IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

PHYTOCONSTITUENTS SCREENING AND COMPARATIVE EVALUATION OF DIETHYL PHTHALATE ADSORPTION IN *ANDROGRAPHIS ECHIODES* USING GAS CHROMATOGRAPHY–MASS SPECTROMETRIC ANALYSIS. (GC–MS)

Clara Preethi Rodrigo.B [1, 2], Dr.K.Murugan[*] Kalavathi.K[3]and S.Abirami[4]

[1]*Resesarch Scholar,Reg.No :17214012272022 Manomaniam Sundaranar University, Tirunelvel, India.*

[2]*Assistant Professor, PG department of microbiology, Kamaraj College, Thoothukudi, India*

Department of Biotechnology, Manomaniam Sundaranar University,Tirunelveli, India

[4]*Assistant Professor, PG department of microbiology, Kamaraj College, Thoothukudi, India*

ABSTRACT:

Plastics being a major issue in environment from past few decades, Diethyl phthalate is a plasticizer, used in manufacturing wide range of materialsdue to its durability, flexibility and for long shelf life. The phthalate polymers have great adverse effects and health hazards while exposed in environment. This present investigation reveals the phthalate esters are absorbed by plants, the green medicine being the alternative source for the antimicrobial drug resistance, should have to face the upcoming sneak attack of this phthalate proliferation in all biome. In this study the *Andrographis echioides* is subjected to grow in two varying environment, TPS1 pollutant free plant and TPS 2 grown in pollutant exposed area, the extract obtained using polar and non polar solvents. Purification was done by column chromatography using various solvents. Based on high activity the eluted fraction was further investigated to GCMS, FTIR, HPLC and UV spectroscopy. Results show that the diethyl phthalate compound present in pollutant exposed plant (TPS 2)in high abundance and nil in the plant grown on pollutant free environment (TPS 1). This study implies that phthalate ester starts to penetrate in all livings organisms as an emerging environmental hazard.

Keywords: Diethyl phthalate, *Andrographis echioides,* antimicrobial drug resistance, plasticizer

INTRODUCTION:

Diethyl phthalate (C12 $H_{14}O_4$)is an emerging environmental hazard. It's a clear, colorless,(NIOSH 2005) odorless liquid ,viscous than water and miscible substance. The other names are phthalic acid diethyl ester, Diethyl 1,2-benzenedicarboxylate, o-Benzenedicarboxylic acid diethyl ester, diethyl ester1,2-Benzenedicarboxylic acid. Molecular weight is 222.24 g/mol, Boiling point 568 °F , melting point 27 °F (NTP, 1992). Phthalates a xenobiotic component since 1920, used in making flexibility plasticizers , cosmetics additives ,construction, cables, wires, food packaging materials, medical devices, toys ,blood bags, perfumes, shampoo, fabric, arthropod repellent, and as paints.(Andersson et al. 2007)(Calafat and Mckee 2006) Due to its ubiquitous and numerous applications , these compound tend to highly exposed to environment resulting the inhalation, adsorption by living organisms.(Weschler et al. 2015)the use of low-molecular-weight phthalate doesn't covalently bonded to their products, resulting oozing out in to environment.(Latif et al. 2020).In human these compounds have a wide adverse effects was unproven due tothe risk assessment study, the cream formulation of phthalate on animal studies shows after uptake of compound through dermal converted to monoethyl phthalate (MEP) degraded into residual alkyl chain which increases solubility of water and facilitates excretion. Absorption of phthalate was fast as well asin high rate.(Andersson et al. 2007) Based on rodents testing results show liver effects, histological changes, including increased weights, elevated enzyme levels and tumors, associated with peroxisomal proliferation. Phthalates are chemicals which are hazardous to human health and fertility as they can be readily absorbed through the skin. They have been linked to birth defects, such as hormonal damage, infertility and cancer. They are also known to be endocrine-disrupting compounds.(Suer and Bayram 2017)

This present study investigates the phthalate adsorption by using *Andrographis echioides* commonly called false water willow an annual herb,(Agnel Ruba and Mohan 2016) Acanthaceae family possess various medicinal applications like dissolving kidney stones, prevent hair loss, nerve related disorders, wound healing activity, antipyogenic, curing of dental infections also reported for magic illusionary tricks. Possess antibacterial effect against *S. mutans, Lactobacillus acidophilus, S.salivarius.*(Sharma et al. 2017)The components from *A.echioides* such as Silane dimethyl, 13-Octadecadienol are activity against larvicidal and acaricidal activity.(Mathivanan, Gandhi, et al. 2018) used for treating pyresis(Alagesaboopathi 2009)Four medicinally compounds reported are Androgechoside **B**, Androgechoside A. *Andrographis echioides* is used for the degradation studies. In this work the phytochemical, comparativescreening the presence of phthalate compound in two different plants grown in varying environments.

MATERIALS AND METHODS

The plant *Andrographis echioides* was collected from two different environments. Test plant (TPS 1) grown in farm free from herbicide and fertilizer, Test plant (TPS 2) collected from the 8º33'22.35''N, 77o58'15.64''E from train tracks, due to its chasmophytes perennial nature. After six weeks the plants are harvested, washed and subjected to shade dry. The plant materials were shade dried at 31°C for 10 days; 100 g (1:6 w:v) of each sample preparation was powdered by using an electric blender. They were loaded into an empty glass column added along with methanol, chloroform, ethyl acetate, for solvent extract by means of cold percolation method.

PHYTOCONSTITUENTS SCREENING

The extracts of the dry powdered leaves of *Andrographis echioides* were analyzed for the presence of diverse phytochemical constituents like Alkaloids, Flavonoids, Terpenoids, Steroids, Tannins, Saponins,Phenols, Carbohydrates, Proteins and amino acids, and fixed oil and fat were identified using standard phytochemical procedures.

CHARACTERIZATION OF *Andrographis echioides*EXTRACT BY GC-MS ANALYSIS.

For the bioactive and phthalate components screening the methanolic extracts TPS1, TPS 2and TPS 2 chloroform extracts were identified by GC–MS. Compounds were separated by GC. Peaks and retention time were identified by computer searching in a commercial mass spectral reference library (MassHunter library/ NIST library).GC-MS analysis of methanol, ethyl acetate and chloroform solvents of *Andrographis echioides.* The analysis was done in Agilent GC-MS – 5975C fitted with a DB - 5 MS. For carrier gas the helium with flow rate1.51ml/min and temperature injector port 240° was used. By split injection mode the sample was injected. The NIST- 11 Library was used for standard comparisons.. Optical rotations were recorded using a Jasco digital polarimeter (chloroform) used to measure for optical rotations.

SEPERATION OF PHTHALATE:

Column chromatography technique is used to separate phytochemicals form a mixture of compounds even in small amounts. The column was packed with silica gel using hexane, a low polar solvent together the admixture was added. The column was eluted with initial solvent followed by chloroform, methanol etc.

Ultra-Violet and Visible Spectroscopy:

The UV- Visible spectra were obtained in MeOH on a Genesys 10 UV spectrophotometer. By keeping the concentration of fractionated compound (1mgml−1), the absorption spectra at different concentrations (1–5w/v%) were recorded by the UV-1800 Shimadzu spectrophotometer with 10mm quartz cell. The steady-state fluorescence was measured using Shimadzu RF-6000 Spectrofluorometer.

Fourier Transform Infra-Red Spectroscopy. (FT-IR)

Dried and powdered *Andographic Echioides* were pelleted with potassium bromide (KBr) (1 : 10 proportion). The spectra wave number range of 450-2500 cm-1 and analyzed by subtracting the spectrum of pure KBr. FT-IR analysis in diffuse reflectance mode operated at a resolution of 4 cm-1 in the range of 400 to 4,000 cm-1 was carried out to evaluate the functional groups that might be involved in nanoparticle formation.

The IR region spectrum used in organic chemistry is 4000-500cm-1this far infra-red region. Absorption in infrared region corresponds to vibration and corresponding rotational energies in the molecule. The vibration modes include sym stretching, unsym-stretching and sketatal vibrations. Sketatal vibrations include bending, rocking, wagging etc. The Infra red spectrum is useful in detecting functional groups with characteristics vibrational frequencies. e.g. the hydroxyl groups in a compound absorbs at 3600-3200 cm-1 due to OH stretching.

