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GC-MS Study and Pharmacognostic Study of *Pithecellobium Dulce* Leaves

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ABSTRACT

Pithecellobium dulce is a species of flowering plant in the pea family Fabaceae, that is native to the Pacific Coast and adjacent highlands of Mexico, central America, and northern south America. *Pithecellobium dulce*. It is used as medicinal plant. An extract of the leaves is used for gall ailments and to prevent miscarriage. The methanolic extract of leaves was obtained by Soxhelt extractor followed by concentration in rotary evaporator. Separation of bioactive chemicals was carried out by column chromatography while studies by GC-MS which shows presence of following bioactive chemicals Phytol, Anthracene, 9(3butenyl), mm Diisooctyl phthalate, 13Docosenamide,, 3,6,9triethyl3,6,9trimethyl Formic acid, Cyclotetrasiloxane, octamethyl, 1(+), Ascorbic acid 2,6dihexadecanoate. The leaf extract of *Pithecellobium dulce* showed good inhibition against gram positive Organisms. The highest inhibition was noted *S.epidermidis* (24mm), *P. acne*(14mm) and *s. aur*(11mm).

Keywords: GC-MS, Soxhlet, Chemical Composition, Antimicrobial.

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INTRODUCTION

Pithecellobium dulce is a species of flowering as well as fruit bearing plant has family fabaceae Often planted for living fence or thorny hedge, eventually nearly impenetrable, guamachil furnishes food, forage, and firewood, while fixing a little nitrogen. The pods, harvested in Mexico, Cuba, and Thailand, and customarily sold on roadside stands, contain a thick sweetish, but also acidic pulp, eaten raw or made into a drink similar to lemonade. Pods are devoured by livestock of all kinds; the leaves are browsed by horses, cattle, goats, and sheep; and hedge clippings are often gathered for animal feed¹ Reported to be abortifacient, anodyne, astringent, larvicidal, guamachil is a folk remedy for convulsions, dysentery, dyspepsia, earache, leprosy, peptic ulcers, sores, toothache, and venereal disease² The bark of *P. avaremotem*, the "avaremo-temo" from Brazil, is a folk cancer elixir³ *Pithecellobium dulce* is a tree that reaches a height of about 10 to 15 m (33 to 49 ft). Its trunk is spiny and its leaves are bipinnate . Each pinna has a single pair of ovate-oblong leaflet that are about 2 to 4 cm (0.79 to 1.57 in) long. The flowers are greenish-white, fragrant, sessile and reach about 12 cm (4.7 in) in length, though appear shorter due to coiling. Pods contain a pulp that is variously sweet and acid, commonly white but also red. The seed and pulp are made into a sweet drink similar to lemonade and also eaten roasted or fresh. The seeds are used fresh in curries in India.

MATERIALS AND METHOD

Collection of plant material

The fresh leaves of *Pithecellobium dulce* plant were collected from Melghat region Dist-Amravati (Maharashtra) The experimental site is located between coordinates 20.91° N, 77.75°E and an altitude of 312 m in foothills of Central India experiencing the subtropical climate during winter season in the month December 2014 and the Authentication of plant was confirmed by botanist (Prof.S.K Tippat, Department of Environment Science , Art, Commerce & Science College Amravati).

Chemicals and microbial cultures

All the chemicals and standard antibiotics used in this work were purchase from Sigma Aldrich, Merck and Hi-media, Mumbai India. The reference bacterial strains used in this study were obtained from American Type Culture Collection (ATCC) and Microbial Type Culture Collection (MTCC) Institute of Microbial Technology, Chandigarh, India. They were selected from gram positive and gram negative bacteria to represents a broad spectrum of potential pathogens that pose significant threats in the medical field (Table 1)

Preparation of plant extract

The plant were dried over ambient temperature and the dried sample were grind properly and dried powder sample was extracted in Methanol at 65°C, by using soxhlet apparatus ⁴ and extracts were concentrated by gradually evaporating the respective solvent on rotary evaporator . The concentrated extract was collected in sterile bottles and kept in a cool and dark place prior to analysis.

