IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Spectro Chemical Analysis And Evaluation Of Anticancer Activity Of Ethanolic Leaf Extract Of *Tabernaemontana Divaricata*

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ABSTRACT

Herbs are resource with therapeutic properties. *Tabernaemontana Divaricata* a native of India a many other tropical regions is a common garden plant. It has been used traditionally for treatment of number of diseases. The main aim of the present work the ethanol extract of the leaves of the plant have been tested for anticancer activity. Characterization studies were carried out using UV Spectrophotometer and FTIR analysis. The chemical composition of the plant leaf extract of *T. Divaricata* was investicated using Gas chromatography – Mass spectrometry analysis was conducted to determine the types of different phytochemical compounds in ethanolic leaf extract of *Tabernaemontana Divaricata*. The ethanolic extract revealed the presence of several bioactive compounds such as 1,2-benzenedicarboxylic acid, diethyl ester . Squalene, Hydroperoxide, 1,4- dioxan-2-yl.

Keywords : MTT assay, IC_{50}, *Tabernaemontana Divaricata*. UV Spectrophotometer, FTIR , Gas chromatography- Mass spectrometry analysis.

INTRODUCTION

Some medicinal plants have been used for a wide variety of applications such as food preservation, pharmaceutical, alternative medicine, and natural therapies for thousands of years [1] . People now changed their lifestyle to use herbal medicine instead of synthetic medicine because of less or no side effects. In different countries, several plants are used for herbal preparations as indigenous system of medicine [2] .

Cancer is a disease that has always been a major threat and has been characterized by proliferation of abnormal cells. Though Chemotheraphy is now Being used as a standard treatment method, search for anticancer agents from natural products has increased. In order to annotate the mechanism of prevention of cancer and to identify new anticancer activity numerous plants has been explored [3].

Tabernaemontana Divaricata is a plant under the family Apocynaceae [4]. The plant is an evergreen shrub growing to a maximum height of six feet [5], distributed in Coast forests of Bengal, Myanmer, Mangrove forests of China and Japan [6]. The flowers are white coloured [7] the leaves, flowers, roots, stem of the plant have medicinal value and used in curing various physiological disorders of human being like epilepsy, abdomial tumors, eye infections, fractures, fever, headache, inflammation, edema, diarrhea etc [8]. Other medicinal properties of plant include antinoceptive, antioxident, anti- inflammatory activities [9].

Figure 1: The whole plant of *Tabernaemontana Divaricata*.

MATERIALS AND METHODS

Plant collection

Tabernaemontana Divaricata was indentified and mature plants were collected in the garden of Arulmigu Palaniandavar College of Arts and Culture, Palani in the month of March. The plants were washed throughly with tap water to remove dust. Then the plants were shade dried to avoid the loss of bio-active compounds. After complete drying, each part of the plant were subjected to mechanical grinding and collected in a air tight container.

Extract Preparation

100g of each plant part were extracted using different organic and aqueous solvents which have varying polarity (Petroleum ether, EtOH,water). Each 250ml of the solvent used, with the help of the soxhelt apparatus the extract was prepared and stored in clean beakers.

Gas Chromatography Mass Spectrometry Analysis

GC-MS analysis of the *T. divaricat*a extract The ethanolic extract of *T. divaricata* underwent gas chromatography-mass spectrometry (GC-MS) analysis (GC-MS - QP-2020)with the thermal desorption (TD) system .

Experimental conditions of the GC-MS system were as follows:

Trace-5 mass spectrometry capillary standard non-polar column, dimension: 30 meters; internal diameter: 0.25 mm; film thickness: 0.25 µm. The flow rate of the mobile phase (carrier gas: helium) was set at 1.2 ml/min. In the gas chromatography phase, the temperature programme (oven temperature) was 50°C, which was raised to 250°C at 10°C/min, and the injection volume was 1 µl. Samples dissolved in chloroform were run fully at a range of 50-500 mass-to-charge ratio (m/z) .

In vitro evaluation of anticancer activity by MTT assay

Cell culture

Chronic Myelogenous leukemia cell line (CML) was provided by National Centre for Cell line (NCCS), Pune and was grown in Minimum essential medium (MEM) supplemented with fetal bovine serum (FEM). All cells were maintained at 37 ^{0} C, with 5% CO_2 ,95% air.

