Plant of Zhizhiphus Mauritania

2.1 Taxanomical Classifications

Table 1: Taxonomical Classification

Domain:	Eukaryota
Kingdom:	Plante
Phylum:	Spermatophyta
Subphylum:	Angiospermae
Class:	Dicotyledonae
Order	Rhamnales
Family:	Rhamnaceae

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Genus:	Zhizhiphus Mill-Jujube
Species	Zhizhiphus mauritiana- Indian jujube

- Preferred Scientific Name : Zhizhiphus mauritiana
- Preferred Common Name : Indian Jujube Taxonomic Tree :
- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae

2.2 Distribution

Found in India as well as other countries like Burma, Iran, Syria, Afghanistan, Europe, Australia, America and Africa as a traditional system of medicine. In India commonly it is known as a ber in Hindi and Badrah in Sanskrit. It is distributed originally from the Middle East or the Indian subcontinent, but now cultivated throughout the tropics and subtropics for its nutritious. In India it is found throughout the country to an altitude of approximately 1500 m in the Himalayas, typically on or near old village sites, and very commonly on black cotton soils in the central India.

III. PHARMACOGNOSTIC EVALUATIONS

3.1 Morphological Evaluation

The plant is an evergreen shrub with small spines and a teeny plant contains many hanging branches with pointed stipules. Generally, the leaves are 2.5–4.0 cm long and 1.8–3.8 cm wide, alternate, ovate or oblong-elliptic, entire, with three prominent basal veins, with a rounded apex

- Color: Green
- Odour: Characteristic
- Taste: Bitter

3.2 Microscopic Evaluation

The microscopic study of the fresh leaf section showed presence of upper and lower epidermis and vascular tissues i.e. xylem and phloem. Lignified pink colored components were clearly observed in the section. The powdered material showed presence of xylem vessels, covering trichomes and starch grains in it.

3.3 Physical Evaluation of Powder

The collected leaves were dried by using shade drying method for complete removal of moisture. It was further powdered by hand crushing and finally passed through sieve no 40 # size to obtain uniform sized powdered particles. Physical evaluation of this dry powder was done for following parameters.

1. Solubility
2. Moisture content
3. Ash value

IV. MATERIAL AND METHODS

4.1 Morphological Evaluation

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4.3 Analytical Parameter

A. Ash Values

Theory

Used to determine quality and purity of a crude drug and to establish the identity of it. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. These are present in definite amount in a particular crude drug hence, quantitative determination in terms of various ash values helps in their standardization. Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug Total ash value'. Such variables are removed by treating with acid (as they are affects soluble in hydrochloric acid) and acid insoluble ash value is determined e.g. Rhubarb, Liquorice etc. Used to determine foreign inorganic matter present as an impurity.

Total Ash Value

Procedure

- Weigh and ignite flat, thin, porcelain dish or a tared silica crucible.
- Weigh about 2 g of the powdered drug into the dish/crucible.
- Support the dish on a pipe-clay triangle placed on a ring of retort stand.
- Heat with a burner, using a flame about 2 cm high and supporting the dish about 7 cm above the flame, heat till vapours almost cease to be evolved; then lower the dish and heat more strongly until all the carbon is burnt off.
- Cool in a desiccator.
- Weigh the ash and calculate the percentage of total ash with reference to the air dried sample of the crude drug
- If a carbon free ash cannot be obtained in this way then any one of the following method can be used.