The carbonyl group of ketones would give a strong band at 1710cm-1 due to stretching of the CO band. COOMe would give a band around 1720 cm-1 due to C=O stretching and a band around 1230 cm-1 due to COC bending. Structural changes like α, β unsaturation hydrogen bonding, etc, modify the absorption frequencies. Hydrogen bonding α, β unsaturation cause decrease in absorption frequencies. FTIR band intensities in different regions of *A.echioides* leaf powder.

HPLC ANALYSIS

To analysis the water phyto-constituents found in plant extracts the HPLC analysis was done using Perkin Elmer 200 Series HPLC with UV–Visible detector (192–700 nm) and a 200 Series pump. The sample was eluted using a mobile phase containing 0.1M KCl and 32% acetonitrile (pH was adjusted to 3.0 with dil. HCl) using Brownlee Analytical C-18 (150 × 4.6mm 5 lm110 Å) column packed with silica particles. The detection was carried out using the UVisible detector at 260 nm with a flow rate of 1mL min^{-1}. The obtained peaks were compared and matched with external standards.

RESULTS AND DISCUSSION

PLANT PHYTOCONSTITUENTS SCREENING

The methanol, ethylacetate and chloroform extracts of the whole plant powder showed the presence of alkaloid anthraquinone, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein. Table .1

Table .1Phytoconstituents screening of *A.echioides*

S.No	TEST	Methanol	Ethyl acetate	Chloroform
1.	Alkaloid	+	+	+
2.	Anthraquinone	_	_	_
3.	Catechin	_	_	_
4.	Saponin	-	+	+
5.	Phenol	+	+	+
6.	Coumarin	+	+	+
7.	Quinone	+	+	+
8.	Flavonoid	+	+	+
9.	Phenol	+	+	+
10.	Steroid	+	+	+
11.	Glycoside	+	+	+
12.	Carbohydrate	+	+	+
13.	Terpenoid	+	+	+
14.	Tannin	+	+	+
15.	Xanthoprotein	+	+	+

+ Present, - Absence

CHARACTERIZATION OF *ANDROGRAPHIS ECHIOIDES* EXTRACT BY GC-MS ANALYSIS.

The phytochemical compounds were obtained from the crude extract of both the samples methanol TPS 1, TPS2 and TPS 2 chloroform extract of *Andrographis echioides* extract was obtained by GCMS. In over all components the certain components were screened based its abundance and area, is tabulated.

Figure 1 . GC MS chromatogram methanolic extract of *Andrographis echioides* TPS 1

In Methanol (TPS 1):

Methyl vinyl ketone, Cyclopropane, 1,2-dimethyl-, trans- ,Cyclopropane, 1,2-dimethyl-, cis-,5H-Tetrazol-5-amine,2 Oxazole, 4,5-dihydro-2-methyl-, 4-Cyclopentene-1,3-diol, trans-,Propanedinitrile, methylene-,Propanedinitrile, methylene-, Fumaronitrile, Propanedinitrile, methylene-, 1,5-Hexadiyne, 2,4-Hexadiyne, 2-Propenenitrile, Difluoramine, 3-Hexen-2-one, 3,4-dimethyl-, (Z)-, Cyclobutanone, 2-methyl-2-oxiranyl- ,4-Cyclopentene-1,3-diol, trans-,Furan, 2,5-dimethyl-,2 s(+)-1-Cyano-2-methyl-azetidine, 2-Butenal, 2-ethenyl-,Acetonitrile, 2,2'-iminobis- ,2 1,2-Butadiene, 3-methyl-, 2-Pentyne, 4-Cyclopentene-1,3-diol, trans-,2 Bicyclo[1.1.0]butane, Propiolamide, Propiolamide,2 2(3H)-Furanone,3 Cyclobut-1-enylmethanol, 2-Propenenitrile, Furan, 2,5-dimethyl-,2 2,4-Dimethylfuran,3 2-Cyclopenten-1-one, 3-methyl-,2(1H)-Pyridinone,3 1,3,5-Triazine, 2-chloro-4,6-bis..., n-Decanoic acid,

2 Dodecanoic acid, Maltose, [1,2,4]Triazolo[4,3-a]pyrimidine..., 2 (1-Cyanocyclohexyl) carbamate,3 trans-2-Dodecen-1-ol, trifluoroa..., 5-Acetamido-4,7-dioxo-4,7-dihydr..., [1,2,4]Triazolo[1,5-a]pyrimidine, N-Methyl-1-adamantaneacetamide, Cyclotrisiloxane, hexamethyl, Purine-2,6-dione, 8-(3-ethoxypro..., 1,4-Bis(trimethylsilyl)benzene, Silane, trimethyl[5-methyl-2-(1-... , Propiophenone, 2'-(trimethylsilo...,1,4-Bis(trimethylsilyl)benzene, Benzo[h]quinoline, 2,4-dimethyl- ,1H-Indole, 2-methyl-3-phenyl-,3 Benzo[h]quinoline, 2,4-dimethyl-,

Table .2GCMS ANALYSIS OF *Andrographis echioides* IN SOLVENT METHANOL - TPS 1.

SL.NO	NAME OF THE COMPOUND	MOLECULAR FORMULA	MOLECULAR WIEGHT	AREA %	LOG P	HYDROGEN DONOR	HYDROGEN ACCEPTOR	ROTATION BOND
1.	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	252.27 g/mol	30.17	4.2	0	1	0
2.	1H-Indole, 2-methyl-3-phenyl-	$C_{15}H_{13}N$	207.27 g/mol	30.17	4.1	0	0	1
3.	Silane, trimethyl[5-methyl-2-(1-...	$C_{14}H_{20}OSi$	222.4 g/mol	31.06	-	0	1	3
4.	Propiophenone, 2'-(trimethylsilo...	$C_{12}H_{18}O_2Si$	252.5 g/mol	31.06	-	2	2	4
5.	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222.46 g/mol	31.06	-	0	3	0
6.	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	150.1 g/mol	23.65	-0.8	1	2	0
7.	Purine-2,6-dione, 8-(3-ethoxypro...	$C_{12}H_{19}N_5O_3$	266.53 g/mol	23.65	-	2	2	4
8.	Furan, 2,5-dimethyl-	C_6H_8O	96.13 g/mol	2.30	1.1	0	2	0
9.	s(+)-1-Cyano-2-methyl-azetidine	$C_5H_8N_2$	96.13 g/mol	2.30	1.3	0	1	2