MTT assay

After 24h of incubation to each well 10 µl of MTT (1mg /ml) in Phosphate- buffered saline (PBS) (p^H 7.2) was added and incubated at 37 ^{0} C for 3-4 h. The medium with MTT was flicked and the formed

formazen crystals were solubilized in 100% of DMSO. Using microplate reader the absorbance was measured at 570 nm.

The imaging were done using Inverted Phase Contrast Microscope.

RESULTS AND DISCUSSION

UV – Ultraviolet Visible spectroscopy

UV– Visible spectrometer were recorded in wave number (cm^{-1}). (UV – Visible spectrophotometer , model UV- 1800), UV- Vis spectra was analysis data taken as Wave number of 664 cm^{-1} and 607 cm^{-1} .

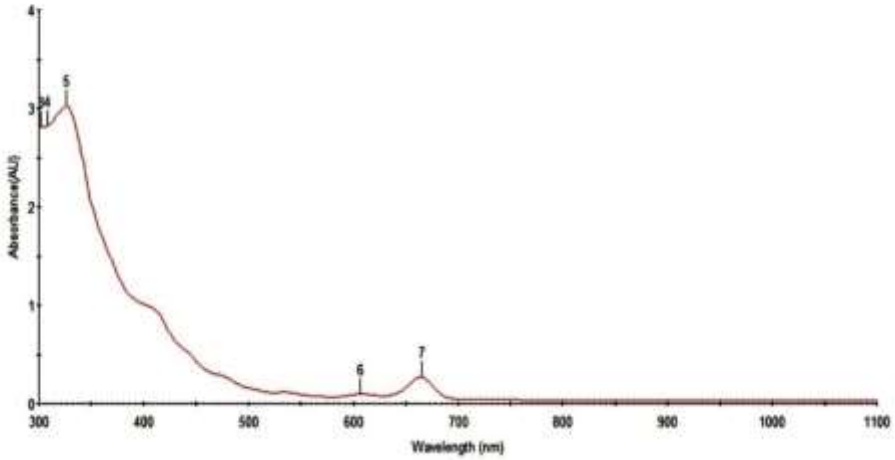


Figure : 2 UV – Visible spectrum of *Tabernaemontana Divaricata*

Spectrum :664 cm^{-1} colour of absorbing in blue green (N=O), 607 cm^{-1} of colour of absorbing Green blue (N=O), 236 cm^{-1}– 325 cm^{-1} of colour of absorbing ultraviolet colour **(**Poly- unsaturated and aromatic(C=O, H-CH=O).

Table : 1 Description of UV – Visible Spectrum

S.No	Wave number	Description
1	200 nm to 380 nm	Poly- unsaturated and aromatic (C=O, H-CH=O)
2	664nm	Blue- green (N=O)
3	607nm	Green - blue (N=O)

FTIR- Fourier Transform Infrared Spectroscopy

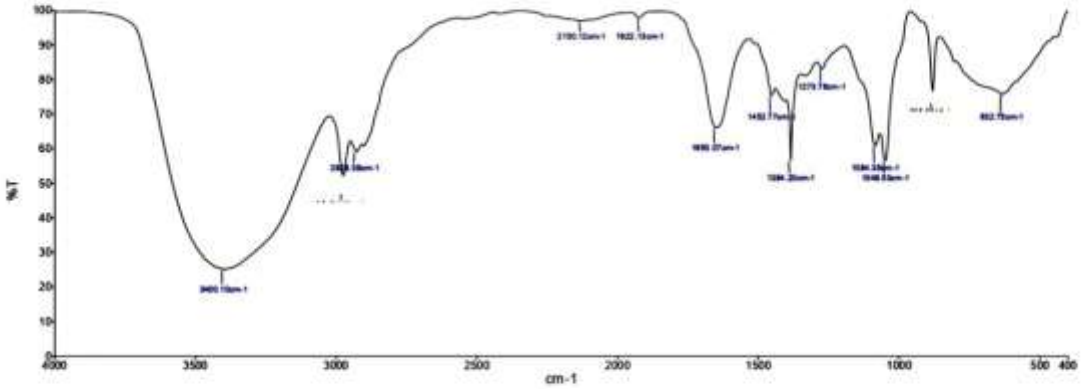


Figure : 3 FTIR Spectrum of *Tabernaemontana Divaricata*

FTIR spectral measurements were performed to find out the potential functional group in *Tabernaemontana Divaricata* leaf extract showed stretch of the bond.

