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Original Research Article

Chemistry for New drug discovery

Isolation and Study of Structural Analogue of Bioactive Chemical Dibutyl Phthalate from Leaves of *PithecellobiumDulce*Plant

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Abstract: *Pithecellobiumdulce* is a species of the family fabaceae. The methanolic extract of leaves was obtained by a Soxhlet extractor followed by concentration in rotary evaporator. Separation of bioactive chemicals was carried out by column chromatography and thin layer chromatoghraphy while studies by GC-MS which shows presence of six major fractions were collected by eluting the column with ethyl acetate: formic acid: acetic acid: water at 100:11:11:26 proportions. Major fractions arePhytol, Dibutylphthalate, Anthracene, 9(3butenyl), 1,3Docosenamide, 3, 6, 9Triethyl,3 6, 9trimethyl Formic acid. The major component was subjected to further purificationby using preparative thin layer chromatography. The bioactive component recovered from TLCanalyses by GC-MS &NMR. The ^{1}H-NMR spectrum shows the peak at δ 4.08- 4.13 corresponds to the protons of the cyclic ring of aromatic hydrogen appear as multiplet. The CH_2-O nearer to phthalate group gives signal near δ 2.11-2.16 ppm is appeared as doublet, then butyl groupaliphaticCH_2 gives signal nearδ 1.91-1.96 is appeared as doublet also but δ 1.31-1.28 value showing CH_3 of the di-butyl group. On the basis of above spectral data of GC-MS,^{1}HNMR& FT- IR assign structure of isolate *Pithecellobiumdulce* leaves.

Keywords: GC-MS, Soxhlet, Rotary evaporator, Column Chromatography, TLC, NMR, Dibutyl phthalate

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1. INTRODUCTION

Pithecello biumdulce is a species of flowering as well as fruit bearing plant under family *fabaceae.* Often planted for living fence or thorny hedge, eventually nearly impenetrable, guamuchil furnishes food, forage, and firewood, while fixing a little nitrogen. The pods, harvested in Mexico, Cuba, and Thailand, and customarily sold on roadside stands, contains a thick sweetish, but also acidic pulp, eaten raw or made into a drink similar to lemonade[1]. *Pithecellobium dulce* is the only species that has become widespread outside its origin. In India and tropical Africa, especially along coasts, it is notably weedy in the Caribbean islands (including Cuba, Jamaica, Puerto Rico, and St. Croix), and in Florida and Hawaii, USA, but less so where population and animal pressure keep it contained[2]. Reported to be abortifacient, anodyne, astringent, larvicidal, guamuchil is a folk remedy for convulsions, dysentery, dyspepsia, earache, leprosy, peptic ulcers, sores, toothache, and venereal disease[3]. The methanolic extract of the leaves of the plant. *Pithecellobium dulce* has the highest larval mortality[3]. *Pithecellobium dulce* is a tree that reaches a height of about 10 to 15 m (33 to 49 ft). It's trunk is spiny and its leaves are bipinnate . Each pinna has a single pair of ovate-oblong leaflets 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 sour 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. Proteins and peptides with the potential to combat protein malnutrition are richly in seeds and its leaves are also used as a feed for goats because of its good nutritional contents[4].Seeds have been reported to contain steroids,saponin,lipids, glycosides and polysaccharides. Bark yields 37% tannins of the catechol. Leaves yield quercitin,Kaemferoldulcitol and afezilin[5]. The active compound of the plant includes flavonoids, sterols, tannins, triterpenoids etc. The health promoting properties due to the presence of proteins, carbohydrates, steroids etc. and diseases preventing properties such as antioxidant, antifungal, antiviral, antibacterial[6].

2 MATERIALS AND METHODS

2.1 Collection of plant materials

The fresh leaves of *Pithecellobiumdulce* 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 342 m in foothills of Central India experiencing the subtropical climate during winter season in the month Jan-2014 and the authentication of plant was confirmed by botanist Prof. S.KTippat, Department of Environment Science, Art, Commerce & Science College Amravati.India

2.2 Preparation of plant extract

The plant was dried over ambient temperature and the dried samples were grounded properly and extracted in methanol at 65°C, using soxhlet apparatus[7]and methanol extracts were concentrated by gradually evaporating the respective solvent on a rotary evaporator. The concentrated extract was collected in sterile bottles and kept in a cool and dark place prior to analysis[8].

