



DETERMINATION OF ANTIMICROBIAL ACTIVITY USING METHANOLIC EXTRACT OF PITHECELLOBIUM DULCE PLANT LEAVES.

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ABSTRACT Present days due to the pathogenic bacteria and fungi, the human mortality rate has increased in number. The synthetic antibacterial and antifungal are used in the treatment of various fungal and bacterial diseases, but have various side effects which limit's their use. Traditionally Pithecellobium dulce plant has been used for treatment of antifungal and antimicrobial infection. In this article we are comparing the activity of Antibacterial and antifungal properties of Pithecellobium dulce plant using leaves extract on gram negative bacteria, gram positive bacteria and fungi. Methanolic extraction of Pithecellobium dulce leaves was carried out using Soxhlet method and the phytoconstititional analysis was carried out for presence of alkaloids, flavonoids, glycosides, saponins, phytosterols, and triterpenoids. The inhibitory effect was determined by Cup plate method, by the measurement of zone of inhibition. The outcome was the leaves extract consisted of active phytoconstituents which were phenols, glycoside, sugar and flavanoid due to which the extract showed significant zone of inhibition, by which property of antibacterial and antifungal was listed. It can be concluded that the leaves of Pithecellobium dulce consist of active phytoconstituents which can be used as potent antimicrobials

KEYWORDS : Phytoconstituents, Antibacterial, Antifungal, Pithecellobium Dulce, Soxhlet, Antimicrobial Agents.

INTRODUCTION

Pithecellobium dulce is a species of flowering plant in the Pea family, Fabaceae. It is used as an effective traditional medicine by the tribal people of Srilanka and India since 1994. The leaves of this plant is used in poultice with alcohol to treat bile as well as to prevent abortion. Traditional medicine also claims to treat ulcer and used as astringent and haemostatic. Due to failure or development of microbial resistance to antibiotics, it has resulted in emergence of multiple clinical situations in the treatment of infection. Plants produce wide variety of secondary metabolites such as terpenoids, tannins, phenol, flavonoids, and glycosides etc, which have claimed to be effective antimicrobials agents. The plant extract or their active constituents are used as folk medicine Pithecellobium dulce plants extract has been selected to conclude their antimicrobial properties. These plants consist of active constituents which can be a future scope for treatment of bacterial and fungal infection. Pithecellobium dulce in relation with antimicrobial properties, it also has antiviral, anti diabetic, antiepileptic properties which broaden areas towards research of other pharmacological properties. In the current research, we have worked upon the methanolic extract of Pithecellobium dulce plant leaves screening it against pathogenic bacteria and fungi to detect new sources of antimicrobial agents.



Pithecellobium dulce Tree.



Pithecellobium dulce leaves.

MATERIALS AND METHOD.

COLLECTION OF PLANT MATERIAL

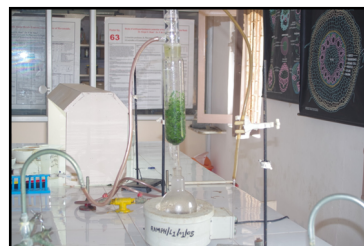
The fresh leaves of Pithecellobium dulce were collected from Rampura (Godhra) region, Dist-Panchmahal, (Gujarat) in the month of October 2019 and were authenticated by botanist (Department of Botany, Rampura, Gujarat)

PREPARATION OF EXTRACT

The collected leaves of the Pithecellobium dulce were dried under ambient temperature and the sample was grinded properly in to fine

powder. Extraction of the powder was carried out in Soxlet extractor by using methanol as a solvent 65 °C for 24 hours. The extracts were then filtered using Whatman filter paper 1 and concentrated by evaporating on rotary evaporator. Dark greenish color extract was obtained and collected in sterile bottles and kept in a cool and dry place.

Fig.no.1-Methanolic extraction of Pithecellobium dulce leaves using Soxlet extractor.



PRELIMINARY PHYTOCHEMICAL SCREENING.

Air dried powdered plant material were screened for presence of saponins, tannins, alkaloids, phenols, flavanoid, glycoside, triterpenoids, starch, protein, carbohydrates, reducing sugar and amino acid as described in the literature [15-16].