GC-MS Analysis of *Pithecellobium dulce*

Gas Chromatography and Mass Spectroscopy:-

A JEOL GCmate II benchtop double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000¹ software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Identification of chemical constituents:-

Identification of the chemical constituents was done on the basis of retention index (RI) using a mass spectra library search NIST and by com-paring the mass spectral and retention data with literature ¹⁷. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

Antimicrobial Activity of *Pithecellobium dulce* leaves

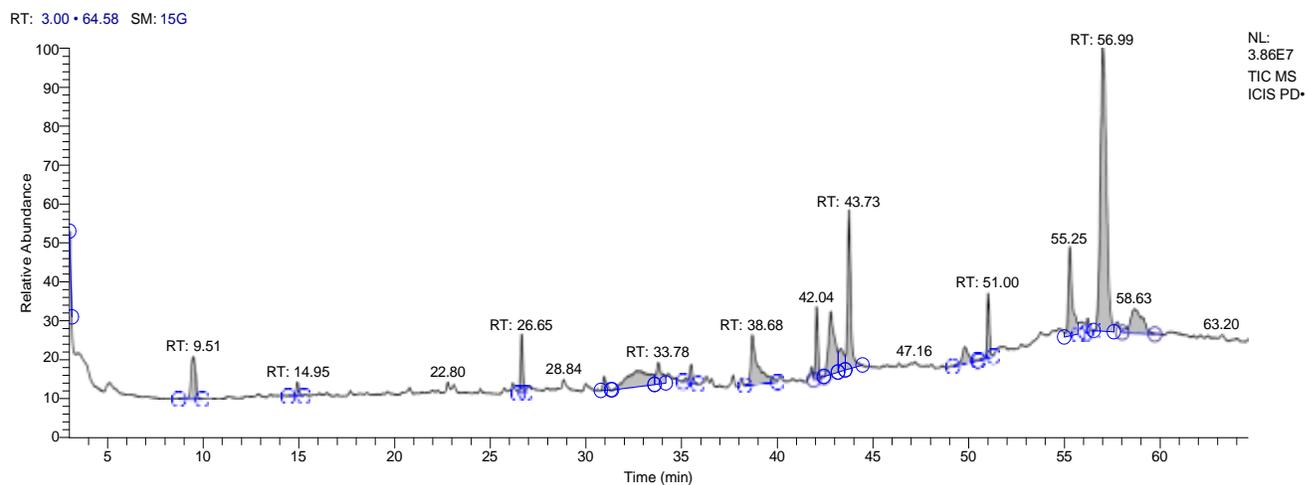
Antimicrobial activity organic extracts methanolic of *Pithecellobium dulce* leaves were determined by Agar disc diffusion assay according to the Manual of antimicrobial susceptibility testing ¹⁸ was used to assay the various antibiotics for bactericidal activity against test strains of E. coli, K. pneumonia, S.epidermidis , S. aureus, P. acnes, S.typhi.

RESULTS AND DISCUSSION

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Sample Header

Data File:	PD•
Original Data Path:	C:\GCMS•DATA\YEAR 2015\JAN•15\28
Sample Type:	Unknown
Sample ID:	1
Sample Name:	
Acquisition Date:	01/28/15 11:57:51 AM
Run Time(min):	61.58
Injection Volume(µl):	1.00
Scans:	3681
Low Mass(m/z):	30
High Mass(m/z):	400
Instrument Method:	C:\GCMS•data\instrument method\GERNAL•gcms•METHOD•OIL.meth

Figure 1: Gas Chromatogram of Leaves extract of *Pithecellobium dulce*

Gram positive bacteria



S.aureus



P.acne

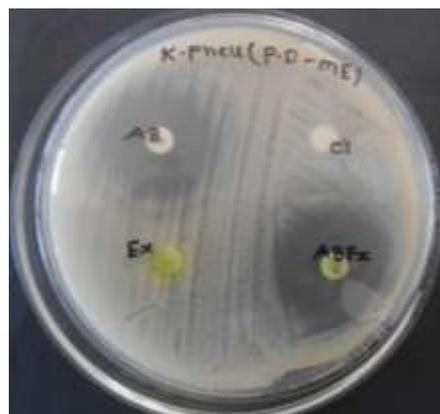
**S.epidermidis****Gram negative bacteria -****E.coli****K. pneumonia****S. typhi**

Figure 2: Antibacterial activity of *Pithecellobium dulce* Leaf against six bacteria, in each image: AB- Antibiotic disk, CI - Sterile disk (control), Ex- Extract disk, AB+ Ex- Antibiotic + Extract disk.