2.3 GC-MS Analysis of Pithecellobium dulce

Gas Chromatography as Chromatography analysis of the plant extract was carried out on a 6890 Gas Chromatography model 5765 equipped with direct injector and split ratio set to 10:1. (DB-5) (5% phenyl polysiloxane, 30m length 250u internal diameter; 0.25um film coating) fused capillary column. Helium was the carrier gas at 1.0 ml min. The oven temperature program was programmed to start at35^0c hold for 2 min then temp at 20^0C per min to 300^0C and hold for 5 min. Injector and detector temperature were 220°C and 230°C respectively .Injection size was 0.02 µl neat.

2.4 *Gas Chromatography and Mass Spectroscopy*

A JEOL GCmate II benchtop double-focusing magnetic sector mass spectrometer operating in electron ionization(EI) mode with TSS-2000[1] 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/s 25 to m/z 700 at 0.3 second 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.

2.5 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 comparing the mass spectral and retention data with literature. The relative amounts of individual component were calculated based on the GC peak area (FID response) without using a correction factor.

CIL/ SAIF Panjab University Chandigarh

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

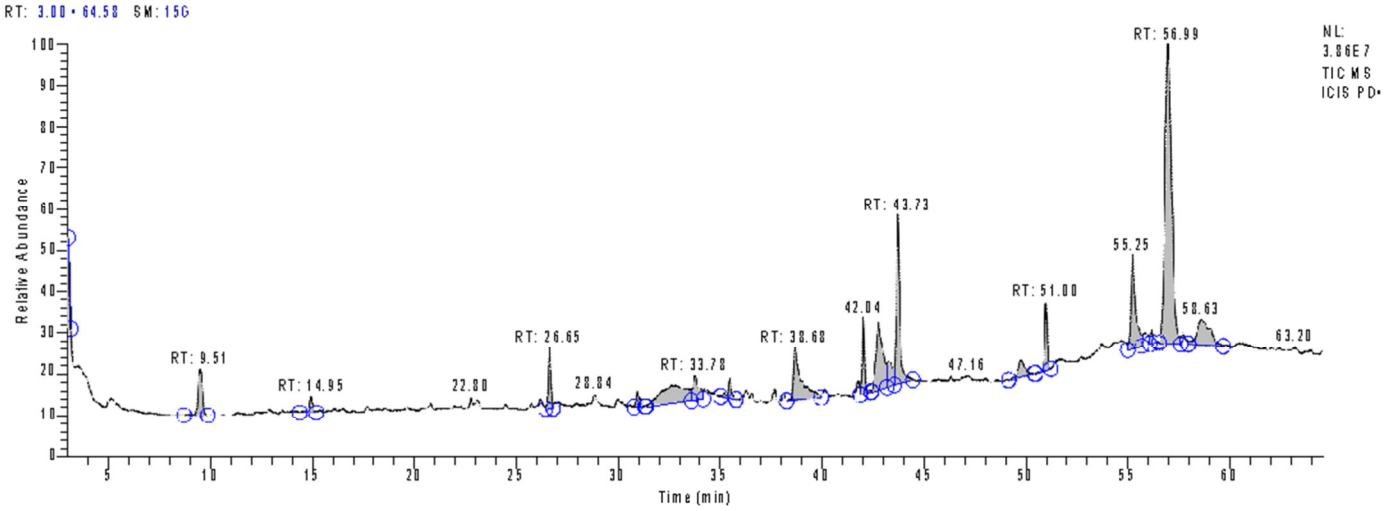


Fig1.GC-MS Chromatogram of methanol extract of *Pithecellobium dulce* leaves

3 Isolation of major chemical component by column and thin layer chromatography

3.1 Preparation of extract

Powder sample 1g was extracted with 10 ml methanol for 5 minute heat on a water bath at about 60^{0}C and then filtered, 500µlwas used for column chromatography.