TEST FOR MICROORGANISM AND GROWTH MEDIA.

Microorganism used in this study represents pathogenic species associated with nosocomial infections. They were obtained from Microbial Technologies Chandigarh, which consisted of one gram positive bacteria-*Staphylococcus aureus* (MT Cc96), one gram negative bacteria-*Salmonella typhimurium* (MTCC3224), and two fungal strains- *Aspergillus niger* (MTCC282), *Candida albicans* (MTCC227).

The bacterial stock cultures were incubated on nutrient agar medium at 37 °C for 24 hours and the fungal stock cultures were incubated on potato dextrose agar medium at 37 °C for 24 hours followed by refrigeration at 4 °C. The bacterial strains were grown in blood agar medium at 37 °C, where yeast and moulds were grown in potato dextrose agar medium at 28 °C. The stock cultures were maintained at 4 °C.

ANTIMICROBIALACTIVITY

DETERMINATION OF ZONE OF INHIBITION METHOD.

The agar plates are simply drilled with a stainless steel cylindrical chamber in the middle area and the plant extract is added inside the drilled area and allowed to diffuse and interact with freshly seeded test organism. Resulting zone of inhibition is formed uniformly due to antimicrobial agents present in the leaf extract, which inhibit the growth of microorganism. The diameter of zone of inhibition is measured in millimeters.

METHOD

Stainless steel cylindrical chamber (measuring 2.5cm in length and 1.5cm in diameter) is priority sterilized on heating flame and is used to cut a circular shaped disk in the center of agar plates. The leaves extract of *Pithecellobium dulce* about 20µl each, of five different concentrations (5µl, 25µl, 50µl, 100µl, 250µl) are added on 5 individual cut portion of agar plates. The inoculating loop is sterilized on heating flame and the test culture is streaked on the plated with streak plate method for fungus culture & pour plate method for bacterial culture and allowed to stay at 37 °C for 3 hours.

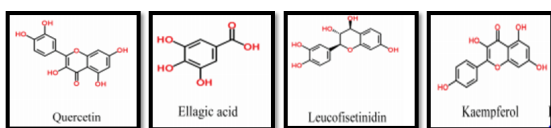
The control experiment was carried out in similar condition with standard drug Chloramphenicol for antibacterial assay and Nystatin for antifungal assay. The zone for growth of inhibition around the circular disk was measured after 36-48 hours in incubation at 37 °C.

RESULTS& DISCUSSION.

PRELIMINARY PHYTOCHEMICAL SCREENING.

It was found that methanolic extract of *Pithecellobium dulce* leaves consisted of the following active phytoconstituents, they were Flavanoids, Glycosides, Phenols, Sugar.

Fig no.2-General structure of compounds present in *Pithecellobium dulce* leaves extract.



Antimicrobial activity

The antimicrobial activity of the *Pithecellobium dulce* leaves extracts was studied under different concentration of extract and standard drug, against two pathogenic bacteria, one gram positive bacteria-*Staphylococcus aureus* & one gram negative bacteria-Salmonella typhimurium and two fungal strains- *Aspergillus niger* & *Candida albicans*.

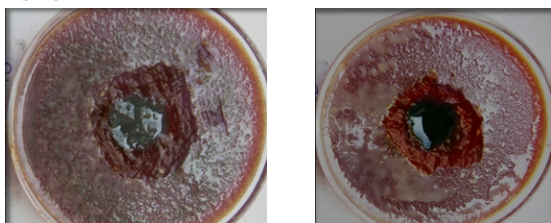
The antibacterial potential of *Pithecellobium dulce* leaves extract in terms of zone of inhibition with comparison to standard drug is presented in table 1.1 and Graph-1.

The antifungal potential of *Pithecellobium dulce* leaves extract in terms of zone of inhibition with comparison to standard drug is presented in table 1.2 and Graph-2.

Table 1.1 Antibacterial activity of methanolic extract of pithecellobium dulce leaves and standard drug against test organism.