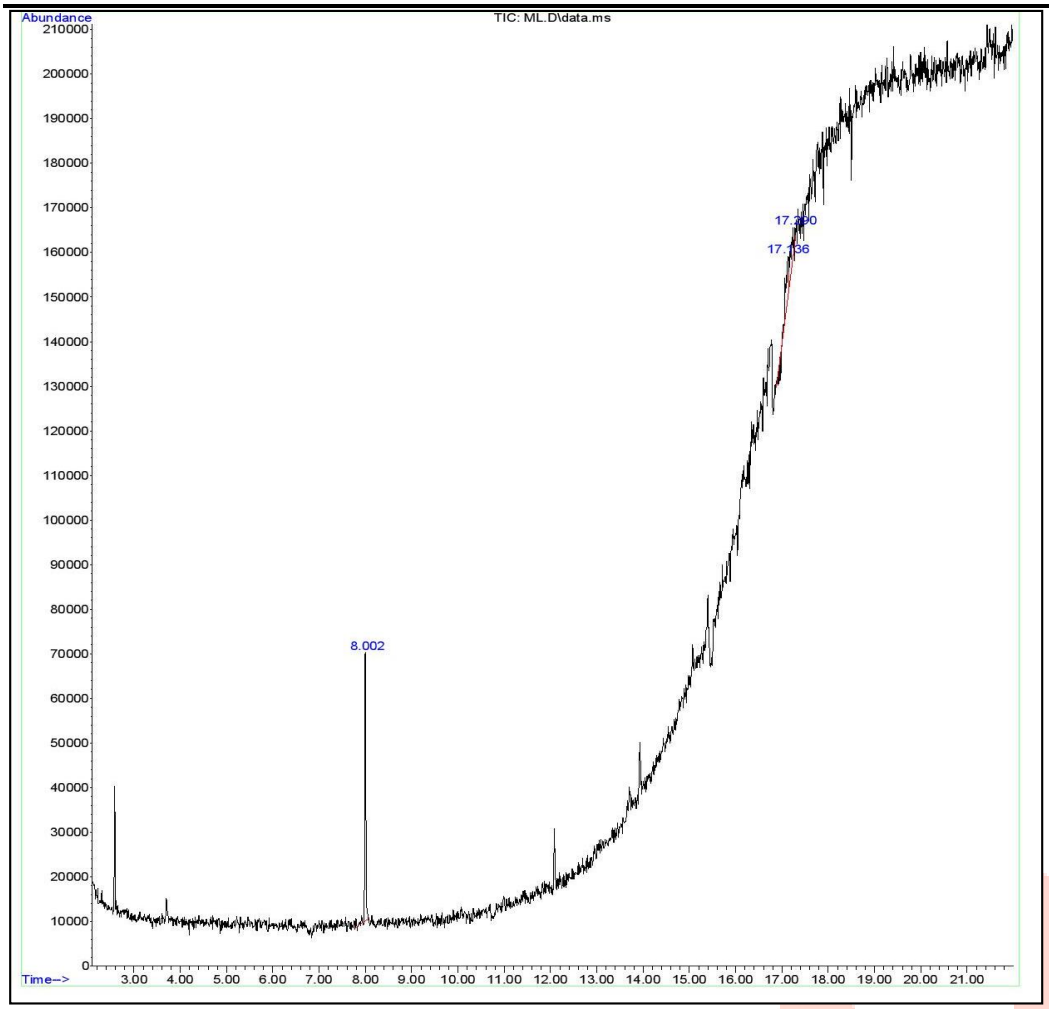

Figure 2.GC - MS chromatogram of methanolic extract of *Andrographis echioides* TPS 2

In Methanol (TPS 2) :

The components obtained in methanolic extract TPS 2 (obtain in environment for phthalate absorption) Fig .2, the phtyo constituents are Diethyl Phthalate, Benzene, 2-[(tert-butyldimethyls..., Tetrasiloxane, decamethyl-,Trimethyl[4-(2-methyl-4-oxo-2-pe..., 2-Ethylacridine, Cyclotrisiloxane, hexamethyl-,Benzo[h]quinoline, and 2,4-dimethyl-

Table .3 GCMS ANALYSIS OF *Andrographis echioides* IN SOLVENT METHANOL - TPS 2

SL.NO	NAME OF THE COMPOUND	MOLECULAR FORMULA	MOLECULAR WIEGHT	AREA %	LOG P	HYDROGEN DONOR	HYDROGEN ACCEPTOR	ROTATION BOND
1.	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24g/mol	66.28	2.5	0	4	6
2.	Benzene, 2-[(tert-butyldimethyls...	$C_{12}H_{18}O_2$	194.27 g/mol	15.23	3.5	0	2	3
3.	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$	310.68 g/mol	15.23	-	0	3	6
4.	Trimethyl[4-(2-methyl-4-oxo-2-pe...	$C_{15}H_{24}O_2Si$	264.43 g/mol	15.23	-	0	2	5
5.	2-Ethylacridine	$C_{15}H_{13}N$	207.27 g/mol	18.49	-	0	1	1
6.	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46 g/mol	18.49	4.2	0	3	0
7.	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207.27 g/mol	18.49	4.2	0	1	0

In Chloroform (TPS 2):

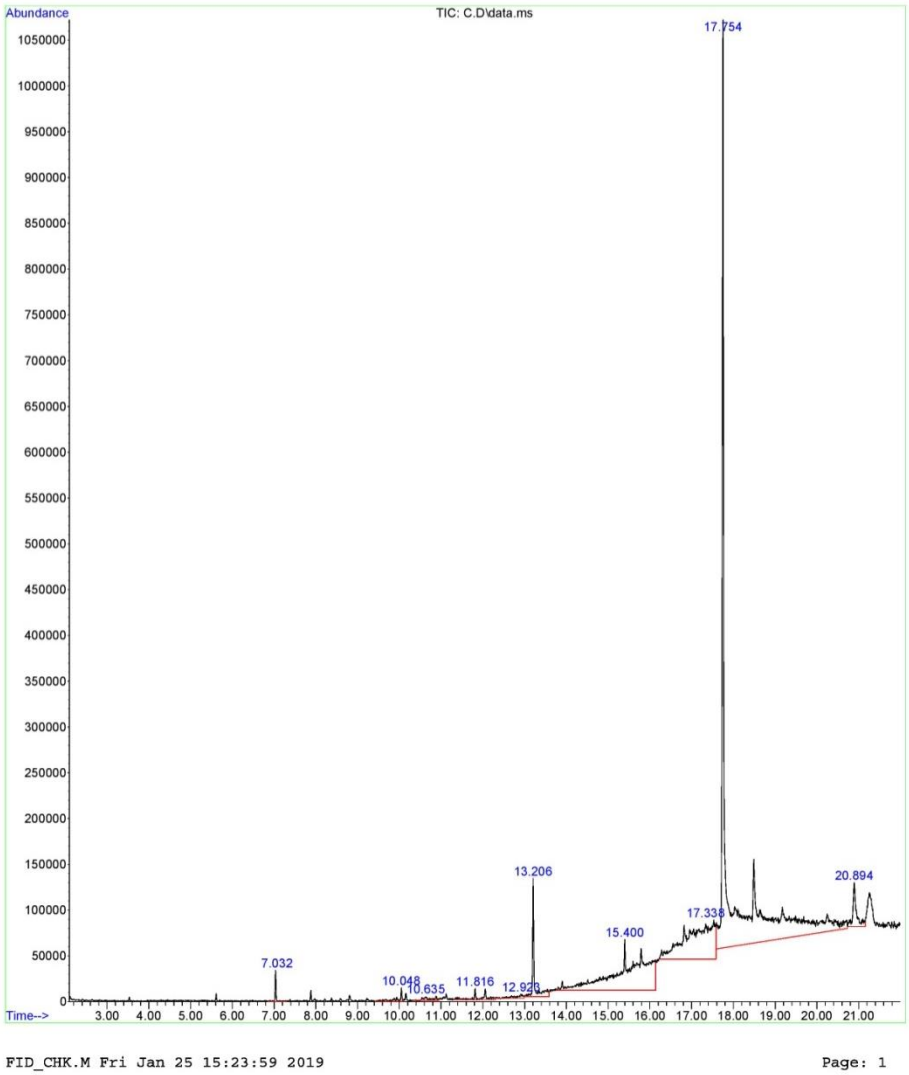

Figure 3 .GC - MS chromatogram of chloroform extract of *Andrographis echioides* TPS 2

They are Phenol, 2,4-bis(1,1-dimethylethyl)-, Phenol, 2,4-bis(1,1-dimethylethyl)-, Phenol, 3,5-bis(1,1-dimethylethyl)-, 1-Tricosene, Oxirane, [(dodecyloxy)methyl]-, Cycloeicosane, 9-Octadecynoic acid, methyl ester, Acetic acid, 3-(2-methoxyethyl)heptyl ester, 9-Octadecen-1-ol, (Z)-, Phthalic acid, ethyl 2-propylpentyl ester, 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester, Phthalic acid, ethyl 2-ethylbutyl ester, 2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha., (1R,2S,8As)-8-oxo-1-carboxymethyl-1,2,5,5-tetramethyl-trans-decalin, Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester, Phytol, Ethanone, 1-(3-ethylcyclobutyl)-, Oxirane, tetradecyl-, [1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-, Benzenamine, 4,4'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis-, Stigmasta-4,6,22-trien-3.beta.-ol, 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene 207.0, 2-Ethylacridine, Benzo[h]quinoline, 2,4-dimethyl-, 4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl-, Naphthalene, 1-(2-naphthalenylmethyl)-, 13H-Dibenzo[a,i]carbazole, Purin-2,6-dione, 1,3,7-trimethyl-8-[2-(3-aminophen)ethyl]-, Silane, dimethyl(2-chloro-5-methylphenoxy)octyloxy-, Pyridine-3-carbonitrile, 1,2-dihydro-4-[4-(1,1-dimethylethyl)phenyl]-6-phenyl-2-oxo-.

In over all above components the certain components was screened based its abundance, area, and their drug usage properties.