3.2 Column Chromatography

Slurry was prepared by mixing 60gm of silica gel-120mesh with ethanol and then the column was packed with the slurry. Then the 500 µl sample was applied on top of the column and six fractions were collected by eluting the column with ethylacetate: formic acid: acetic acid: water at 100:11:11:26 proportions. Out of these six fractions, the third major fraction which contained the major component was subjected to further purification by using preparative thin layer chromatography[9].

3.3 Thin layer Chromatography

The third fraction (20-30µl) obtained from column chromatography was applied in the form of a band, on preparative silica gel plates. The solvent system, ethyl acetate: formic acid: acetic acid: water at 100:11:11:26 proportions was used as a developing solvent system[10]. The required band *Pithecellobium dulce* leaves extract was scraped off and collected in a beaker to which ethyl acetate was added and filtered. The filtrate was then concentrated under reduced pressure and the residue was kept in a refrigerator for further investigation for GC-MS analysis, ^{1}H NMR .

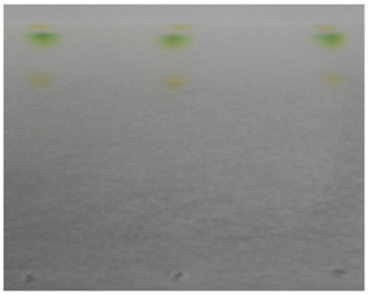
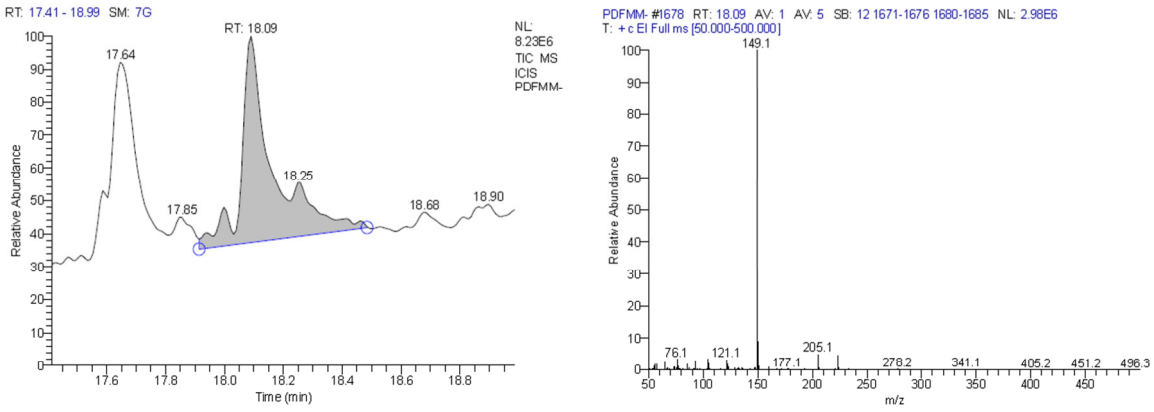


Fig2.TLC image for fraction second isolated Pithecellobium dulce leaves extract

3.3.1 Gas Chromatogram and Mass Spectrum of isolated *Pithecellobiumdulce*leaves extract.



Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
Dibutyl phthalate	18.09	C16H22O4	84-74-2

Fig3.GC-MS of TLC isolated comound from *Pithecellobium dulce* leaves extact.

3.3.2 ^{1}H-NMR Spectrum of isolated compounds from *Pithecellobiumdulce*leaves extract.