Table : 2 Functional group present in leaf extract

S.No	Wave number (cm^{-1})	Functional group
1	3400.10	N-H stretching of amines
2	2928.38	O-H stretching of carboxylic acids
3	2130.12	C≡C stretching
4	1922.13	C-H stretching of alkanes
5	1650.07	C=C stretching

Gas chromatography- Mass spectrometry analysis

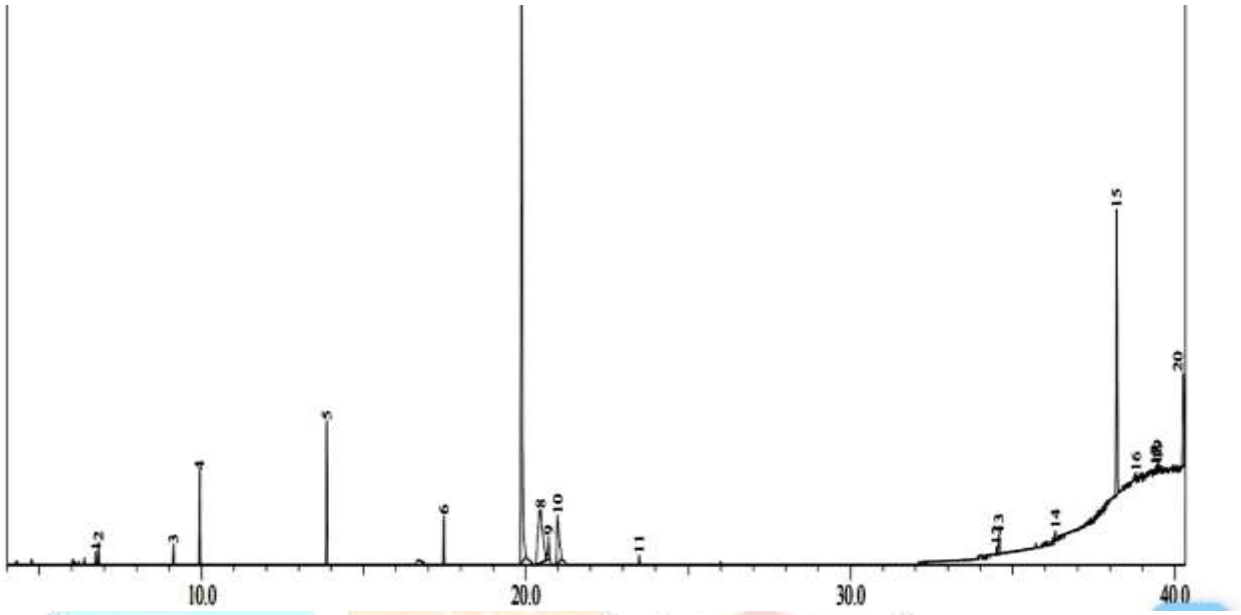


Figure : 4 Chromotogram of ethanolic extract of *Tabernaemontana divaricata*

The bioactive compounds present in the ethanolic extract *T. Divaricata* coupled with massspectroscopy (GC-MS)reports are given in fig 4.

