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GC MS ANALYSIS OF PETROLEUM ETHER EXTRACT OF *PTEROBRYOPSIS PILIFOLIA* (DIXON) MAGIL TO IDENTIFY CHEMICALS THAT OFFER ANTIFEEDANT PROPERTIES IN BRYOPHYTES

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ABSTRACT

Phytochemicals from the bryophyte *Pterobryopsis pilifolia* was extracted using Soxhlet apparatus with petroleum ether as solvent. The extract was concentrated using rotary evaporator. TLC analysis of extract displayed several fluorescent bands under UV Light. GC MS analysis of the extract revealed the presence of several volatile compounds, many of which are not seen in higher plants. Bryophytes thus can be used as a source of novel phytochemicals of potential applications. Many of these chemicals, especially phthalates have several mutagenic and foetal toxic properties. Presence of these compounds might be the reason for the antifeedant property of *Pterobryopsis pilifolia* and that of bryophytes in general.

KEY WORDS : Bryophyte, Soxhlet extraction, *Pterobryopsis pilifolia*, Petroleum ether, GC-MS

INTRODUCTION

Plants are a rich source of phytochemicals and are extensively used in the treatment of various ailments. With the dawn of synthetic chemistry and docking studies, a large array of chemicals have been characterized as potential drugs. Even under this scenario, according to WHO, 11% of 252 drugs considered as basic and essential are derived from flowering plants (Veeresham, 2012). Not only flowering plants, the gymnosperms and vascular cryptogams are widely used in Ayurveda, Homeopathy and modern medicinal systems.

Bryophytes are the second largest group of land plants, but are least explored due to low biomass and difficulty in collection and identification of taxa. Bryophytes include around 20,000 species (Shaw *et al*., 2011). Though this diversity and complexity when compared to angiosperms are low, bryophytes possess a broad physiological and biochemical adaptations to survive in diverse habitats and to protect itself from microbial and herbivore attack (Glime, 2007). According to Botanical Survey of India, 2562 taxa of bryophytes occur in the country with 2 genera and 168 species of liverworts, 19 species of hornworts and 547 species of mosses being endemic (http://www.bsienvis.nic.in/Database/Bryophytes_22589.aspx).

Studies indicate bryophytes to be a rich source of phytochemicals. More than 2200 chemical constituents are reported from bryophytes and their number is increasing owing to the recent interest in the taxa and better phytochemical screening methods (Horn *et al*., 2021). Many of these compounds are novel and exhibit several bioactivities like antimicrobial, anticancerous, antioxidant, piscicidal, larvicidal, antiviral, cytotoxic, plant growth inhibitory, vasopressin antagonistic, muscle relaxing, neurotrophic and antiobesity activities (Asakawa, 2007). Bryophytes are not been eaten by most of the larvae, insects or higher animals. This may be due to an array of chemicals, many might being new to already identified phytochemicals. India being diverse in climatic and edaphic factors can substantially contribute to the hoard of biochemical from plant

sources, especially from bryophytes.

*Pterobryopsis*is a genus coming under Pterobryaceae family of mosses.There are 52 species of *Pterobryopsis* (http://www.theplantlist.org/). *Pterobryopsis pilifolia* is a corticolous species with primary stem being rhizomatous and blackish. Secondary stem often drooping or erect. Leaves broad and plicate, apex slightly dentate. Natural specimens seemed to be perfect with no signs of herbivore attack. This may be due to the antifeedant chemicals present in the thallus. The present study deals with extraction of dried and powdered *Pterobryopsis pilifolia* using petroleum ether solvent. The extract was subjected to TLC analysis and then GC MS analysis was performed to analyse the volatile compounds present.

MATERIALS AND METHOD

Collection and identification of Specimen

Pterobryopsis pilifolia (Dixon) Magil, was collected during March from Idukki District, Kerala. The plant was collected in sterile containers and brought to the lab and identified based on gametophytic characters.

Extraction of phytochemicals

The gametophytic plant material was washed well to remove soil particles using running tap water and then dried under shade. The dried thallus was powdered. 30 g of powdered thallus was taken in soxhlet extraction apparatus and was extracted with petroleum ether. The extract so obtained was filtered using filter paper and then concentrated by rotary evaporator.