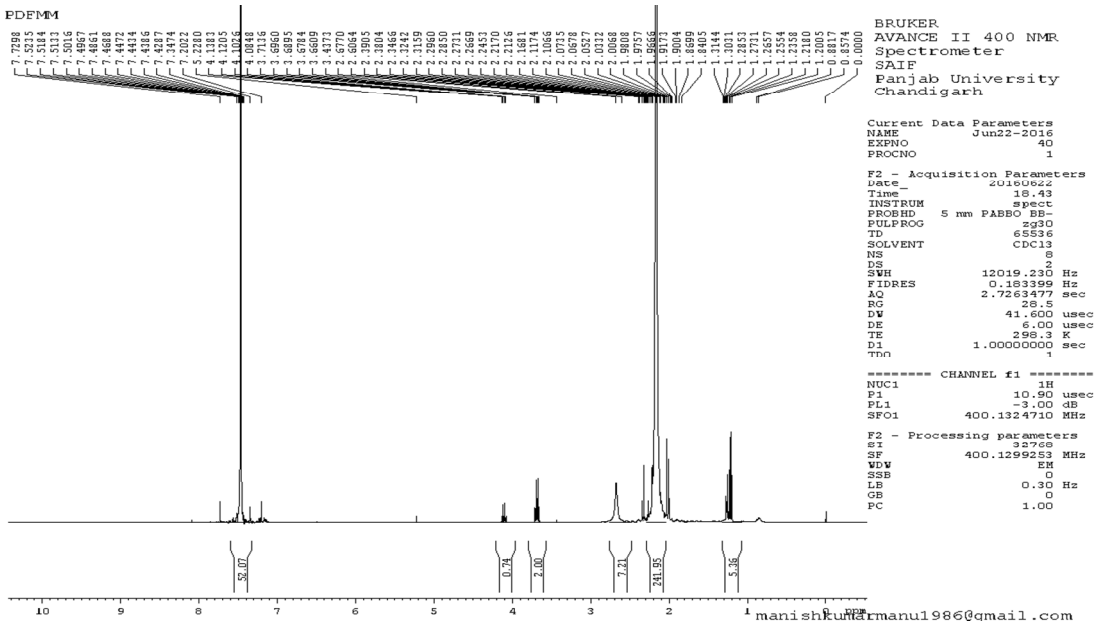


Fig 4. ^{1}H-NMR spectrum of TLC isolated comound from *Pithecellobium dulce* leaves extact.