Table 1: Reference microbial strains and their clinical implications.

Organism	Reference	Clinical implication
Gram positive bacteria Staphylococcus aureus	ATCC-33591	Staphylococcal infections ⁵ Superficial or localized infections ⁶ Osteomyelitis, Scalded skin syndrome ⁷
Gram positive bacteria Propionibacterium acnes	ATCC-1951	CNS shunt infections ⁸ , Cardiovascular, Chronic pseudophakic, endophthalmitis ⁹
Gram negative bacteria Escherichia coli	ATCC-14948	Cholecystitis ¹⁰ , bacteremia ¹¹ , cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical infections such as pneumonia and neonatal meningitis ¹²⁻¹³
Gram negative bacteria Klebsiella pneumonia	MTCC-4030	Cholangitis, UTI ¹⁴ , Nosocomial disease and pneumonia ¹⁵
Gram negative bacteria Salmonella typhi	ATCC-25812	Typhoid fever, enteritis ¹⁶

Table 2 :Chemical Composition of *Pithecellobium dulce* leaves

Sr. No	Retention Time	Name of chemical constituent	Molecular Formula	Peak Area %
1	9.51	Cyclotetrasiloxane, octamethyl	C ₈ H ₂₄ O ₄ Si ₄	3.58
2	26.65	Phenol, 2,4bis(1,1dimethylethyl)	C ₁₄ H ₂₂ O	2.65
3	33.78	9,10Dimethyltricyclo[4.2.1.1(2,5)]decane9,10diol	C ₁₂ H ₂₀ O ₂	2.35
4	38.68	l(+) Ascorbic acid 2,6dihexadecanoate	C ₃₈ H ₆₈ O ₈	6.63
5	42.04	Phytol	C ₂₀ H ₄₀ O	7.55
6	43.73	Anthracene, 9(3butenyl)	C ₁₈ H ₁₆	9.79
7	51.00	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	2.80
8	55.25	13Docosenamide,	C ₂₂ H ₄₃ NO	7.06
9	56.99	3,6,9triethyl3,6,9trimethyl Formic acid	C ₁₈ H ₃₀	32.69
10	58.63	Benzoic acid, 3,5dicyclohexyl4hydroxy, methylester	C ₂₀ H ₂₈ O ₃	5.77

Table 3 Antimicrobial activity of *Pithecellobium dulce* leaves

Organisms	Test Samples (Growth inhibition ^a) mm			
	AB	Ex	AB-Ex	Cl
S.aureus	16±0.3	11±0.4	16±0.3	00
P.acne	29±0.1	14±0.3	29±0.1	00
S.epidermidis	31±0.3	24±0.4	34±0.3	00
E.coli	29±0.2	00	29±0.2	00
K. pneumonia	24±0.3	00	24±0.3	00
S.typhi	26±0.3	00	26±0.3	00

GC-MS chromatogram analysis of the Methanolic extract of *Pithecellobium dulce* Fig-1 showed major ten peaks which indicating the presence of various phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library. The various phytochemicals which contribute to the medicinal activities like antimicrobial, antifungal, antiviral and antioxidants.. The mass spectra of all the phytochemicals identified in the whole plant the most

prevailing compounds were phytol a diterpene (7.55%) , 13Docosenamide has a amide compound (7.06%), l(+) Ascorbic acid 2,6dihexadecanoate is a fatty acid (6.63%) and Phenol, 2,4bis(1,1dimethylethyl) is tannins compound. In addition to biological activity, the phytol was also reported to have antimicrobial, anti-inflammatory, anticancer and diuretic¹⁹ 13Docosenamide which shows antimicrobial activity²⁰. l(+) Ascorbic acid 2,6dihexadecanoate was reported as Anti-oxidant, anti-inflammatory, anti- nociceptive, anti-bacterial, enhances sperm motility²¹ Phenol, 2,4bis(1,1dimethylethyl) which showed Antioxidant, antibacterial activity²².

CONCLUSION

The presence of various bioactive compounds in the *Pithecellobium dulce* justifies the use of whole plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give fruitful results. From the results, it could be concluded that *Pithecellobium dulce* contains various bioactive compounds. Therefore, it is recommended as a plant of phytopharmaceutical importance.

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