Thin Layer Chromatography

Petroleum ether extracts wasspotted on TLC plate (silica gel coated on aluminium sheet, Merck). The plate was run using a solvent mixture consisting of hexane, petroleum ether and ethyl acetate (2:1:1) and visualized under UV light. The florescent bands were photographed.

GC MS Analysis

The extracts were centrifuged and the clear supernatant was subjected to GC MS analysis. Agilent make GC MS was used (GC 7890A with MS 5975). Peaks along with retention time were searched with available library (NIST 14.L) to identify the volatile compounds.

RESULTS AND DISCUSSION

Gametophyte plant was collected from bark of trees. Plant was green and leafy. Stem was dark brown, creeping twisted and wiry with pinnate branching. The branches were either erect on pendant. Leaves crowded ovate cordate and cucullate, costa small. Based on the morphological and anatomical characters, the plant was identified as *Pterobryopsis pilifolia* (Figure 1).

Fig. 1. *Pterobryopsis pilifolia* in natural habitat

Petroleum ether extract of *Pterobryopsis pilifolia* was dried and weighed 0.18 g. The dried extracts were stored under refrigeration and diluted for TLC and GC MS. TLC analysis showed several fluorescent bands in UV light (Figure 2). TLC method using silica gel as adsorbent is a non-quantitative method that involve visual detection of colourless substances by observing the plate under UV illumination or by spraying chromogenic or fluorogenic reagents (Marsh and Hiekane, 1991). Usually UV active chemicals possess some levels of

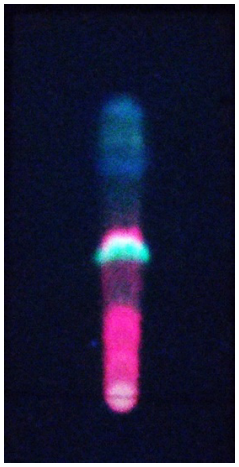

Fig. 2. TLC of the extract viewed under UV Light

conjugation, which most commonly occur in aromatic compounds. For example coumarins and flavonoids show blue white and yellow fluorescence under UV light (Kovac-Bensovic and Duric, 2003). In the present chromatogram, 14 spots were detected of which one showed white-yellow fluorescence, two spots showed blue fluorescence and the rest showed red fluorescence.

GC MS analysis revealed several volatile compounds (Figure 3). Many of these compounds were also detected in several higher plants and have potential bioactivities. 36 compounds were identified in petroleum ether extract. Percentage peak area, retention time, compound name and toxicological data is given in Table 1.

The major component in petroleum ether extract was octyl phthalate, also known as diethyl hexyl phthalate or Bis (2-ethylhexyl) phthalate. The chromatogram of the chemical and the corresponding NIST chromatogram is shown in Figure 4. The compound is a carbocyclic acid and show severe reproductive toxicity. In addition to this, a similar phthalate calleddibutyl phthalate was also detected.

Octyl phthalate is widely used in medical devices, building products, children's product and