3.3.3 FT-IR Spectrum of isolation Pithecellobium Dulce leaves.

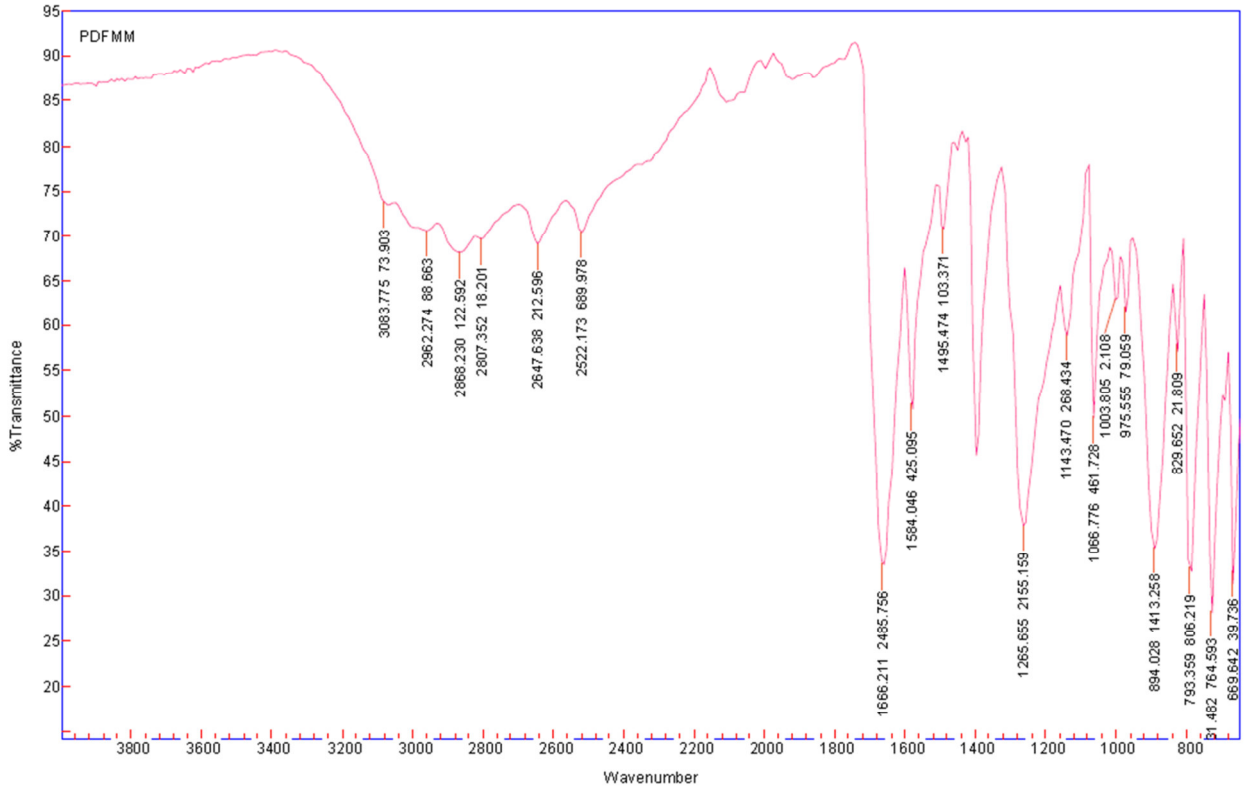


Fig 5.FT-IR Spectrum of isolation Pithecellobium Dulce leaves methanol extract

4. RESULTS AND DISCUSSION

Phytochemical evaluation confirms the presence of various chemical constituents present in plants. Phytochemical analysis listed in Table No.1.Due to higher polarity of methanolic extract revealed the presence of maximum phytochemical composition these phyto constituents independently responsible for the broad range of medicinal properties. GC-MS chromatogram analysis of the methanolic extract of *Pithecellobium dulce* Fig-1showed major 10 peaks which indicates the presence of various phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST library[11].

Table 1:- Chemical Composition of Pithecellobiumdulceleaves

Sr.No	Retention Time	Name of chemical constituent	Molecular Formula	Peak Area %	Biological Application
1	9.51	Cyclotetrasiloxane, octamethyl	$C_8H_{24}O_4Si_4$	3.58	Antimicrobial, antioxidant, antibacterial[12]
2	26.65	Phenol,2,4bis(1,1dimethylethyl)	$C_{14}H_{22}O$	2.65	Antibacterial and anti-inflammatory activities[13]
3	33.78	9,10 Dimethyl tricyclo[4.2.1.1(2,5)]decane9,10diol	$C_{12}H_{20}O_2$	2.35	AntifungalActivity[14]
4	38.68	l(+) Ascorbic acid 2,6dihexadecanoate	$C_{38}H_{68}O_8$	6.63	Antioxidant, antiscorbutic, anti inflammatory, anti nociceptive, anti mutagenic, wound healing property[15]
5	42.04	Phytol	$C_{20}H_{40}O$	7.55	Cytotoxic, antioxidant, anti-Inflammatory,antimicrobial[16]
6	43.73	Anthracene, 9(3butenyl)	$C_{18}H_{16}$	9.79	No Activity reported
7	51.00	3,6,9trimethyl Formic acid	$C_{18}H_{30}$	19.69	No Activity reported
8	55.25	13 Docosenamide,	$C_{22}H_{43}NO$	7.06	Anti- Inflammatory, anti-inflammatoryactivity[17]
9	56.99	Dibutyl phthalate	$C_{16}H_{22}O_4$	21.02	Antibacterial activities[18]
10	58.63	Benzoic acid, 3,5dicyclohexyl4hydroxy,methyl ester	$C_{20}H_{28}O_3$	5.77	Antifungal and antibacterial[19]

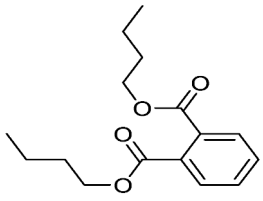
The various phytochemicals which contribute to the medicinal activities like biological applications, the Cyclo tetrasiloxane, octamethyl are used as antimicrobial, antioxidant, antibacterial Phenol, 2,4bis(1,1dimethylethyl) also were reported to have antibacterial and anti-inflammatory activities. 9,10 Dimethyltricyclo[4.2.1.1(2,5)]decane 9,10 diol as antifungal;l(+)