1. Exhaust the charred mass with hot water, collect the residue on an ash less filter paper incinerate the residue and filter paper, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450°C.
2. Cool the crucible; add 15 ml of alcohol, break up the ash with glass-rod burn off the alcohol and again heat the whole to a dull red heat. Cool, weigh the ash

Acid Insoluble Ash Value

Procedure:

1. Using 25 ml of dilute hydrochloric acid, wash the ash from the dish used for total ash into 100 ml beaker and add a crude drug. Further
2. Place a mere gauze over a Bunsen burner and boil for five minutes.
3. Filter through an ash less filter paper, wash the residue twice with hot water.
4. Ignite a crucible in the flame, cool and weigh.
5. Put the filter-paper and residue together into the crucible; heat gently until vapours cease be evolved and then more strongly until all carbon has been removed.
6. Cool in a desiccator.
7. Weigh the residue and calculate acid-insoluble ash of the crude drug with reference to the airdried sample of the crude drug

Water Soluble Ash Value

Procedure:

Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug

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1. Using 25 ml of water, wash the ash from the dish used for total ash into 100 ml beaker .a crude drug. Further
2. Place a mere gauze over a Bunsen burner and boil for five minutes.
3. Filter through an ash less filter paper, wash the residue twice with hot water.
4. Ignite a crucible in the flame, cool and weigh.
5. Put the filter-paper and residue together into the crucible; heat gently until vapours ceas be evolved and then more strongly until all carbon has been removed.
6. Cool in a desiccator.
7. Weigh the residue and calculate acid-insoluble ash of the crude drug with reference to the air-dried sample of the crude drug

V. EXTRACTION BY MACERATION

Medicinal plants are extracted and processed for direct consumption as herbal or traditional medicine or prepared for experimental purposes. The concept of preparation of medicinal plant for experimental purposes involves the proper and timely collection of the plant, authentication by an expert, adequate drying, and grinding. This is followed by extraction, fractionation, and isolation of the bioactive compound where applicable. Extraction of medicinal plants is a process of separating active plant materials or secondary metabolites such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides from inert or inactive material using an appropriate solvent and standard extraction procedure. Several methods were used in the extraction of medicinal plants such as maceration, infusion, decoction, percolation, digestion and Soxhlet extraction,

The solvent used for the extraction of medicinal plants is also known as the menstruum. The choice of solvent depends on the type of plant, part of plant to be extracted, nature of the bioactive compounds, and the availability of solvent. In general, polar solvents such as water, methanol, and ethanol are used in extraction of polar compound, whereas nonpolar solvents such as hexane and dichloromethane are used in extraction of nonpolar compounds. Furthermore, solvent used in extraction is classified according to their polarity, from *n*-hexane which is the least polar to water the most polar. The following are various solvents of extractions arranged according to the order of increasing polarity.

	Solvent	Polarity
1.	n-Hexane	0.009
2.	Petroleum ether	0.117
3.	Diethyl ether	0.117
4.	Ethyl acetate	0.228
5.	Chloroform	0.259
6.	Dichloromethane	0.309
7.	Acetone	0.355
8.	n-Butanol	0.586
9.	Ethanol	0.654
10.	Methanol	0.762
11.	Water	1.000

VI. MACERATION

- This is an extraction procedure in which coarsely powdered drug material, either leaves or stem bark or root bark, is placed inside a container;
- the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days.
- The content is stirred periodically, and if placed inside bottle it should be shaken time to time to ensure complete extraction.
- At the end of extraction, the micelle is separated from marc by filtration or decantation.

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- Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath .
- This method is convenient and very suitable for thermolabile plant material.

VII. PHYTOCHEMICAL ANALYSIS

Phytochemical Screening or Preliminary Test is the first thing to be done before major discoveries of molecules or drug entities are known. It is used to provide concrete knowledge and research to what plant active constituents have potential to benefit mankind.

Preliminary Phytochemical Screening of the Extracts

The qualitative chemical test ethanolic extract of Z.mauritiana leaves was carried out using standard procedure.