TABLE.4 GC- MS ANALYSIS OF *Andrographis echioides* in CHLOROFORM EXTRACT

SL.NO	NAME OF THE COMPOUND	MOLECULAR FORMULA	MOLECULAR WIEGHT	AREA %	LOG P	HYDROGEN DONOR	HYDROGEN ACCEPTOR	ROTATION ON BOND
1.	Phenol,2,4,bis 1,1-dimethyl)	$C_{22}H_{30}O$	310.481g/mol	0.55%	-	0	1	4
2.	1-Tricosene	$C_{23}H_{46}$	322.621g/mol	0.62%	12.7	0	0	20
3.	9-octadecynoic acid, methyl ester	$C_{19}H_{34}O_2$	294.479g/mol	0.44%	7.3	0	2	14
4.	Phthalic acid ethyl 2-propylpen	$C_8H_6O_4$	166.132g/mol	0.96%	5.2	0	4	11
5.	2H 3, 9a –methano-1-benzoxepin	$C_{15}H_{26}O_4$	238.366g/mol	0.49%	6.9	0	2	15
6.	Phytol	$C_{20}H_{40}O$	296.539g/mol	2.89%	8.2	1	1	13
7.	(1,1,Bisphenyl)-2,3-diol,3,4	$C_{12}H_{10}O_2$	186.21g/mol	19.32 %	2.7	2	2	1
8.	2-(Acetoxymethyl)3 methoxycarb	$C_{11}H_{15}NO_5$	241.243g/mol	15.93 %	1.2	0	4	5
9.	4H-Benzopyran-4-one,5-hydroxy	$C_{11}H_{10}O_3$	190.198g/mol	56.27 %	3	2	5	2
10.	Purine 2-6 dione,1,3,7, trimethyl	$C_8H_{10}N_4O_2$	193.194g/mol	20.894 %	-0.5	1	6	4

PHTHALATE SEPERATION:

The bioactive compound was done by various methods like chromatography, ultrasound methods(NOGUERA, OKABE, and PICIOREANU 1999). The column was successively eluted with

individual and in combination of solvents in the following ratios sequentially during the elution process as follows: methanol (100%); methanol : chloroform (80:20, 40:60, 60:40,20:80), chloroform (100%); chloroform : ethyl acetate (60:40, 40:60, 20:80,80:20), ethyl acetate (100 %); ethyl acetate : methanol (90 :10), and methanol (100%) In total eluted fraction based on its high antibacterial activity fraction VI was collected. Obtained pure compound was subjected to GCMS, FTIR, HPLC and further UV for structural determination.

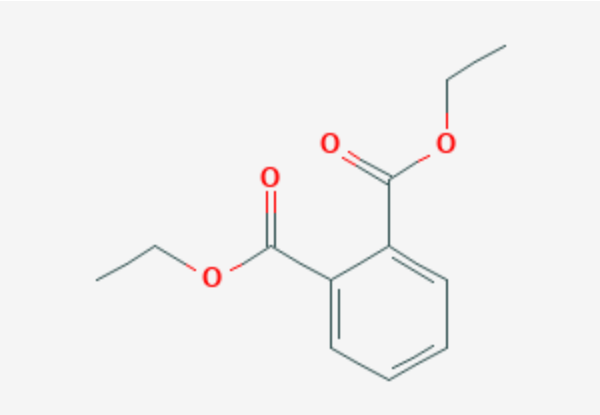

Figure .5Diethyl phthalate

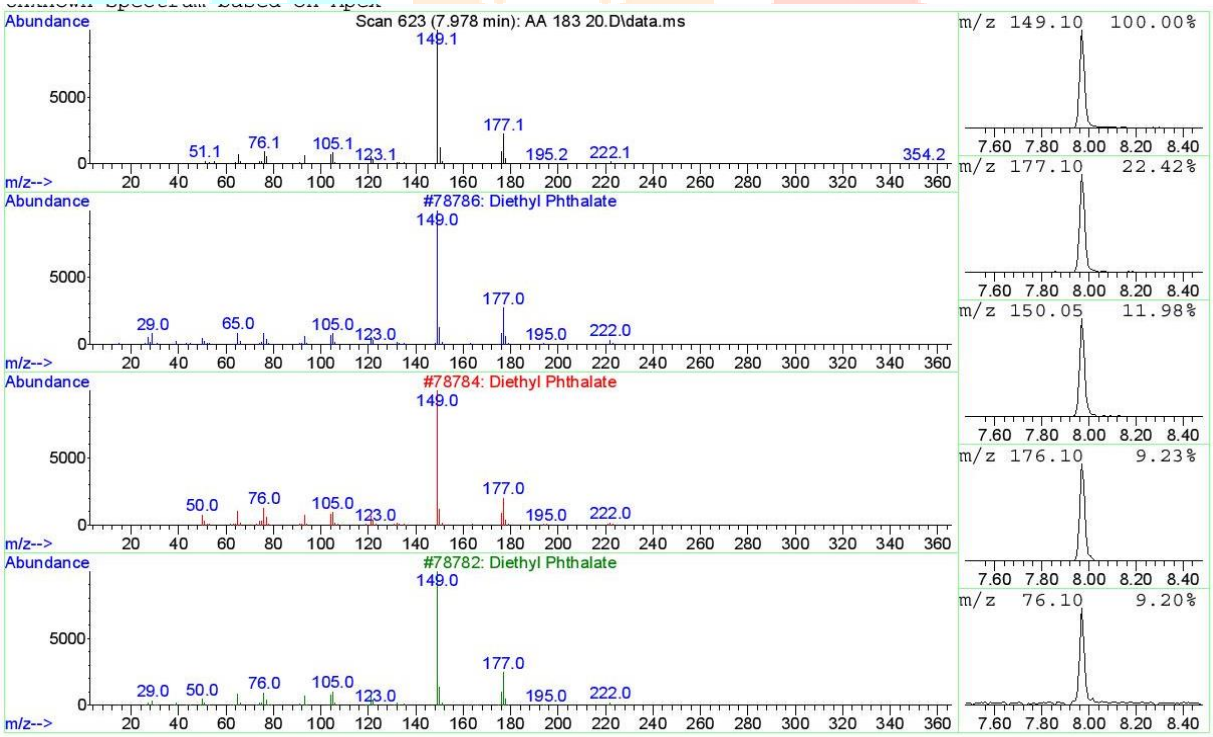

Figure .4.1 Gas chromatography–mass spectrometry (GC–MS) peak of Compound IV

Among the various eluted components in *Andrographis echioides* the fraction IV of the methanolic activity shows high zone of clearance against the four clinical isolates such as *E.coli*, *Pseudomonas* sp, *Klebsiella* sp and *Staphylococcus* sp. The fraction purified through column chromatography was further subjected to Gas chromatography Mass spectroscopy; the result shows that obtained compound was Diethyl phthalate in TPS 2 methanolic extract. Also the crude chloroform extract of *A.echioides* also posses phthalate in form of Phthalic acid ethyl 2-propylpen, indicates the phthalate absorption in pollutant exposed environment.