Ascorbic acid 2,6dihexadecanoate was reported as antioxidant, antiscorbutic, anti-inflammatory, anti nociceptive, anti mutagenic, wound healing Property. Dibutyl phthalate also reported to have antibacterial activities, antifungal, antimicrobial agent, anti-maleria and Benzoic acid, 3,5 dicyclohexyl4hydroxy, methylester antifungal and antibacterial. GC-MS chromatogram analysis of the methanolic extract of *Pithecello biumdulce showed a high p*ercentage of Dibutyl phthalate - an oily ester. After stage wise analysis, we succeeded in isolation of pure components from crude extract of *Pithecellobium dulce* leaves which is further confirmed by GC-MS,^{1}H NMR. The GC-MS,NMR result revealed thePresence of Dibutyl phthalate which is confirmed in structural elucidation by 1H NMR.The ^{1}H-NMR spectrum in Fig.3shows the peak at δ 4.08- 4.13 corresponds to the protons of the cyclic ring of aromatic hydrogen that appears as a multiple. The CH_2-O nearer to phthalate group gives signal near δ 2.11-2.16 is appeared as doublet, then butyl group aliphatic CH_2gives signal near δ 1.91-1.96 is appeared as doublet also but δ 1.31-1.28 value showing CH_3 of di-butyl group. On the basis of above spectral data of GC-MS,^{1}HNMR and FT-IR spectral analysis of isolate *Pithecello biumdulce* leaves methanol extract showed the presence of following absorption band.

Table 2 FT-IR spectral analysis of isolate absorption band of methanol extract *Pithecellobiumdulce* leaves.

Sr.No.	Absorptioncm^{-1}	Assignment for group	Literature value cm^{-1}
1	2868.230	Ar-C-H	2800-3000
2	1495.474	Ar- C=C	1450-1550
3	1265.655	Ar-C-C	1000-1350
4	1666.211	C=O	1515-1570
5	1066.776	C-O	2110-2160
6	3083.775	Ali-C-H	3000-3100

The FT-IR spectrum table2 analysed data showed a characteristics absorption frequency at 2868 cm^{-1} alkane C-H stretching,1495cm^{-1}multiplesharp peaks C-H bending,1265 cm^{-1}strong appearance C-O stretching alkyl aryl ether 1666 cm^{-1}weak appearance C=C,C=O Stretching alkene, 1066 cm^{-1} strong appearance C-O stretchingprimary alcohol, 3083 cm^{-1}aromatic having alkyl C-H stretching.



Dibutyl phthalate

$C_{16}H_{22}O_4$
Exact Mass: 278.15
Mol. Wt.: 278.34

Fig 6.Isolated Structural analogue of Dibutyl Phthalate (NIST Chemistry Web Book,SRD69)

In Fig.6 assign structural analogue of isolated *Pithecellobium* dulce leaves extract is Dibutyl phthalate reported to have antibacterial activities[20], antifungal, antibacterial, antimicrobial agent and antimalerial[21].

5. CONCLUSION

The presence of various bioactive components in the *Pithecellobium dulce* plant leaves justifies the use of for various Element s(ailments) by traditional practitioners. However, isolation of individual phytochemical constituents we succeed in isolation of pure components from crude extract of *Pithecellobium dulce* leaves is further confirmed by GC-MS,^{1}HNMR and FTIR . Subjecting the pure compounds to biological activity will definitely give fruitful results. From the results, it could be concluded that *Pithecellobium dulce*conta in various bioactive compounds. Therefore *Pithecellobium dulce* plant is recommended as phytopharmaceutical importance,

6. FUNDING ACKNOWLEDGEMENT

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7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCE

1. Perry LM, Metzger. Medicinal plants of East and Southeast Asia: attributed properties and uses. Cambridge: MIT Press; 1980. p. 622.

2. ShwetaS, Mehta BK. A review on pharmacological activities of *PithecellobiumDulce* extract, and their effective doses. J MedPharm Allied Sci. 2013;05:35-45.