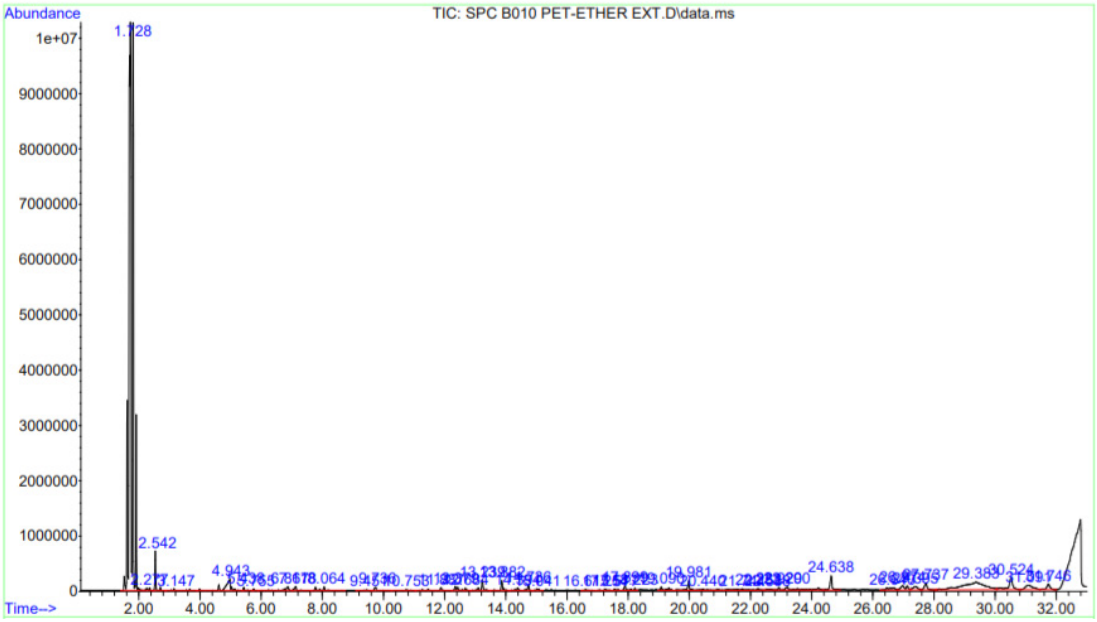

Fig. 3. Total Ion chromatogram of petroleum ether extract of *Pterobryopsis pilifolia*