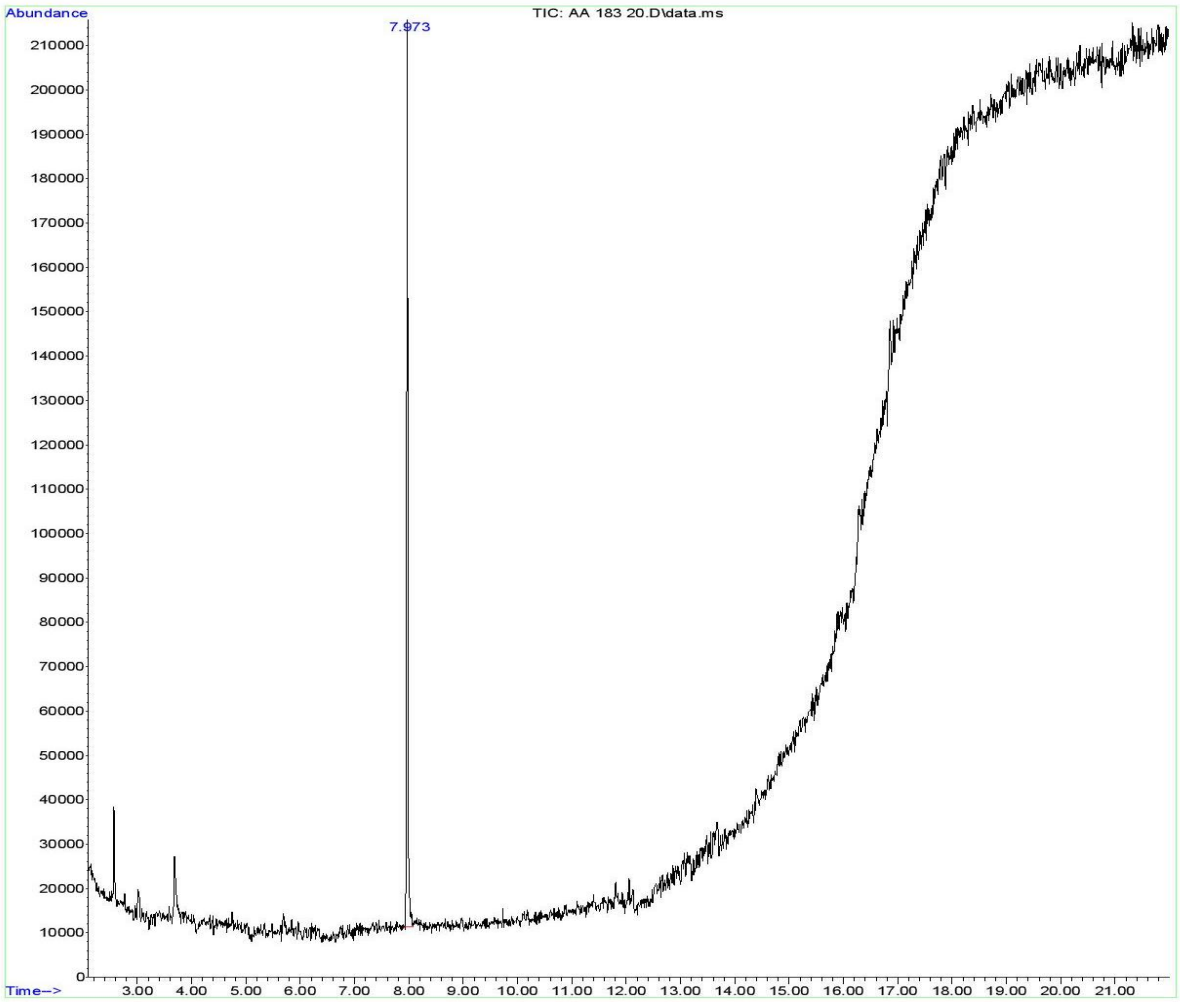

Figure 6.GC - MS chromatogram of methanolic extract of *Andrographis echioides* TPS 2 (purified fractionated compound by column)

FOURIER TRANSFORM INFRA-RED SPECTROSCOPY (FT-IR) AND HPLC:

FTIR measurement was used to predict the role of reducing and stabilizing capacity of *A. echioides* leaf extract. The FTIR spectra was recorded for the leaf extract of eluted TPS 2 *A. echioides*, purified fractionated extract were observed at 3151.47 cm-1, 1743.53 cm−1, 1640.78 cm−1, 1406.35 cm−1, 1195.78 cm−1,1094.53 cm−1, 754.12cm−1, 602.71 cm−1 and 602.71 cm−1 (Fig.5). Characteristically, the region between 3700 and 3050 cm−1 was noticeable, 3151.47 cm-1which is attributed to the bond vibrations of carboxyl, hydroxyl or phenol groups, and to amides' N-H vibrations.Additionally, peaks within the 1800–1700 cm−1 region1743.53 cm−1, caused by the C -O bonds, typically from lipids, and 1640.78 cm−1 peaks found between 1700 and 1500 cm−1, caused by amides in proteins, were also clearly present.Carbon-Nitrogen bonds (C-N) found in proteins contributed to the detected 1406.35 cm−1peak 1500–1400 cm−1, the two peaks (1195.78 cm−1, 1094.53 cm−1) between 1200 and 1100 cm−1 is frequently attributed to the vibrations of C-O bonds, Lastly, found in carbohydrates, as well as the peak at 1080–1000 cm−1,

754.12cm−1, 602.71 cm−1 corresponds to aromatic stretching vibrations (Figure.7) and the intensity, area are given in the (Table.5).The analyzed HPLC and UV results was shown in (Figure.8, Table.6) and Figure.9

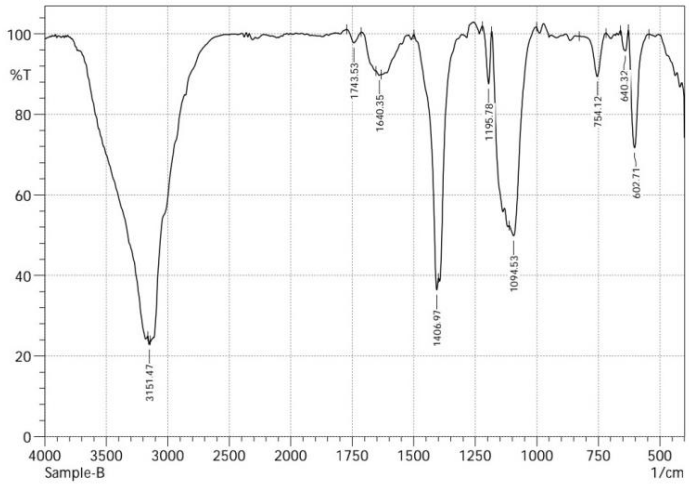

Figure.7. FT-IR Analysis of purified methanolic extract of *Andrographis echioides (Compound IV)*

Table.5 The peak value of FTIR were given in the below table.

No.	Peak	Intensity	Corr.Intensity	Base(H)	Base (L)	Area	Corr.Area
1.	602.71	71.716	29	628.75	544.85	4.236	4.414
2.	640.32	95.787	5.197	659.61	628.75	0.324	0.45
3.	754.12	89.395	10.522	828.37	719.4	1.668	1.561
4.	1094.53	49.849	10.615	1112.85	1000.99	13.449	-1.841
5.	1195.78	87.578	13.448	1220.86	1183.25	0.83	1.031
6.	1394.44	36.436	7.516	1499.55	1399.26	12.383	-8.005
7.	1640.35	89.705	0.538	1653.85	1653.85	0.962	0.028
8.	1743.53	97.761	3.046	1772.46	1713.64	0.179	0.383
9.	3151.47	22.88	1.489	3163.04	3141.82	13.258	0.265

Figure .8 - HPLC Analytical result for the *Andrographis echioides*

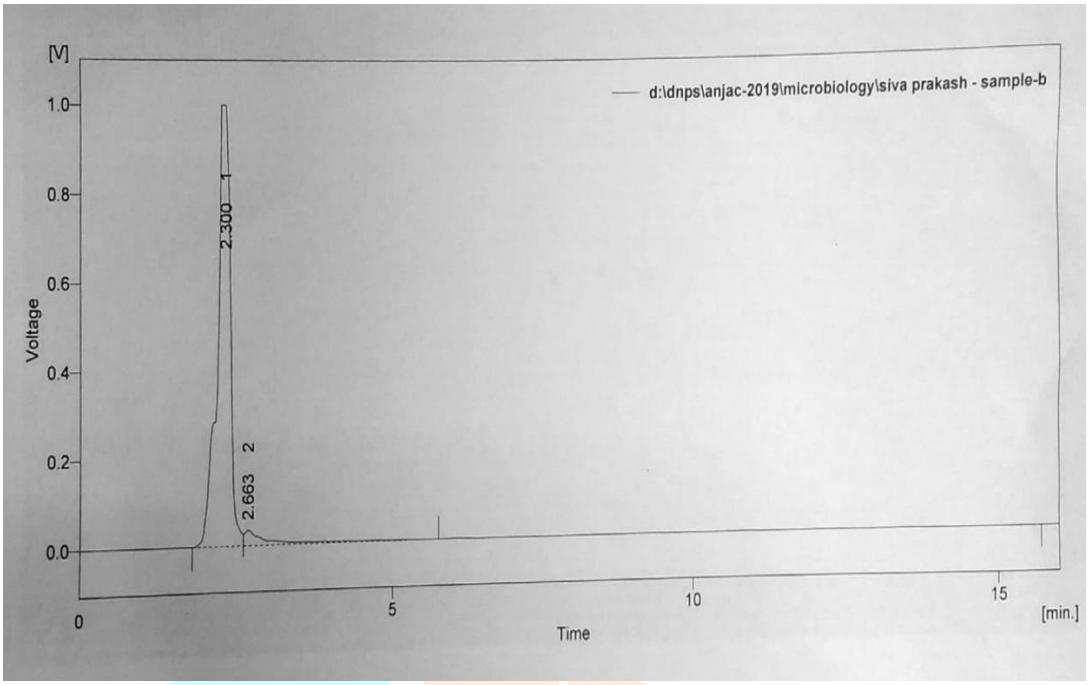

Table .6 HPLC analysis of purified methanolic extract of *A. echioides (Compound IV)*