Tests for Carbohydrates and Reducing Sugars:

The extracts were dissolved in water and filtered. The filtrates were divided intoseveral portions and were tested as follows:

1. Molisch's test: A small quantity of aqueous extract was subjected to Molisch's reagent (naphthol in alcohol). Shake and add conc. H_2SO_4 from sides of the test tube.A violet ring is formed at the junction of two liquids. This indicates the presence of carbohydrates.
2. Fehling's test: A small quantity of aqueous extract was subjected to Fehling's A and B reagents ($CuSO_4$ solution), boil on water bath. A brick red precipitate is formed. This indicates the presence of reducing sugars.
3. Benedict's test: To a set of filtrates of various extracts, added equal volumes of Benedict's reagent and heated in boiling water bath for 5min. The appearance of green yellow or red colour indicated the presence of reducing sugars.

Test for Alkaloids

Small quantities of extracts were treated with few drops of diluted hydrochloric acid and filtered. The filtrates of each extract were divided into four portions and the following tests were carried out.

1. Dragendroff's test: With Dragendroff's reagent (solution of potassium bismuth iodide) formed orange brown precipitate.
2. Mayer's test: With Mayer's reagent (potassium mercuric iodide solution) formed creamy precipitate.
3. Hager's test: With Hager's reagent (saturated picric acid solution) formed yellow precipitate
4. Wagner's test: With Wagner's reagent (solution of iodine in potassium iodide) formed reddish-brown precipitate. The formation of respective precipitates indicated the presence of alkaloids.

Tests for Glycosides

The following tests were carried out to detect the presence of different types of glycosides

1. Legal's test: To the extracts, added pyridine and sodium nitroprusside and development of pink or red colour indicated the presence of cardiac glycosides.
2. Borntrager's test: The extracts were boiled with dilute sulphuric acid and filtered. To the cold filtrates equal volumes of chloroform were added. After thorough shaking the organic solvent layers were separated and ammonia solution was added. 96 The change of ammonia layer to pink or red color indicated the presence of Anthraquinone glycosides.
3. Foam test: Small quantities of drugs were shaken vigorously with water. Formation ofpersistent foam indicated the presence of saponin glycosides.
4. Grignard reaction or sodium picrate test:

Soaked filter paper strips first in 10% picric acid and then in 10% sodium carbonate and dried. Drugs were taken in small bottles and the strips were suspended from the mouth of the container and the lids were tightly closed with portion of the strip stuck in the lid. The strips did not turn brick red or maroon indicating the absence of cyano genetic glycosides Extracts when made alkaline did not show blue or green fluorescence indicating the absence of coumarin glycosides

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Test for Steroids and Triterpenoids

1. Salkowski reaction: To the extracts, chloroform and concentrated sulphuric acid were added and shook well. The appearance of reddish-blue colour in the chloroform and green fluorescence in acid layer indicated the presence of steroids
2. Liebermann-Burchard reaction: To the extracts added chloroform, mixed and then added acetic anhydride followed by concentrated sulphuric acid from the sides of the tubes.Appearance of first red, then blue and finally green colour indicated the presence o steroids and triterpenoids.

Test for Flavonoids:

1. Sulphuric acid test: extract was added to 66% or 80% of sulphuric acid. Deep yellow solution is formed which indicates the presence of flavones and flavonols Orange to red colour solution is formed which indicates the presence of flavones. Red or reddish blue solution isformed which indicates the presence of aurones and chalcones.
2. Shinoda test: Extract + 95% ethanol + conc. Hydrochloric acid + Mg turnings. Orange, pink red to purple colour is observed. This indicates the presence of flavonols, dihydro derivatives and xanthenes
3. Test for lead acetate: Test residue was subjected to lead acetate solution. A yellow colour precipitate is formed. This indicates the presence of Flavonoids.

Tests for Phenolic compounds and Tannins:

Small quantities of the extracts were treated with the following reagents and the appearance of corresponding endpoints indicated the presence of phenolic compounds and tannins

1. With 5% Ferric chloride solution: Deep blue-black colour.
2. With 10% lead acetate solution: White precipitate..
3. Salkowski test: Extract + chloroform + conc. H2SO4, shake well. CHCl3 layer shows red colour which indicates the presence of steroids, acid layer shows greenish yellow colour which indicates the presence of triterpenoids.
4. Liebermann's test: Extract + acetic anhydride, heat, cool and add conc. H2SO4. Blue colour is appeared. This indicates the presence of steroids.