Name of test Microorganism	Concentration of the solution.				
	5µl	25µl	50µl	100µl	250µl
<i>Staphylococcus aureus</i>	-	10mm	12mm	16mm	18mm
<i>Salmonella typhimurium</i>	-	10mm	11mm	14mm	16mm
Chloramphenicol	12mm	14mm	17mm	19mm	20mm

Fig. no.3-Antibacterial sensitivity test – plates showing zone of inhibition

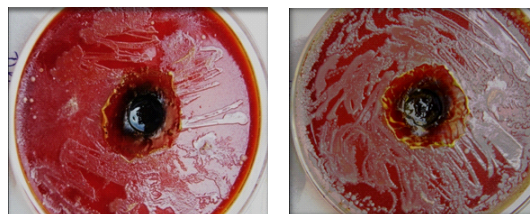


Salmonella typhimurium *Staphylococcus aureus*

Table 1.2 Antifungal activity of methanolic extract of Pithecellobium dulce leaves and standard drug against test organism.

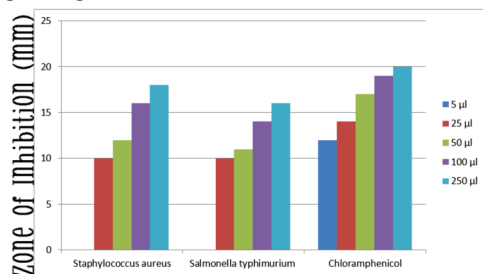
Name of test Microorganism	Concentration of the solution.				
	5µl	25µl	50µl	100µl	250µl
<i>Aspergillus niger</i>	-	10mm	12mm	14mm	16mm
<i>Candida albicans</i>	-	9mm	10mm	12mm	14mm
Nystatin	10mm	12mm	15mm	18mm	20mm

Fig.no.4-Antifungal sensitivity test – plates showing zone of inhibition.

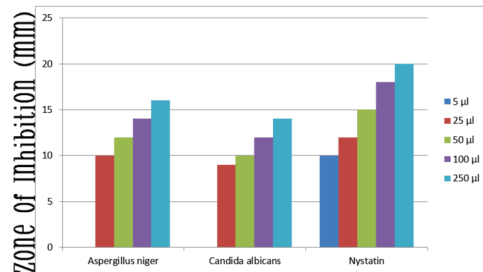


Aspergillus niger

Candida albicans



Graph- 1.Antibacterial activity of methanolic extract of pithecellobium dulce leaves and standard drug against test organism.



Graph- 2.Antifungal activity of methanolic extract of Pithecellobium dulce leaves and standard drug against test organism.

The antimicrobial activity of the extract increased gradually on increasing the concentration of the extract, as compared with standard drug. The result revealed that the *Pithecellobium dulce* leaves extract had more antibacterial activity on *Staphylococcus aureus* compared to *Salmonella typhimurium* and the growth inhibition zone was from range 10-18mm, while the antifungal activity on *Aspergillus niger* was more compared to *Candida albicans* and the growth inhibition zone was from range 10-16mm. The result showed that the methanolic extracts of *Pithecellobium dulce* were found to be effective against the microbial tests. Results shows that plant rich in flavanoids and phenol constituents possess more antimicrobial properties against wide number of microorganisms.

CONCLUSIONS

In the current investigation, the methanolic extract of leaves of *Pithecellobium dulce* were used to determine the antibacterial and antifungal properties in which comparison of microbial activity using most tested bacterial and fungal strains was carried out, which concluded that active phytoconstituents such as phenol and flavanoids are present in *Pithecellobium dulce* leaves which can be used as effective antimicrobials. Further investigation should be carried out on *Pithecellobium dulce* plants and various active constituents should be implemented in modern antimicrobial preparation.

REFERENCES

1. Medicinal uses of *Pithecellobium dulce* and its health benefits by Kaushik V Kulkarni and Varsha R Jamakhandi, Journal of Pharmacognosy and Phytochemistry 2018; 7(2): 700-704.