	RETEN.TIME [MIN]	AREA [MV.S]	HEIGHT [MV]	AREA [%]	HEIGHT [%]	W05 [MIN]
	2.300	14521.336	999.706	88.5	96.0	0.20
	2.663	960.170	34.510	5.9	3.3	0.27
	16.223	6.336	0.231	3.861e-02	2.218e-02	0.59
	17.737	25.102	0.871	0.2	0.1	0.45
	21.417	121.475	0.777	0.7	0.1	1.05
	28.317	480.060	2.699	2.9	0.3	3.08
	31.303	297.335	2.295	1.8	0.2	2.36
	Total	16411.816	1041.089	100.0	100.0	

UV- visible spectrophotometer:

Concentrations were followed at 230nm in water and in 1M H2SO4 solutions, since the predominant phthalate and/or neutral phthalic acid in these methanolic solvents, give a strong characteristic UV absorption band maximum at this wavelength. In 0.005M NaOH, the conversion of all the phthalic acid into phthalate anion causes the band maximum at 230nm to disappear. Therefore, the concentrations of phthalic acid in 0.005MNaOH solution were followed at 272nm where the phthalate anion shows relatively weak UV

absorption band maximum. The UV result showed that 36.9%, 0.23% was achieved within 120 min by P200/UV and P400/UV respectively.

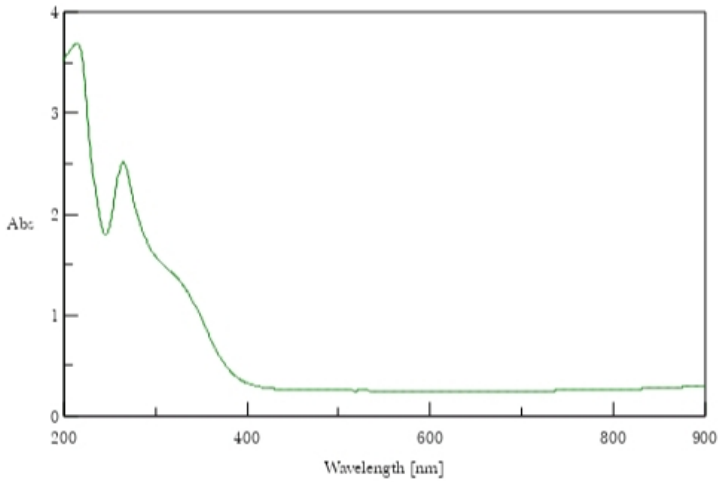

Figure. 9., UV- visible spectrophotometer adsorption behavior of compound IV

DISCUSSION:

Phthalate esters (PAEs) being their wide convention and easy production reflect as a ubiquitous contaminants and are frequently detected in environment. PAEs are certified as endocrine disrupter chemicals (EDCs), which exhibit in vitro estrogenic potential for human, especially harmful to male's reproductive system.(Lei et al. 2020) These diethyl phthalate (DEP), a ubiquitous organic pollutant, is emerging as a global threatening molecule. Many studies reported that it is absorbed by soil, water and animals and cause lethal damage.(Yu et al. 2012) This present investigation states that absorption of diethyl phthalate in plants by growing diethyl exposed environment (TPS 2) and control as grown in a free pollutant environment (TPS 1). The *Andrographis echioides* plant reported phytoconstituents flavanone, dihydroechioidinin,glucoside. echioidinin S-O- 3-D-glucopyranoside.(Jayaprakasam et al. 1999)(Jayaprakasam et al. 2001)For pyresis, antihelmintic, gastrointestional, and antidote purpose, false willow plant was used in earlier days.(Alagesaboopathi 2009; Bassey et al. 2020). The test plant (TPS 1) was grown in farm which is a pollutant free environment. The test plant sample (TPS 2) is harvested near railway lines, due to litho adhering chasmophytic nature the plant grown well in that environment. Train engines uses diesel as a fuel,spills of hydraulic fracturing fuel fluids,(Drollette et al. 2019) was absorbed by the plants, results the presence phthalate compounds in the plants under pollutant environment due to exposure. In TPS 1 it doesn't have the phthalate compound. Its reported that the phthalate compound was observed in animals, in this current study states it's also observed in plants, due to the emerging drug resistance, people are focusing to the green herbal medicine, phthalate components being a soluble, partially miscible of many organic molecules for fragrance easily absorb and adsorbed by both plants and animals.DEP causes conjunctival, dermal irritation while tested in rabbits(Api 2001).(Luxminarayan et al. 2017; Qin et al. 2020), The

chloroform extract of *Chaetomorpha media* posses 1H-Indole, 2-methyl-3-phenyl- similar to the methanolic extract of *A.echioides*.(Kumar and Ritika 2020; Luxminarayan et al. 2017), the Silane, trimethyl[5-methyl-2-(1- shows activity against anti bacterial and fungal activity, also reported in *Chaetomorpha media* algae. The ethanol extract of *A. karoo* leaves possess the same compound have broad-spectrum antimicrobial activity against clinical isolates of bacteria.(Priyanka et al. 2015).For 1,4-Bis(trimethylsilyl)benzene, the relevant compound was reported in 1,2-bis(trimethylsiloxy)ethane, were observed in barberry bark extracts (Hosseinihashemi et al. 2015),the extractives of *P. lasiocarpa* poplar wood contained Cyclotrisiloxane, hexamethyl- rich and rare drug and biomedical activities.(Peng et al. 2017)*Tropaeolum majus* flower contains Purine-2,6-dione, 8-(3-etho…(Valsalam et al. 2019)s(+)-1-Cyano-2-methyl-azetidine was as a bioactive compound from microalgae. The major phyto constituents in the methanolic extract are (Silane dimethyl- and 2-Methyl-Z, Z-3,13-Octadecadienol) responsible for that larvicidal and acaricidal activities.(Mathivanan et al. 2017)

In TPS which is grown in exposed environment possess high percent of Diethyl Phthalate (66.28 %) ,While the same plant is grown in farm doesn't have phthalate content in methanolic extract, diesel in train fuel may emit the phthalate resulting enormous presence of compound, also study shows (Drollette et al. 2019) the elevated level of phthalates in ground water. Due to the presence of toxic benzene carboxylic group, PAEs resist biological wastewater treatment.Diethyl phthalate (DEP) predicted to have substantial dermal uptake directly from air. In experimentally evaluate transdermal uptake of DEP by using human volunteers.Although DEP has been measured at high levels in skin wipes, concluded that Diethyl phthalate was absorbed by the skin. (Yu et al. 2012).Phthalate concentration from 0.01 mg L-1 to 1mg L-1 could cause chloroplasts disintegrating and disorganizing, which could damage the ultrastructures of *Arabidopsis thaliana* leaves, also cause decrease in the Vitamin C in capsicum fruit. Thus DEPs are emerging slowly as a sneak attack to global organisms, from past few decades the struggling towards drug resistance is a known fact, taking green medicine as a key alternative weapon against these drug resisters,this is also going to fade because of the phthalates incorporation in flora. *Albizia amara* possesses phytochemical constituents, Tetrasiloxane, decamethyl- having significant pharmacological activities.(Sedahmed et al. 2021) 2-Ethylacridine (Hosseinihashemi et al. 2015; Sani et al. 2015),Cyclotrisiloxane, hexamethyl(Luxminarayan et al. 2017; Priyanka et al. 2015;)these biologically active molecules and are considered to act in the plants' defense system, as part of a large group of protective molecules called "phytoanticipins" or "phytoprotectants. Benzo[h]quinoline, 2,4-dimethyl- one of the major compound reported in the *Mangifera indica* (Sani et al. 2015).Tricosene is an antimicrobial compound used for comparative studies for the formulation of toothpastes and for disinfectants.(Ellepola et al. 2011). 9-octadecanoic acid is saturated fatty acid present in *A.echiodes* (Mathivanan, Gandhi, et al. 2018) active againstlarvae of *Aedes aegypti* .(Sedahmed et al. 2021) The compound 9-octadecanoic acid, methyl ester found in *Albizia amara* possesses pharmacological activities. (Sani et al. 2015) Phthalic acid in the methanol extract of *T. bellerica*,in*P.*