Test for Saponins

1. Foam Test: The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam indicated the presence of saponin
2. Haemolysis Tests: - Add leaves extract to one drop of blood placed on glass slide. Hemolytic zone appears.

VIII. DETERMINATION OF TOTAL FLAVONOID CONTENT

Folin-Ciocalteu method was used to estimate total flavonoid contents. In this experiment, 2 mL of each extract solution was mixed with 2 mL of aqueous AlCl3.6H2O (0.1mol/L). The mixture was incubated at room temperature for 10 minutes. Absorbance of the final reaction mixture was measured at 417nm. The content of flavonoids was expressed as µg quercetin equivalents (QEs) using standard calibration curve

Thin Layer Chromatography:

Sample solutions (10 ml) were applied against standard solutions on silica gel plates. Development of the plates was done using the solvent system that consisted of butanol:water:acetic acid (glacial) (55:30:15, v/v/v). Plates were dried and visualized under UV light and then sprayed with 80% Folin-C phenyl reagent in water. Standard solutions were prepared by dissolving (0.025 g) of powder samples in 50% methanol–water (10 ml)

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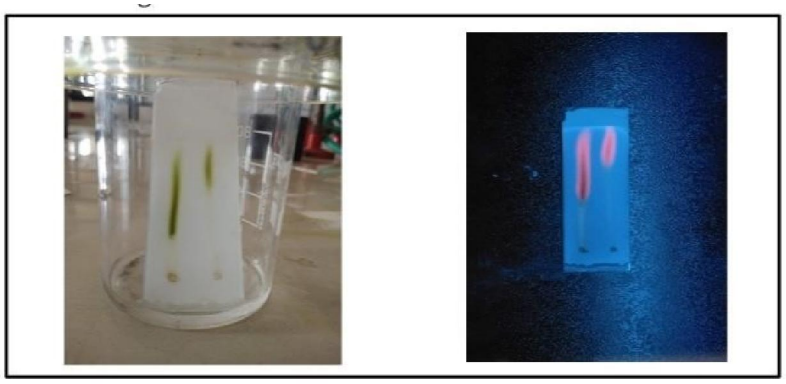

Fig No 2 : TLC

IX. ACID NEUTRALISING CAPACITY

A. Selection of samples

Commonly consumed commercial antacid drugs and natural antacid remedies were identified based on a literature survey and their availability.

B. Preparation of samples

Commonly consumed commercial antacids drugs and natural antacid remedies were collected and the juice was extracted from the samples.

C. Analysis of the antacid content of the selected sample

Analysis of Acid neutralizing capacity was done following the Rossett-Rice test. Back titration method is the basis of this test. In this method, an antacid is dissolved in an excess of acid and is titrated against a known concentration of base until an endpoint is reached. The molarity of neutralized acid is equal to the difference between the moles of acid added and therefore the moles of base required for the back titration.

Procedure

1. The antacid formulation is dissolved in a known amount of surplus HCl and is titrated with an alkali, NaOH(aq) until enough OH– (from the NaOH solution) has been added to completely react with the excess H+ (from the excess HCl in the solution).
2. A portion of the added acid is neutralized by the antacid, the remainder is neutralized by the NaOH added. An equilibrium is reached when the number of moles of NaOH added is equal to the number of moles of HCl remaining after the reaction with the antacid. HCl acts as the source of H+(aq) and NaOH as the source of OH–(aq). At the endpoint of the titration, the acid will be totally neutralized by the base. 1 g of each formulation was weighed and crushed using mortar and pestle.
3. It was then transferred into a conical flask and 25ml of 0.1 molarity of HCL was added to each sample, 2-3 drops of phenolphthalein indicator were added. The samples were then titrated against 0.1 M NaOH. The titrations were repeated until concordant values were obtained. The experiments were undertaken under standard laboratory conditions. Standardization of HCl and NaOH was carried out as per the USP method.