In the ethanolic sample , 20 compounds were identified and the highest percentage compound content a peak area 41.26 (1,2-benzenedicarboxylic acid, diethyl ester with RT 19.875), followed by the peak area of 14.91(squalene with 38.12 RT) and peak area of 20.67 (hydroperoxide, 1,4-dioxan-2-yl with RT 20.467.) and the lowest percentage of content areaof 0.2 (tert-butyl trans-3-methyl-5-0xopyrrolidine-2-carboxylate with RT 34.498). Phytochemical with their retention time (RT), molecular formula and molecular weight(MW) in the ethanolic extract present in table 3

Table : 3 Phytochemical constituents in ethanolic extract of *Tabernaemontana divaricata*

S. No	RT	Name of the compound	Molecular formula	MW	Area%
1	6.743	2-(2-Oxo-2-phenyl-ethyl)-1,3-Dioxolane	$C_{10}H_9Cl_4$	228	0.54
2	6.822	3-Ethyl-4-methyltetrahydrofuran-3-0l	$C_7H_{14}O_2$	130	1.05
3	9.138	Pentadecane	$C_{15}H_{32}$	212	0.85
4	9.946	Cyclopentasiloxane, Decamethyl	$C_{10}H_{30}O_5Si_5$	370	3.78
5	13.867	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	444	6.44
6	17.477	(1z)-1,3-Diphenyl-1-Pentenyljoxy)(trimethyl)silane	$C_{20}H_{26}OSi$	310	2.01
7	19.875	1,2-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222	41.26
8	20.467	Hydroperoxide, 1,4-dioxan-2-yl	$C_4H_8O_4$	120	12.72
9	20.712	Tri-o-trimethylsilyl, n-trifluoroacetyl derivative of terbutaline	$C_{23}H_{42}F_3NO_4Si_3$	537	1.52
10	20.987	2-(2-Methyl-1,3-dioxolan-2-yl)ethyl acetate	$C_{18}H_{12}D_2O_4$	176	5.67
11	23.507	Phosphonous dibromide, [2,2,2-trifluoro-1-(trifluoromethyly1((trimethylsilyl)oxyiethyl]	$C_6H_9Br_2F_6OPSi$	428	0.39
12	34.498	Tert-butyl trans-3-methyl-5-Oxopyrrolidine-2-carboxylate	$C_{10}H_{17}NO_3$	199	0.2
13	34.575	9-Phenanthrenemethyl propanoate	$C_{18}H_{16}O_2$	264	1.36
14	36.314	(22s,23r,25r)}-23,26-epoxy-5.alpha.-furostan-3.beta.-ol	$C_{27}H_{44}O_3$	416	0.48
15	38.12	Squalene	$C_{30}H_{50}$	410	14.91

16	38.799	3,4-Dihydroxyphenylglycol, 4tms derivative	$C_{20}H42O_4Si_4$	458	0.31
17	39.405	Succinic acid, 2,2,3,3,4,4,4-heptafluorobutyl 2-methylhex-3-yl ester	$C_{15}H_{21}F_7O_4$	398	0.26
18	39.433	4-(Dimethylamino)azoestrone 3-methyl ether	$C_{21}H_{29}N_3O_2$	355	0.35
19	39.475	Acetamide, n-(acetyloxy)-n-[2-chloro-3-nitro-5- (trifluoromethyljphenyl]	$C_{11}H_8ClF_3N_2O_5$	340	0.13
20	40.265	lbogamine-18-carboxylic acid, 12-methoxy-, methyl ester	$C_{22}H_{28}N_2O_3$	368	5.81

Table : 4 Highest percentage of compound in the ethanolic leaf extract of *T. Divaricata*

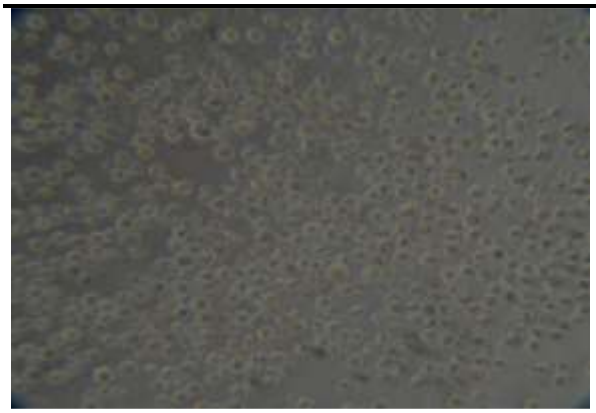
S.No	RT	Name of the compound	Molecular formula	MW	Area %
1	19.875	1,2-Benzenedicarboxylic acid, diethyl ester	$C_4H_8O_4$	222	41.26
2	38.12	Squalene	$C_{30}H_{50}$	410	14.91
3	20.467	Hydroperoxide, 1,4-dioxan-2-yl	$C_{12}H_{14}O_4$	120	12.72