tomentosa .(Peng et al. 2017; Sankar Ganesh and Ravishankar Rai 2018). Phthalic acid is a primary degradative component of diethyl phthalate compound. The plant collected for testing is TPS 2 which is a pollutant exposed. (Kingdom 2003).The *A. paniculata* methanolic extract posses phytol compound as like *A.echioides.*(Murugan, Sekar, and Al-Sohaibani 2002; Velayudham and Murugan 2012)*Tropaeolum majus* also posses the compound Purine 2-6 dione as that of test plant.(Valsalam et al. 2019)The screened gas chromatographic analysis in various solvents posses many compound, further it was shortlisted based on their percentage area availability and their effects on biome callus. Among the various phyto-constituents the extracts were purely obtained by column chromatography and to determine their activity it was subjected to Muller Hinton antibacterial assay. Based on their high activity among the eluted fraction IV was further analyzed for active compound results of GCMS, FITR, HPLC and UV spectrum. The active compound is Diethyl phthalate (DEP), the ftir results are 3151.47 cm-1, 1743.53 cm−1, 1640.78 cm−1, 1406.35 cm−1, 1195.78 cm−1, 1094.53 cm−1, 754.12cm−1, 602.71 cm−1 and 602.71 cm−1. DEP in FTIR results for phthalate with clay material shows alkane CAH stretching vibration of 2983.1 cm-1 and ester C@O stretching of 1718.4 cm-1 in DEP was red shifted to 2925.9 cm-1 and 1710.8 cm-1 in adsorbed DEP spectrum at 79.7 mmol L-1 loading, and a new peak at 2852 cm-1 appeared, while ester CAO stretching of 1272.8 cm-1 in DEP blue shifted to 1301.9 cm-1.(Valsalam et al. 2019; Wu et al. 2015).A study on*A.echiodes* shows spectra showed sharp and strong absorption band at 3290.93 to 3331.43, 1668.12 to 1565.20, 1402.96 to 1375.96 and 1038.84 to 1037.52 cm-1.(Elangovan et al. 2015)The leaf extract of test plant shows bands of absorbance at around 3697, 1409,1562,1080,1120, 871 and 453 cm-1.(Mathivanan and Suseem 2017)The HPLC for the *A.echioides* TPS extract shows the high peaks indicates the present of bioactive compound(Mantzouris, Karapanagiotis, and Panayiotou 2014), the UV spectrumreports showed that 36.9%, 0.23% was achieved within 120 min by P200/UV, and P400/UV respectively. DEPs were characterized by its maximum wavelength.(Valsalam et al. 2019)Phthalates, diesters of phthalic acid, in dehydration yields phthalic esters ,which is used as a plasticizer for manufacturing medical devices for intravenous tubing's and catheter kits, paints, wall and floor covering, food packing materials. Due to its multitasking usage randomly exposed to human, animals and also in plants. Mainly human got exposed through ingestion,phthalates metabolized to hydrolytic monoester further to hydrophilic oxidative substances .This is converted to glucuronide conjugates. In rodents it causes increased body weight, raised in hormonal levels, changes in tissue components,peroxisomal proliferation and tumors.(Calafat and Mckee 2006; Weschler et al. 2015),Also causing a major pollutant in ground water.(Drollette et al. 2019).This study indicates that plant absorbs DEP's,the test weed plant.*Andrographis echioides* reported have many antimicrobial components.(Mathivanan, Kirankumar, et al. 2018). These phthalate in plants cause disintegration of chloroplasts which affects the photosynthesis and finally plant bereavement.

CONCLUSION:

In this present work,*A. echioides* grown in different environment TPS 1 and TPS2 used for the comparative study of pollutant absorption in plants. the investigation states the pollutant exposed plants(TPS 2-methanol and chloroform solvent) contain pollutant constituents in their extract, the major components was analyzed and identified by using GCMS,FTIR,HPLC and UV spectrum . The diethyl phthalate found to be the major component in pollutant exposed plant where as the plant grown in pollutant free area (TPS 1) doesn't contain the phthalate components.Many studies revealed that phthalate are reflecting in their tissue organization; in ground water level.The phthalates are slowly penetrating and proliferating in all organisms, this becoming a great threat in upcoming future.

REFERENCES :

Agnel Ruba, A. and V. R. Mohan. 2016. "Pharmacognostical Studies and Phytochemical Investigation of Andrographis Echioides (L). Nees (Acanthaceae)." *International Journal of Pharmacognosy and Phytochemical Research* 8(6):941–48.

Alagesaboopathi, C. 2009. "Ethnomedicinal Plants and Their Utilization by Villagers in Kumaragiri Hills of Salem District of Tamilnadu, India." *African Journal of Traditional, Complementary and Alternative Medicines* 6(3):222–27.

Andersson, Anna-maria, Brian Kongshoj, Niels E. Skakkebæk, and Hans Christian Wulf. 2007. "Systemic Uptake of Diethyl Phthalate , Dibutyl Phthalate , and Butyl Paraben Following Whole-Body Topical Application and Reproductive and Thyroid Hormone Levels in Humans." 41(15):5564–70.

Api, A. M. 2001. "Toxicological pro ® Le of Diethyl Phthalate : A Vehicle for Fragrance and Cosmetic Ingredients." 39.

Bassey, Kokoette, Patience Mamabolo, Mmamosheledi Mothibe, and Freddy Muganza. 2020. "Phytochemical Profiling and Chemical Marker Compounds Identification in Helichrysum Caespititium: A Chemometrics and 2D Gas Chromatography Time of Flight Mass Spectrometry (GCxGC-TOF-MS) Perspective." *Pharmacognosy Journal* 13(2):486–94.

Calafat, Antonia M. and Richard H. Mckee. 2006. "Integrating Biomonitoring Exposure Data into the Risk Assessment Process : Phthalates [Diethyl Phthalate and Di (2-Ethylhexyl) Phthalate] as a Case Study." 114(11):1783–89.

Drollette, Brian D. et al. 2019. "Correction : Elevated Levels of Diesel Range Organic Compounds in Groundwater near Marcellus Gas Operations Are Derived from Surface activities(Proc Natl Acad Sci U

S A. (2015) 112:43 (13184-9) DOI: 10.1073/pnas.1511474112).” *Proceedings of the National Academy of Sciences of the United States of America* 116(18):9135.

Elangovan, K., S. spectra showed sharp and strong absorption band at 3290.93 to 3331.43, 1668.12 to 1565.20, 1402.96 to 1375.96 and 1038.84 to 1037.52 cm?1Anupriya, R. Shenbhagaraman, P. K. Kaleena, and K. Murugesan. 2015. “Phyto Mediated Biogenic Synthesis of Silver Nanoparticles Using Leaf Extract of Andrographis Echioides and Its Bio-Efficacy on Anticancer and Antibacterial Activities.” *Journal of Photochemistry and Photobiology B: Biology* 151:118–24. Retrieved (http://dx.doi.org/10.1016/j.jphotobiol.2015.05.015).

Ellepola, Arjuna N. B., Zia U. Khan, Rachel Chandy, and Leeba Philip. 2011. “A Comparison of the Antifungal Activity of Herbal Toothpastes against Other Brands of Toothpastes on Clinical Isolates of Candida Albicans and Candida Dubliniensis.” *Medical Principles and Practice*.

Hosseinihashemi, Seyyed Khalil, Hamidreza Anooshei, Hamed Aghajani, and Mohamed Z. M. Salem. 2015. “Chemical Composition and Antioxidant Activity of Extracts from the Inner Bark of Berberis Vulgaris Stem.” *BioResources* 10(4):7958–69.

Jayaprakasam, B., A. G. Damu, D. Gunasekar, A. Blond, and B. Bodo. 1999. “Dihydroechioidinin, a Flavanone from Andrographis Echioides.” *Phytochemistry* 52(5):935–37.

Jayaprakasam, B., D. Gunasekar, K. V. Rao, A. Blond, and B. Bodo. 2001. “Androechin, A New Chalcone Glucoside from *Andrographis Echioides*.” *Journal of Asian Natural Products Research* 3(1):43–48. Retrieved (http://www.tandfonline.com/doi/abs/10.1080/10286020108042837).

Kingdom, United. 2003. “Concise International Chemical Assessment Document 52 DIETHYL PHTHALATE.”