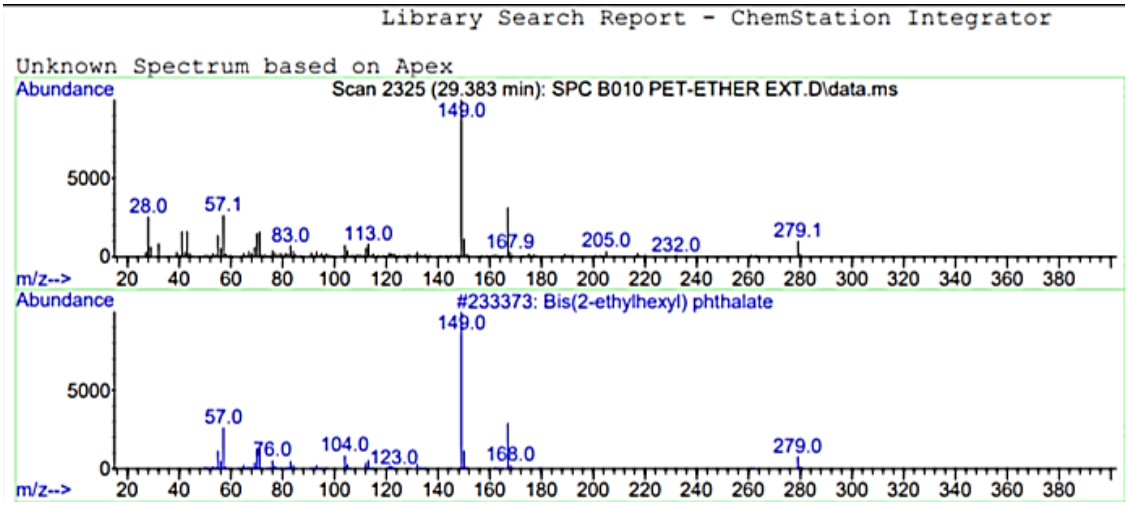

Fig. 4. Chromatogram of Octyl phthalate

Table 1. GC MS data of Petroleum ether extract of *Pterobryopsis pilifolia*

Peak No	Retention time (Min)	Peak Area (%)	Most probable compound	NCBI PUBCHEM Toxicological data	LD50/LCLo value
2	2.276	0.26	1-Pentanol	Skin and eye irritation in rabbit, Mutation in *E. coli*, sex chromosome loss and nondisjunction in hamster, liver and lung cancer, renal failure, lethality	LD50 - 615 mg/kg intraperitoneal dosage for guinea pigs, 200 mg/kg oral dosage for mouse, 2830 µl ml/kg skin dosage for rabbits
3	2.54	1.32	Hexanal	Continuous exposure can lead to discomfort in eyes and nose, headaches, interfere with cholesterol transport, lethality	4890 mg/kg oral dosage for rats, 3700mg/kg oral dosage for mammal, 5 ml/kg skin dosage for guinea pigs
5	4.942	2.15	Hexanoic acid	Skin and eye irritation, mutant, acute toxicity and lethality.	3g/kg oral dosage for rats, 630 µl/kg skin dosage for rabbit, 5 ml/kg skin dosage for guinea pigs
6	5.432	0.19	2,4-Heptadienal, (E,E)-	Acute toxicity oral or dermal, skin and eye irritation	1150g/kg oral dosage for rats, 313 mg/kg skin dosage for rabbit, >5 mg/kg skin dosage for guinea pigs
8	6.866	0.56	2-Octenal, (E)-	Serious eye irritation and eye damage, respiratory tract irritation	Not available
9	7.118	0.43	Heptanoic acid	Lysosomal damage, reduced weight gain in new born rat,	7 g/kg oral dosage for rats, 2000 mg/kg skin dosage for rabbit
10	8.061	0.54	Nonanal	Depressed activity, affects urinary system	>5g/kg oral dosage for rats
11	9.445	0.11	Cyclopropanecarboxamide, N-benzo[b] benzofuran-3-yl-2,2-dimethyl-3-(2-methyl-1-propenyl)-	Information not available	Information not available
12	9.735	0.29	Octanoic acid	Levels are associated in Autism, Crohn's disease, colorectal cancer, ulcerative colitis	10080 mg/kg oral dosage for rats, >5 g/kg skin dosage for rabbit
13	10.753	0.15	2-Octenoic acid	Cause skin burns and eye damage	Not available
14	11.886	0.42	1-Nonene	Irritation to nose, eyes and throat. Can cause dizziness, anaesthetic	Not available
15	12.376	0.45	2-Decenal, E	Information not available	Information not available
16	12.678	0.14	Nonadecane	Inhibitory effect of germination and seedling growth	Information not available
17	13.244	0.76	2,4- Decadienal, (E,E)	Causes skin irritation and dermal toxicity, eye irritation	Information not available
19	14.389	0.24	1H-1,2,4-Triazol-5-amine, 1-propyl	Information not available	Information not available

Table 1. *Continued ...4*

Peak No	Retention time (Min)	Peak Area (%)	Most probable compound	NCBI PUBCHEM Toxicological data	LD50/LCLo value
20	14.741	0.35	2-Pentanone, 3,3, 4,4-tetramethyl	Information not available	Information not available
21	15.043	0.17	Bimethallyl	Eye, skin and respiratory irritant	Information not available
25	17.898	0.41	9-Oxononanoic acid	Information not available	Information not available
26	18.225	0.11	Heneicosane	Skin, eye and respiratory irritation. Not teratogenic or embryo toxic in rats	LD 50- 5g/kg skin dosage for mice.
27	19.093	0.58	Calamenene	Information not available	Information not available
28	19.986	0.74	Dodecanoic acid	Skin and eye irritation for rats, 121 mg/kg intravenous dosage for mouse	LD 50- 12 g/kg oral dosage
29	20.439	0.27	Benzofuran, octahydro-6-methyl-3-methylene-	Information not available	Information not available
30	21.735	0.64	Methyl 10-oxo-8-decenoate	Information not available	Information not available
31	22.251	0.16	Limonene oxide	Skin and eye irritation, eye damage	LD 50- 2700µL/kg oral dosage for mouse, 100 mg/kg intramuscular dosage for mouse
32	22.553	0.21	Oxalic acid, cyclobutylpentadecyl ester	Information not available	Information not available
33	22.93	0.18	Muurol-5-en-4-one	Information not available	Information not available
34	23.194	1.08	Heptadecane	May become fatal if swallowed or enters airways	LDLo intravenous in mouse is 9821 mg/Kg
35	24.641	1.22	Tetradecanoic acid	skin irritation and eye damage, environmental hazard	LD50 >10 g/kg oral dosage for rats and 43 mg/kg intravenous dosage for mouse
36	26.641	0.61	1-Cyclododecanone, 2-ethylidene	Information not available	Information not available
37	26.968	0.93	9,12-Octadecadienoic acid, methyl ester	Information not available	Information not available
38	27.396	0.66	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	Information not available	Information not available
39	27.735	0.73	Pentadecanoic acid	Skin, respiratory and eye irritation	LD50 54 mg/kg intravenous dosage for mouse
40	29.383	6.6	Octyl phthalate	Reproductive toxicity, may damage fertility. Can cause skin and eye irritation, mutation and DNA damage in mammals, mutation in microbes and yeast, changes in sperm morphology, pre-	LD50 10 g/kg skin dosage for guniea pigs, 25g/kg skin dosage for rabbits, 30 g/kg oral dosage for rats,29.5 g/kg oral dosage for mouse