Calculation

Eq.(1) moles of acid neutralized = moles of acid added – moles of alkali required

= (VolumeHCl x MolarityHCl) – (VolumeNaOH x MolarityNaOH)

Eq. (2) acid neutralizing capacity per gram of antacid = moles of HCL neutralized grams of antacid.

D. Data Analysis

The antacid values of the commercial and traditional antacids were expressed as mean ± standard error of the mean

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(SEM). An independent t test was administered to check the difference between the different categories of antacids to check their efficacy.. Data were analyzed using the Statistical Package for Social Sciences (SPSS) (version 19.0) software. The significance level for the difference between was set at $p<0.05$

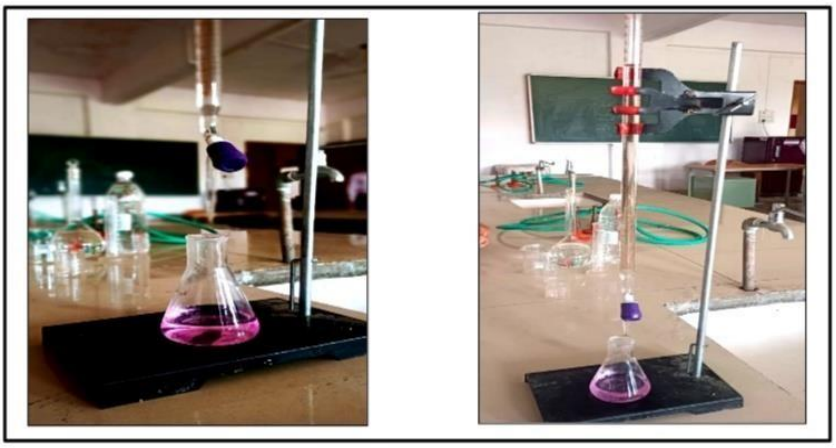

Fig 3: ANC

X. ANTIMICROBIAL ACTIVITY

Antimicrobial Screening

- The leaf extracts of Z.mauritiana were tested for antimicrobial activity against the selected bacteria and fungi.
- Nutrient agar media were used for bacterial growth and Potato dextrose agar media were used for fungal culture.
- The media were prepared and then autoclaved at 121°c for 15 minutes and were poured on petriplates and allowed to solidify.

Well Diffusion Method

- Well diffusion method is used to evaluate both antibacterial and antifungal activity
- The prepared culture plates were inoculated with selected strains of bacteria and fungus using spread plate method.
- The wells were made on the agar surface with sterile cork borer.
- The extracts were poured into the well using micropipette with the concentration of 20, 40 and 60µl/ml.
- Fluconazole was used as positive reference standard to determine the sensitivity of each microbial species tested.
- The bacterial culture plates were incubated at 37°C for 24 hours and fungal culture plates were incubated at room temperature for 1 week.
- The zone of inhibition was calculated by measuring the diameter of the zone around the well in millimeters (mm).

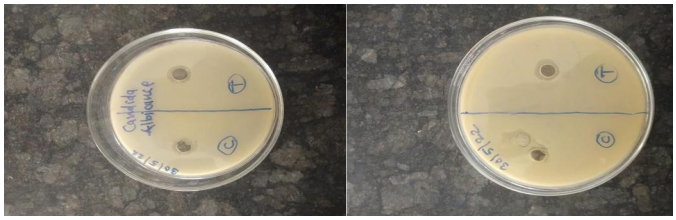

Fig 4: Antimicrobial activity

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X. RESULT AND DISCUSSION:

Morphological evaluation

Dendrocalamus strictus was evaluated for morphological parameters showed in the Table 1. The color of formulation was green . The odor of of the crude drug is chacteristic and taste was found to be bitter.