Kumar, Sunil and Ritika. 2020. “A Brief Review of the Biological Potential of Indole Derivatives.” *Future Journal of Pharmaceutical Sciences* 6(1).

Latif, Maria, Mehwish Faheem, Seyed Hossein Hoseinifar, and Hien Van Doan. 2020. “Ur L P Re of.” *Aquaculture* 736065. Retrieved (https://doi.org/10.1016/j.aquaculture.2020.736065).

Lei, Yu et al. 2020. “Radical Chemistry of Diethyl Phthalate Oxidation via UV/peroxymonosulfate Process: Roles of Primary and Secondary Radicals.” *Chemical Engineering Journal* 379(April 2019):122339. Retrieved (https://doi.org/10.1016/j.cej.2019.122339).

Luxminarayan, Lodha, Sharma Neha, Viswas Amit, and M. P. Khinchi. 2017. “Asian Journal of Pharmaceutical Research and Development.” *Asian Journal of Pharmaceutical Research and Development* 5(2):1–8.

Mantzouris, Dimitrios, Ioannis Karapanagiotis, and Costas Panayiotou. 2014. "Comparison of Extraction Methods for the Analysis of Indigofera Tinctoria and Carthamus Tinctorius in Textiles by High Performance Liquid Chromatography." *Microchemical Journal* 115:78–86. Retrieved (http://dx.doi.org/10.1016/j.microc.2014.02.010).

Mathivanan, D., P.Rajiv Gandhi, R.Regina Mary, and S. R. Suseem. 2018. "Larvicidal and Acaricidal Efficacy of Different Solvent Extracts of Andrographis Echioides against Blood-Sucking Parasites." *Physiological and Molecular Plant Pathology* 101:187–96. Retrieved (http://dx.doi.org/10.1016/j.pmpp.2017.03.008).

Mathivanan, D., P.Rajiv Gandhi, R.Regina Mary, and S. R. Suseem. 2017. "Larvicidal and Acaricidal Efficacy of Different Solvent Extracts of Andrographis Echioides against Blood-Sucking Parasites." *Physiological and Molecular Plant Pathology* 1–10. Retrieved (http://dx.doi.org/10.1016/j.pmpp.2017.03.008).

Mathivanan, D., V. S. Kirankumar, S. Sumathi, and S. R. Suseem. 2018. "Facile Biosynthesis of Calcium Hydroxide Nanoparticles Using Andrographis Echioides Leaf Extract and Its Photocatalytic Activity Under Different Light Source." *Journal of Cluster Science* 29(1):167–75.

Mathivanan, D. and S. R. Suseem. 2017. "Phytochemical and Pharmacological Review of Andrographis Echiodies Phytochemical and Pharmacological Review of Andrographis Echiodies." (May).

Murugan, K., K. Sekar, and S. Al-Sohaibani. 2002. "Detoxification of Cyanides in Cassava Flour by Linamarase of Bacillus Subtilis KM05 Isolated from Cassava Peel." *African Journal of Biotechnology* 11(28):7232–37. Retrieved (https://www.ajol.info/index.php/ajb/article/view/102269).

NIOSH. 2005. "NIOSH Pocket Guide to Chemical Hazards & Other Databases CD-ROM." *Centers for Disease Prevention & Control. National Institute for Occupational Safety & Health.* DHHS (NIOS(DHHS (NIOSH) Publication No. 2005-151 (2005)).

NOGUERA, D., S. OKABE, and C. PICIOREANU. 1999. "Biofilm Modeling: Present Status and Future Directions." *Water Science and Technology* 39(7):273–78. Retrieved (http://www.sciencedirect.com/science/article/pii/S027312239900178X).

Peng, Wanxi et al. 2017. "Characteristics of Antibacterial Molecular Activities in Poplar Wood Extractives." *Saudi Journal of Biological Sciences* 24(2):399–404.

Priyanka, C., P. Kumar, Shivakumar P. Bankar, and L. Karthik. 2015. "In Vitro Antibacterial Activity and Gas Chromatography–mass Spectroscopy Analysis of Acacia Karoo and Ziziphus Mauritiana Extracts ." *Journal of Taibah University for Science* 9(1):13–19. Retrieved (http://dx.doi.org/10.1016/j.jtusci.2014.06.007).

Qin, Deqiang et al. 2020. "Different Lethal Treatments Induce Changes in Piperidine (1,1′-(1,2-Ethanediyl)bis-) in the Epidermal Compounds of Red Imported Fire Ants and Affect Corpse-Removal Behavior." *Ecotoxicology and Environmental Safety* 194(November 2019):110391. Retrieved (https://doi.org/10.1016/j.ecoenv.2020.110391).

Rani, A.Anitha et al. 2016. "Journal of Chemical and Pharmaceutical Research , 2016 , 8 (3): 512-519 Research Article Biochemical and Molecular Analysis of Bacteria Isolated from Dental Caries." 8(3):512–19.

Sani, Hassan Lawal et al. 2015. "Effects of Standardized Stem Bark Extract of Mangifera Indica L. In Wistar Rats with 2,4-Dinitrophenylhydrazine-Induced Haemolytic Anaemia." *Pharmacognosy Journal* 7(2):89–96.

Sankar Ganesh, P. and V. Ravishankar Rai. 2018. "Attenuation of Quorum-Sensing-Dependent Virulence Factors and Biofilm Formation by Medicinal Plants against Antibiotic Resistant Pseudomonas Aeruginosa." *Journal of Traditional and Complementary Medicine* 8(1):170–77. Retrieved (https://doi.org/10.1016/j.jtcme.2017.05.008).

Sedahmed, Ahmed Algali et al. 2021. "Phytochemical, in Vivo, and in Silico Anticonvulsant Activity Screening of Albizia Amara Leave's Ethanolic Extract." *Hacettepe University Journal of the Faculty of Pharmacy* 41(1):9–22.

Sharma, Neha, A. M. Jana, Nivedita Pathak, Charu Singh, and Pragya Singh. 2017. "A Preliminary Study on the Antibacterial Effects of Ethanolic Extract of Psidium Guajava Leaves on Bacteria Isolated from Urinary Tract Infection with Special Reference to Escherichia Coli and Staphylococcus Aureus." *International Journal of Biotechnology and Biochemistry ISSN* 13(2):973–2691. Retrieved (http://www.ripublication.com).

Suer, H. and H. Bayram. 2017. "Liposomes as Potential Nanocarriers for Theranostic Applications in Chronic Inflammatory Lung Diseases Hande." *Biomedical and Biotechnology Research Journal* 1(1):1–8.

Surahmaida, Umarudin, Agustin Widia Rani, and Novicalia Citra Dewi1. 2021. "Skrining Fitokimia Senyawa Metabolit Sekunder Ekstrak Metanol Daun Jarak Pagar (Jatropha Curcas) Dengan GCMS Phytochemical Screening of Secondary Metabolite Compounds Methanol Extract of Jatropha Curcas Leaf with GCMS." *Journal of Pharmacy and Science* 6(1):25–30.

Valsalam, Saritha et al. 2019. "Rapid Biosynthesis and Characterization of Silver Nanoparticles from the Leaf Extract of Tropaeolum Majus L. and Its Enhanced in-Vitro Antibacterial, Antifungal, Antioxidant and Anticancer Properties." *Journal of Photochemistry and Photobiology B: Biology* 191:65–74.

Retrieved (https://doi.org/10.1016/j.jphotobiol.2018.12.010).

Velayudham, Shakthi and Kasi Murugan. 2012. "Diversity and Antibacterial Screening of Actinomycetes from Javadi Hill Forest Soil, Tamilnadu, India." *Journal of Microbiology Research*.

Weschler, Charles J. et al. 2015. "Transdermal Uptake of Diethyl Phthalate and Di (N -Butyl) Phthalate Directly from Air : Experimental Verification." (10):928–34.

Wu, Yanhua, Youbin Si, Dongmei Zhou, and Juan Gao. 2015. "Adsorption of Diethyl Phthalate Ester to Clay Minerals." *Chemosphere* 119:690–96. Retrieved (http://dx.doi.org/10.1016/j.chemosphere.2014.07.063).

Yu, Xu biao et al. 2012. "Preparation of Trimethylchlorosilane-Modified Acid Vermiculites for Removing Diethyl Phthalate from Water." *Journal of Colloid and Interface Science* 369(1):344–51. Retrieved (http://dx.doi.org/10.1016/j.jcis.2011.11.061).