Table 1. *Continued ...*

Peak No	Retention time (Min)	Peak Area (%)	Most probable compound	NCBI PUBCHEM Toxicological data	LD50/LCLo value
				implantation mortality, feto- toxicity	
41	30.528	1.99	methyl palmitate	Inhibition of larval development and wing growth of *Gryllodessigillatus*. Inhibitor of recticuloendothelial system in mice	Information not available
42	31.094	1.3	Palmitoleic acid	Skin and eye irritation	Information not available
43	31.748	0.73	Dibutyl phthalate	Irritation to eyes and skin. Damage to unborn child, can affect fertility, very toxic to aquatic life	7499 mg/kg oral dosage for rats, 10 g/kg oral dosage for guniea pig, > 20 ml/kg skin dosage for rabbit

food packaging (Martinez-Razo *et al*., 2021). It is a naturally occurring chemical common in essential oil of several angiosperm plants. It can vary from 1.5 % as in aerial parts of *Asystasiagangetica* to over 41 % in the root exudates of *Liliumbrownii* (Huang *et al*., 2021). Li *et al*. (2012), reported 17.3 % of octyl phthalate in *Cleorodendruminerme*. In essential oil of *Cirsiumjaponicum*, octyl phthalate was found to be 30.8 % (Luoand Yang, 2009). In roots of *Paris polyphylla*, the concentration of octyl phthalate was found to be 4.2 % (Liu *et al*., 2014). In essential oil of *Pyrusussriensis*, the concentration detected was 29.4 % (Xin *et al*., 2004) and in *Ziziphus mauritiana* it was 18 % (Deng *et al*., 2004). Not only higher plants, bacteria like *Brevibacterium mcbrellneri* (Rajamanikyam *et al*., 2017), *Streptomyces bangladeshensis* (Al-Bari *et al*., 2005) and fungi like *Penicilliumolsonii* (Amade *et al*., 1994) were also capable of synthesizing and secreting octyl phthalate. Dibutyl phthalate is seen in bacteria, green algae and higher plants. It can be as high as 32 % in stalks of *Brassica oleracea*, 47.2 % in root exudates of *Beta vulgaris*, 87.2 % in whole plant of *Radix pseudostellariae* (Huang *et al*., 2021).

Octyl phthalate and dibutyl phthalate were detected in the root exudates of *Ageratinaadenophora* (Zhu *et al*., 2014). Studies with phthalates indicate that they have strong allelochemical properties and hence is considered as a valuable environment friendly herbicides (Shao *et al*., 2012). Octyl phthalate and dibutylpthalate showed a broad spectrum of antibacterial activity. The compounds isolated from *Brevibacterium mcbrellneri* could inhibit gram positive bacteria like *Staphylococcus epidermidis* and *Staphylococcus aureus* and gram positive bacteria like *E.coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Rajamanikyam *et al*., 2017). Octyl phthalate isolated from flowers of *Calotropis giganteum* showed inhibitory activity against *Bacillus subtilis, Sarcinalutea, E. coli, Shigellasonnei, Shigellashiga and Aspergillus flavus* (Habib and Karim 2009). Octyl phthalate produced by bacteria like *Tsukamurellainchonensis, Cellulosimicrobium cellulans* and *Corynebacterium nitrilophilus* could inhibit fungal spore germination and affected the production of total proteins and lipids (El-Mehalawy *et al*., 2008). Adsul *et al*. (2012), isolated dibutyl phthalate from leaf of *Ipomeacarnea* and was found to show mosquito larvicidal activity against fourth instar larvae of *Aedesaegypti* and *Culexquinquefasciatus* with LC50 values of 81.43 ppm and 109.64 ppm.

Studies were conducted by Ma *et al.* (2015), on phytotoxicity of phthalates like di-n-buty phthalate (DnBP) and Bis(2-ethylhexyl)phthalate (DEHP)on test plants like *Triticumaestivum, Medicagosativa, Loliumperenne, Raphanussativus, Cucumissativus, Avenasativa* and *Allium cepa*. Root elongation, seedling growth and biomass production was significantly inhibited by DnBP but not DEHP. However plant pigment content (Chlorophyll a and b) was significantly affected by both chemicals. According to Chen *et al*. (2014), DEPH is highly toxic with an LC50 value of 0.5 ppm and can cause toxicity symptoms like tail curvature, necrosis, non-touch response, cardiac edema and embryo mortality in zebrafish. Direct exposure of DEHP to foetal testis or indirect maternal exposure can reduce steroidogenesis in mice as well as humans (Kariyazono *et al*., 2015).