3. Govindarajan M.Adulticidal activity of *PithecellobiumDulce*(*Roxb.*)*Benth*against Culexquin quefasciatus (Say),Asian Pacific Trop Dis. 2012;1:124-8.
4. GovindarajanM,RajeswariM. Repellent properties of *PithecellobiumDulce*(*Roxb.*)*Benth*(family: Fabaceae) against filariasis vector, Culex quinquefasciatus Say(DipteraCulicide). J Med Herbs Ethnomed.2015;1:103-7. doi: 10.5455/jmhe.2015-08-018.
5. Srinivas G,GeetaHpet al.A review on Pithecellobiumdulce: Apotential medicinal tree. IntJ ChemStud. 2018,6(2);5446:540.
6. Selvakumar Murugesan. Nutritional and therapeutic benefits of medicinal plant *Pithecellobium Dulce*(Fabaceae)A review, JAPS; 2019. p. 001-10. doi: 10.7324/JAPS.2018.88017.
7. Khandekar US, Tippat SK.Chemical composition and pharmacognostic study of crude plant extract of *Vernoniaelaeagnifolia*, International Journal of Pharma and Bio Sciences. Int J Pharm BiolSci. 2015;6(3).(B) p. 7-15. Available from: https://ijpbs.net/contact.html[cited5/8/2020].
8. BobadeAF. GC-MSanalysis of bioactivecompound in ethanolicextract of *Pithecellobiumdulce*Leaves,Actascientific. PharmSci. 2019;3(11):8-13. doi: 10.31080/ASPS2019.03.0412.
9. Dinan L, Harmatha J, Lafont R. Chromatographic procedures for the isolation of plant steroids. J Chromatogr A. 2001;935(1-2):105-23. doi: 10.1016/s0021-9673(01)00992-x, PMID 11762770.
10. SatyajitD.Sarker.An Introduction to Natural Product Isolation,Methods in Molecular Biology. Springer protocols.Humana Press; 2012.
11. Mikaia A.& et.al.NIST Standard reference database 1A,Chemdata.nist.gov;2014.
12. Ismail GA, Gheda SF, Abo-shady AM, Abdel-karim OH. In vitro potential activity of some seaweeds as antioxidants and inhibitors of diabetic enzymes. Food Sci Technol. 2019:1-11. doi: 10.1590/fst.15619.
13. Chandrasekar T et al.GC-MS analysis, antimicrobial, antioxidant activity of an ayurvedic medicine, Nimbapatradi Choornam. J Chem Pharm Res. 2015;7(8):124-36.
14. JAVAID A, Qudsia H, Shoaib A.Bioassays Guided Fractionation of Sennaoccidentalis for Identification of Natural Antifungal Constituents against Macrophominaphaseolina. Plantadaninha.2017;35. doi: 10.1590/s0100-83582017350100002.
15. Madhavi P.GC-MSanalysis of phytocomponents in the methanolicextract of *ShoreaRobusta*,International. J Sci Res. 2015;4(6):1935-8.
16. Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, Chandra Shill M, Karmakar UK, Yarla NS, Khan IN, Billah MM, Pieczynska MD, Zengin G, Malainer C, Nicoletti F, Gulei D, Berindan-Neagoe I, Apostolov A, Banach M, Yeung AWK, El-Demerdash A, Xiao J, Dey P, Yele S, Jóźwik A, Strzałkowska N, Marchewka J, Rengasamy KRR, Horbańczuk J, Kamal MA, Mubarak MS, Mishra SK, Shilpi JA, Atanasov AGPhytol: A review of biomedical activities. Food ChemToxicol.2018;121:82-94. doi: 10.1016/j.fct.2018.08.032. PMID 30130593.
17. GorakshJHase&et.al. Phytochemical Studies on *NeriumoleanderL*. Using GC-MS IntJ PharmacognPhytochemRes. 2017;9(6)(10).25258/phyto.v9i6.8195:885-91.
18. ElijaKhatiwora.Antibacterial activity of DibutylPhthalate: A secondary metabolite isolated from *Ipomoea carnea* stem. J PharmRes. 2012;5(1):150-2.
19. Ojinnaka CM, Nwachukwu KI, Ezediokpu MNThe Chemical Constituents and Bioactivity of the seed (Fruit) extracts of BuchholziaCoriaceaEngler (Capparaceae). J ApplSci Environ Manag;19(4). doi: 10.4314/jasem.v19i4.29.
20. Mini Shobi T, GowduViswanathan MBAntibacterial activity of di-butyl phthalate isolated from Begonia malabarica. JABB,2018;5(2)101-104. doi: 10.15406/jabb.2018.05.00123.
21. Akpuaka A.& et.al.Biological activity of characterized isolates of n-hexane extract of Azadirachta A Juss(Neem)leaves,Nature and Science,2013;11(5):141-147 http://www.science.net/nature