Table 2. Morphological evaluation

Sr.no	parameter	observation
1.	Color	Green
2.	Odour	Characteristic
3.	Taste	Bitter

Microscopical evaluation

The microscopic study of the fresh leaf section showed presence of upper and lower epidermis and vascular tissues i.e. xylem and phloem. And determination of partical size of powder.

Analytical paramters

Zhizhiphus Mauritaina was evaluated for analytical parameters was showed in the table 3. The total ash value was found to be 12% w/w. and water soluble and acid soluble ash found to be 4.2 and 6.5% respectively

Table 3. Analytical parameters

Sr. no	Physical parameter	Obtained value (%w/w)
1	Total Ash Value Water soluble ash Acid insoluble ash	7.89 % 3.96 % 2.34 %
2	Moisture content	53.45%
3	Solubility	Ethanol

Phytochemical screening of extract

The Zhizhiphus Mauritaina was evaluated for phytochemical parameters shown in the table It was found to be presence of phytochemicals such as flavonoids, alkaloids , glycosides and other constituents in ethanolic extract.

Table 4. Phytochemical screening of extract

Sr. no	Test name	Observations Ethanolic extract
1.	Flavonoids	
	1. Shinoda Test	+ve
	2. Alkaline test	+ve
	3. NaOH test	+ve
2.	Glycosides	
	1. Keller Killani test	+ve
	2. Modified Borntragers test	+ve
3.	Sterols	
	1. Salwoski test	+ve
	2. Libberman test	-ve

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4.	Saponins 1. Hemolysis test 2. Foam test	 +ve +ve
5.	Alkaloids 1. Wagners test 2. Hagers test 3. Dragondroff test	 -ve -ve -ve
6.	Carbohydrates 1. Molish test 2. Benedicts test 3. Barfoed test	 +ve +ve -ve
7.	Terpenoids 1. Salwoski test	 +ve
8.	Tannins , phenolic compounds: 1. Lead acetatetest 2. Gelatin test	 +ve +ve

Image 5: Test of phytochemical constituent

Total phytochemical content

Table 5: Total phytochemical content

Sr .no	Total content	Conc . of content
1	Total flavonoid content	54 per 100 gm

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TLC

Table 6: TLC

Sr . no	Extract name	Solvent system	
		No of spots	Rf values
1.	Ethanolic extract	2	0.77
			0.84

Acid neutralizing activity

1 gm of each antacid tablet was taken and each of them contained strong base as an active ingredient .The higher the amount of Hydrochloric acid neutralized by antacids, the better is the acid neutralizing capacity.

Table 7: Acid neutralizing capacity

Antacid	Gelusil (aluminium hydroxide)	Dygil (magnesium hydroxide)	Zhizhiphus Mauritaina extract
Weight of sample	1gm	1gm	1gm
Volume of NaOH required	25ml	25ml	25ml
Volume of HCL required	10.2	24.4	19.7
Moles of HCL neutralized	1.4	0.08	0.53

Antimicrobial activity

Table 8: Antimicrobial activity

Antimicrobial agent	Zone of inhibition (mm)	
	Ethanolic extract	Standard (fluconazole)
Staphylococcous aureous	16mm	11mm
Candida albicans	8.4mm	8mm

XI. CONCLUSION

In the present study, it is found that Z.mauritiana leaf extracts possesses significant antimicrobial activity against the tested microorganisms and the plant contains potential antimicrobial components for the therapy of microbial infections. Therefore the results justify the use of the leaf extract of Z.mauritiana in treating these pathogenic strains and these may serve as leads for the development of new pharmaceuticals. According to the research works on Zhizhiphus mauritianaI have concluded that different types of secondary metabolites are present that have effective functions on different type of diseases. It shows very effective function against pathogens.

The plant has rich source of flavonoids and ascorbic acid which has significant importance in wound healing property and can act as against ulcers . The acid neutralizing property of the plant was determined by back titration method which was found useful in neutralizing the HCL .The ANC was found to be 0.53 which can neutralise the acid in the stomatch.

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