Pthalates show insecticidal properties. Synthetic diethylphthalates are widely used as an ingredient of insect repellents (Brown and Hebert, 1997). In

studies conducted by Rajamanikyam *et al*. (2017), octyl phthalate isolated from *Brevibacterium mcbrellneri* had inhibitory effect on acetylcholinesterases and was found to control *Aedesaegypti* by acting against the fourth instar larvae. Ocytl phthalate was found to cause reproductive and developmental toxicity and is thought to be due to endocrine disruption (Latini *et al*., 2004). Though considered as a pollutant, octyl phthalates are easily degraded by microbes like *Sphingomonas, Pseudomonas, Rhodococcus, Arthrobacter, Ochrobactrum, Serratia, Agromyces, Microbacterium, Acinetobacter, Aspergillus parasiticus, Penicillium funiculosum* (Ortiz and Sansinenea, 2018).

GC MS analysis of *Pterobryopsis pilifolia* petroleum ether extract revealed several fatty acids and fatty acid esters like 2-octenoic acid, dodecanoic acid (lauric acid), tetradecanoic acid (myristic acid), pentadecanoic acid, palmitoleic acid and hexadecanoic acid methyl ester (palmitic acid methyl ester). Studies conducted by Mcfarlane and Henneberry (1965), revealed the inhibitory effect of fatty acids and their methyl esters in the growth of *Gryllodessigillatus*. When absorbed through external body wall, lauric acid, myristic acid, stearic acid and behenic acid and the methyl ester of palmitic acid, myristic acid, stearic acid and oleic acid inhibited growth. Through oral route entry, inhibition of growth was seen only with lauric acid at 1% level. Mixture of straight chain saturated fatty acids like octanoic acid, nonanoic acid and decanoic acids (C8, C9, C10) is a well-known repellent against biting and non-biting flies and ticks (Mullens *et al*., 2009). Siegler (1925), have reported insecticidal properties of fatty acids. Spraying of caprylic acid killed morethan 90 % of black chrysanthemum aphids. Lauric acid and myristic acid was effective against green apple aphid. Application of fatty acids resulted in immediate and complete paralysis of aphids. The result so obtained was comparable to nicotine sulphate, however, dead aphids tend to fall off from leaves with nicotine but aphids remained attached by their beaks even after death on spraying with fatty acids. Yawjen and Biechen (2018) reported reduction in aphid population in cucumber with the spray of hexanoic acid. Soaking seeds in hexanoic acid solution and spraying with 0.04 % and 0.08 % hexanoic acid could significantly reduce cotton aphid (*Aphis gossypii*) population in cucumber.

Petroleum ether extract of *Pterobryopsis pilifolia* indicated the presence of terpenoids. Polycyclic sesquiterpenecalamenene was found to be 0.58 % and monoterpene limonene oxide was 0.16%. Terpenes like limonene can be used against adult head lice and to destroy eggs. They act as fumigant and competitively inhibit acetylcholine esterases (Dambolena *et al.,* 2016).

In the present investigation, several phytochemicals toxic to insects, and higher animals were detected. Bioactivities of many of them are not yet studied. Presence of these chemicals especially phthalates at high concentration can be the reason for reluctance of animals in feeding bryophytes, specifically *Pterobryopsis pilifolia.*

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REFERENCES

Adsul, V.B., Khatiwora, E., Torane, R.C. and Deshpande, N.R. 2012. Isolation and characterization of dibutyl phthalate from leaves of *Ipomoea carnea*. *Chem. Nat. Compd*. 48 : 712-713.

Al-Bari, M.A.A., Bhuiyan, M.S.A., Flores, M.E., Petrosyan, P., Garcia-Varela, M. and Ul-Islam, M.A. 2005. *Streptomyces bangladeshensis* spnov., isolated from soil, which produces bis-(2-ethylhexyl) phthalate. *Int. J. Syst. Evol. Microbiol*. 55 : 1973-1977.