In Vitro Anticancer Activity

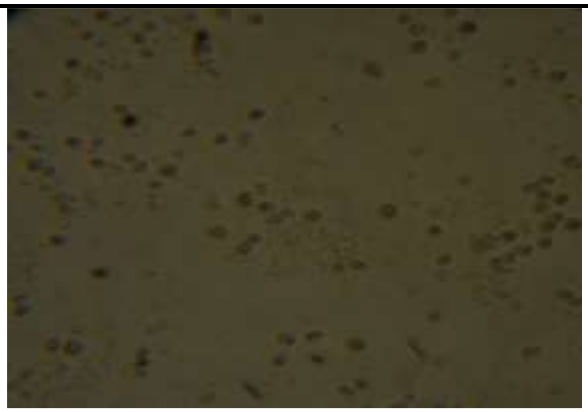
Table : 5 Percentage of Cytotoxicity of ethanolic leaf extract of *Tabernaemontana Divaricata* on K562 cell lines by MTT assay

Concentration	Cytotoxicity (%)	Cell Viability(%)	Cytotoxic Reactivity	With IC50 value
10	30	70	Mild	50.1µg
20	39	61	Mild	
30	46	54	Mild	
40	51	49	Moderate	
50	56	44	Moderate	
60	59	41	Moderate	
70	62	38	Moderate	
80	65	35	Moderate	
90	68	32	Moderate	
100	73	27	Severe	

The results for cytotoxicity by the extract against K562 lines for various concentration shown in table .As the concentration increases with the increases of cytotoxicity increases. The ethanolic extract of *T. Divaricata* showed Mild to Severe cytotoxicity to K562 cells after 24 hrs. The obtained IC_{50} values 50.1 µg. Control showed none cytotoxicity.



(A)



(B)

(C)

(D)

(E)

(F)

(G) **(H)**

(I) **(J)**

(K)

Fig. : 5 Anticancer Activity of T.Divaricata extract against K562 Cell lines from leaf extract of *Tabernaemontana Divaricata* with various concentrations.

(A) Control , (B) 10 µg , (C) 20 µg , (D) 30 µg , (E) 40 µg , (F) 50 µg , (G) 60 µg ,

(B) (H) 70 µg, (I) 80 µg , (J) 90 µg , (K) 100 µg .