2. KasarlaRaju, Jagadeshwar K. Photochemical investigation& and hepatoprotective activity of ripe fruits of Pithecellobium Dulce in albino rats. Scholar'sacademic journal of pharmacy.2014; 449-454.
3. Raghu Praveen A et al. Anti diabetic activity of bark extract of Pithecellobium Dulce benth in alloxan- induced diabetic rats. Natural products. An Indian journal. 2010; 6(4):201-204.
4. JayaramanMegala, PannerDevaraju. Pithecellobium dulce fruit extract. Antiulcerogenic effect by influencing the gastric expression of H⁺-ATPase& Mucosal Glycoprotein's. Journal of young pharmacist. 2015; 7(4):493-499.
5. Hepziban W, Vajida J, Balaji M. Studies on antibacterial activity of Pithecellobium Dulce (Roxb.) Benth against food pathogens – Gram negative bacteria. International journal of novel trends in pharmaceutical science. 2017; 7(3):76-80.
6. WatsikaVichaidrt, PanumartThongyoo. Antioxidant & antibacterial properties of leaf extract of Pithecellobium Dulce, available at www.natpro5.psu.ac.th, 66-68.
7. Erdogru OT. 2002. Anti-bacterial activities of some plant extract used in folk medicine. Pharm, Biol., 2002, 40, 269-273.
8. Zapesochnaya GG, Yarosh EA, Syandize NV and Yarosh GI. 1980. Flavanoids of the leaves of Pithecellobium dulce. Khim. Prir. So ed in., 1980, 2, 252-253 .
9. Agarwal, V.S. 1982. Economic Plants of India, a Pilot Study. Journal of Research in Ayurvedha and Siddha, 10 (1-2): 87-92.
10. Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management by Steven Y. C. Tong, Joshua S. Davis, Emily Eichenberger, Thomas L. Holland, Vance G. Fowler, Jr. Microbiological clinical reviews. DOI: 10.1128/CMR.00134-14.
11. Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections by John A. Crump, Maria Sjölund-Karlsson, Melita A. Gordon, Christopher M. Parry .Microbiological clinical reviews DOI: 10.1128/CMR.00002-15.
12. Aspergillus niger: an unusual cause of invasive pulmonary aspergillosis by A. K. Person, S. M. Chudgar, B. L. Norton, B. C. Tong, and J. E. Stout. J Med Microbiol. 2010 Jul; 59:834–838. DOI: 10.1099/jmm.0.018309-0.
13. Candida albicans: A Model Organism for Studying Fungal Pathogens by Zulfiqar Ahmad, M AnaulKabir, Mohammad Hussain. Internation scholarly research network, volume 23. DOI: 5404/2012/538694.
14. Methanolic extraction and isolation of bioactive compounds from Pithecellobium Dulce leaves by column chromatography and GC-MS studies by AnandBobade. Research Journal of Chemical Science. Volume 7, ISSN 2231-606X.
15. Khandelwal KR. 2nd ed. Pune: NiraliPrakashan; 2009. Practical Pharmacognosy.
16. Kokate CK. Delhi: New Gyan Offset Printers; 2000. Practical Pharmacognosy .
17. Rios JL Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol. 1988.
18. Anand, C. R., H. Gordon, H. Shaw, K. Fonseca, and M. Olsen. 2000. Pig and goat blood as substitutes for sheep blood in blood-supplemented agar media. J. Clin. Microbiol.
19. The United States Pharmacopoeia, 2019, The United States Pharmacopoeial Convention, Rockville, MD.
20. MacFaddin J., 1985, Media for the Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.
21. Growth and Laboratory Maintenance of Staphylococcus aureus by Dominique M. Missiakas and Olaf Schneewind. Curr Protoc Microbiol. DOI: 10.1002/9 78047172 9259.mc09c01s28.
22. Pulvertaft, R. J. V. 1930. Bacterial blood cultures. Lancet i:821-822.
23. Isolation and Identification of Fungi Jane E. Sykes, Shelley C. Rankin, in Canine and Feline Infectious Diseases, 2014.
24. Studies with agar cup-plate method by S. brandit rose and Ruth E Miller. Journal of Bacteriology. 1939 Nov; 38(5): 539-547.
25. Clinical and oral microbiology by AW McCracken, Ra Cawson Publishing Corporation, Washington, Dc 1983.