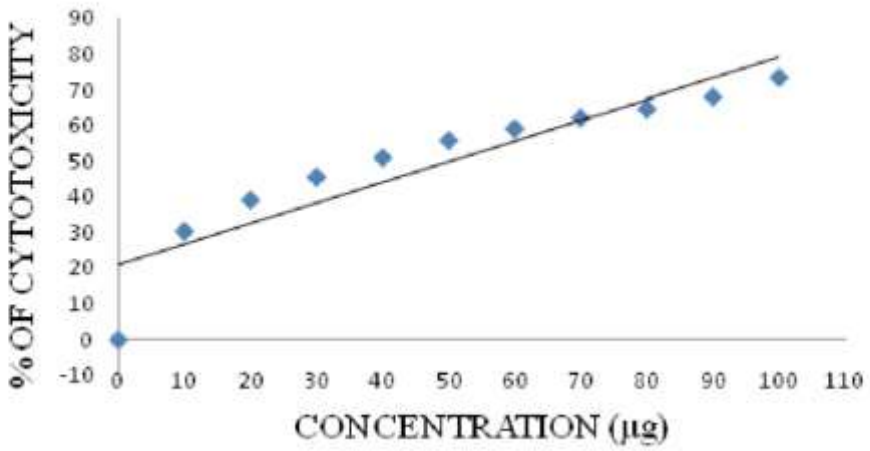


Fig. : 6 Concentration Vs % of cytotoxicity

Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed over generations with in various societies before the era of modern medicine . Traditional medicines are prepared from the single plant or combination of more than one plant. Indian contribution of herbal market and emphasis on novel research is continuously increasing . Phytochemical constituents are responsible for medicinal activity of plant species. Hence the present study phytochemical compounds in ethanolic extract of *T. Divaricata* highest percentage compound content a peak area 41.26 (1,2-Benzenedicarboxylic acid, Diethyl ester with RT 19.875), followed by the peak area of 14.91(Squalene with 38.12 RT) and and peak area of 20.67 (Hydroperoxide, 1,4-dioxan-2-yl with RT 20.467.) and the lowest percentage of content areaof 0.2 (Tert-butyl trans-3-methyl-5-Oxopyrrolidine-2-Carboxylate with RT 34.498).The ethanolic extract of *T. Divaricata* showed Mild to Severe cytotoxicity to K562 cells after 24 hrs. The obtained IC_{50} values 50.1 µ. Control showed none cytotoxicity .

CONCLUSION

The ethanolic leaf extract of *Tabernaemontana Divaricata* having numerous pharmaceutical properties. 1,2- Benzenedicarboxylic acid, Diethyl ester component are present in leaf extract . Hence the leaf extract of *T. Divaricata* could positively used in various ailments for the Herbal Medicine Industry.

In-vitro studies performed using cell lines reveals that the ethanolic leaf extract of *Tabernaemontana Divaricata* has a moderate anticancer activity . Eventhough there was increase in the cytotoxicity when concentration of sample is increased , the IC_{50} value is 50.1µg. Hence the level of cytotoxicity is increases with the increase of volume. Further in future, these components can be isolated and test the pharmacological activity in various metabolites in various pathways.

REFERENCES

1) Rahman Md. Ashikur, Md, Hasanuzzaman, Rahman Md, Mofizur, Shahid Israt Zahan , Roy Sonjoy Muhuri(2011). Evaluation of antibacterial activity of study of Tabernaemontana divaricata (L).,,International research journal of pharmacy., 2(6) 123-127.

2)C. Kalaimagal, G. Umamaheshwari . Evaluation of antibacterial activity from aerial parts of double flower variety of Tabernaemontana divaricata.,International journal of science and research.

(3)Akhila Sravya Dantu, Shankarguru p, Ramya devi D, Vedha Hari BN. Evaluation in Vitro Anticancer activity of Hydroalcolic extract of Tabernaemontana divaricata. Asian Journal of Pharmaceutical and clinical Research (2012) .

(4) Santhana Raut, Poonam , Nupur Gargate and Harshad .Pharmacognostic and Pharmacological aspects on Tabernaemontana Divaricata. Acta scientific Pharmacology. (2022).

(5) Ankita Kulshreshtha, Jyoti Saxena. Alkoloids and Non Alkoloids of Tabernaemontana Divaricata. International Journal of Research and Review.

(6)Shazid Md. Sharker, Samabesh Chakma and Ahmad Rahman . Evaluation of phytochemical analysis and antinoceptive study of leaves of Tabernaemontana divaricata (L) . Journal of medical Plants Research Vol. 5(2) 245-24.

(7)Kalaimagal C. Identification of bioactive compounds in flower of Tabernaemontana Divaricata (L)., using Gas chromatography –Mass spectrometry analysis Asian Journal of Pharmaceutical and Clinical Research (2019).

(8) Pranabesh Ghosh, Susmita Poddar and Sirshendu Chatterjee. Morphological features, Phytochemical and ethanopharmacological attributes of *Tabernaemontana Divaricata* Linn. Journal of Pharmacognosy and Phytochemistry. DOI: https://doi.org/10.22271/phyto.2021.v10.i6a.14253.

9) Anubha Arora, Phytochemical Analysis of Methanolic Extracts of Leaves of some Medicinal plants.

10) Sanjita Das, Anupam Dubey, Divya, Tabernaemontana divaricata: A Herbal Panacea, Journal of Natural Remedies 22(4):549-562 DOI:10.18311/jnr/2022/29962.

11) Kannappan Poornima, Chella Perumal Palanisamy, Sowmya Sundaram, Gopalakrishnan Velliyur Kanniappan Chromatographic Fingerprinting Analysis of Secondary Metabolites Present in Ethanolic Extract of *Tabernaemontana divaricata* (L.) R. Br. by HPTLC Technique, 2017,https://doi.org/10.1080/22297928.2017.1284608.

12) Bindu Rathaur, Meraj Ali, SumitnKumarnand Urmila Nishad , Phytochemical analysis of Tabernaemontana Divaricata, Journal of Pharmacognosy and Phytochemistry, 2020.

13) Md. Omor Faruq, Mst. Shirajum Munira, Sonia Zaman, Sabiha Ferdowsy Koly, Rayhanus Salam, Sonia Rani Das, Md. Anisur Rahaman , Central Nervous System Depressant Effects of the Methanolic Leaves Extracts of Tabernaemontana divaricata, International Journal of Medicinal Plants and Natural Products (IJMPNP), Volume 4, 2018, ISSN No. (Online) 2454-7999 , DOI: http://dx.doi.org/10.20431/2454-7999.0401001.

14) Kalaimagal , In Vitro Antioxidant and Anticancer Potency of Tabernaemontana divaricata (L.) Flowers DOI: https://doi.org/10.18311/jnr/2022/25367

15) Priyanka P. Tadkase, Prof. Dr. Nitin B. Ghiware, Haidarali Shaikh, Vidhya V. Mali, 1Ashwini V. Gowande, comparative evaluation of tabernaemontana divaricata leaves extracts for antidepressant activity, World Journal of Pharmaceutical Research, volume 7, 2018, DOI: 10.20959/wjpr201812-12592.

16) Mohammed Safwan AliKhan, Misbah , Nishat Ahmed , Mohammed Arifuddin, Abdullah Rehman, Mok Pooi Ling, Indole alkaloids and anti-nociceptive mechanisms of *Tabernaemontana divaricata* (L.) R. Br. flower methanolic extract, Food and Chemical Toxicology, Volume118, 2018, https://doi.org/10.1016/j.fct.2018.06.007.

17) Arvind Kumar and S. SelvakumarAntiproliferative efficacy of *Tabernaemontana divaricata* against HEP2 cell line and Vero cell line, doi: 10.4103/0973-1296.157682

.19) Thombre R, Jagtap R, Patil N, Evaluation of Phytoconstituents , antibacterial , antioxident and cytotoxic activity of Vitex negundo andTabernaemontana Divaricata L. International Journal of Pharma and Bio Sciences .

20) Basumatary AR, Preliminary Phytochemical Screening of some compounds from plant stem bark extracts of Tabernaemontana Divaricata Linn, used by Bodo Community at Kokrajhar district , Assam, India, Archives of Applied Science Research 2016; 8(8):47-52.

21) Abubaker IB, Loh Hs, A review on Ethnobotany, Pharmology and Phytochemistry of Tabernaemontana , Journal of Pharmacy and Pharmacology, 2016.

22) Rahman MM, Sayeed MA, Biplab KP, Siddique SA, Antidiabetic cytotoxic activities of methanolic extract of Tabernaemontana Divaricata (L.) leaves of alloxaan induced mice. Asian J Pharm Clin Research.

23) Somnath Mondal1, Koushik Chakrabarty2, Mayukh Bose3, SudipMaity2, Sukanya Saha Chowdhury2, Subhajyoti Das2, Subhajit Samanta2, Sakshar Saha* Exploring The Therapeutic Potential: In-Vitro Assessment of Antioxidant and Anti-Inflammatory Activity Of methanolic extract from Tabernaemontana divaricata Leaves.

24) Merrine Raju, Y. Vasudeva Rao, study of Catalase, Protease, Antioxidant and Antimicrobial Activities of Tabernaemontana divaricata Latex, Journal and medicinal plants and by products, 10.22092/JMPB.2020.127797.1140.

25) Mrinal Kumar Baishya1 , Kandarpa Kr. Saikia1, Naba Kr. Hazarika , Debabrat Baishya1 Deep Jyoti Das, Antimicrobial Potential and In Vitro Cytotoxicity Study of Tabernaemontana Divaricata (L.) Stem Bark Extract Against HEK 293 Cell Line, IOSR Journal Of Pharmacy ,Volume 8, 2018 .