Amade, P., Mallea, M. and Bouaicha, N. 1994. Isolation, structural identification and biological activity of two metabolites produced by *Penicilliumolsonii* Bainier and Sartory. *J. Antibiot*. 47 : 201-207.

Asakawa, Y. 2007. Biologically active compounds from bryophytes. *Pure Appl Chem*. 79 (4) : 557-580.

Brown, M. and Hebert, A.A. 1997. Insect repellents: An overview. *J. Am. Acad. Dermatol*. 36 : 243-249.

Chen, X., Xu, S., Tan, T., Lee, S.T., Cheng, S.H., Lee, F.W.F., Xu, S.J.L. and Ho, K.C. 2014. Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. *Int. J. Environ. Res. Public Health*. 11(3): 3156-3168.

Dambolena, J.S., Zunino, M.P., Herrera, J.M., Pizzolitto, R.P.,Areco, V.A. and Zygadlo, J.A. 2016. Terpenes: natural products for controlling insects of importance to human health- a structure-activity relationship study. *Psyche: A Journal of Entomology*. 2016 : 1-17.

Deng, G., Li, X., Lin, Y., Han, X. and Zhang, X. 2004. Study of the volatile components of the fruit of *Ziziphusmauritiana* Lam. by GC-MS. *Fine Chem*.

21: 318-320.

El-Mehalawy, A., Gebreel, H., Rifaat, H., El-Kholy, I. and Humid, A. 2008. Effect of antifungal compounds produced by certain bacteria on physiological activities of human and plant pathogenic fungi. *J. Appl. Sci. Res*. 4 : 425-432.

Glime, J.M. 2007. Economic and Ethnic Uses of Bryophytes. *Flora of North America*. 27 (1919) : 14-41.

Habib, M.R. and Karim, M.R. 2009. Antimicrobial and cytotoxic activity of Di-(2-ethylhexyl)phthalate and anhydrosophoradiol-3-acetate isolated from *Calotropisgigantea* (Linn.) Flower. *Mycobiology*. 37: 31-36.

Horn, A., Pascal, A., Loncarevic, I., Marques, R.V., Lu, Y., Miguel, S., Bourgaud, F., Thorsteinsdottir, M., Cronberg, N., Becker, J.D., Reski, R. and Simonsen, H.T. 2021. Natural products from bryophytes: from basic biology to biotechnological applications. *Critical Reviews in Plant Sciences*. 40(3) : 191-217.

Huang, L., Zhu, X., Zhou, S., Cheng, Z., Shi, K., Zhang, C. and Shao, H. 2021. Phthalic acid esters: natural sources and biological activities. *Toxins*. 13(495) : 1-17.

Kariyazono, Y., Taura, J., Hattori, Y., Ishii, Y., Narimatsu, S., Fujimura, M., Takeda T. and Yamada, H. 2015. Effect of in utero exposure to endocrine disruptors on fetalsteroidogenesis governed by the pituitary-gonad axis: A study in rats using different ways of administration. *Journal of Toxicological Sciences*. 40(6) : 909-916.

Kovac-Besovic, E.E. and Duric, K. 2003. Thin layer chromatography-application in qualitative analysis on presence of coumarins and flavonoids in plant material. *Bosnian Journal of Basic Medical Sciences*. III (3) : 19-26.

Latini, G., Verrotti, A. and De Felice, C. 2004. Di-2-ethylhexyl phthalate and endocrine disruption: a review. *Curr Drug Targets Immune Endocr Metabol Disord*. 4(1) : 37-40.

Li, D., Wang, Z., Liang, Z., Yang, X. andXu, J. 2012. Comparative evaluation of the chemical composition of essential oil from twig, leaf and root of *Clerodendruminerme* (L.) Gaertn. *Adv. Mater. Res*. 343 : 22-27.

Liu, Z., Liu, Z. and Tian, Q. 2014. Analysis of chemical components of volatile oil from *Paris polyphylla* and their antibacterial activities. *J. Chin. Med. Mater*. 37: 612-616.

Luo, X. and Yang, Z.R. 2009. Studies on chemical components and antibacterial activity of essential oil from *Cirsiumjaponicum* DC. *J. Sichuan Univ. Nat. Sci. Ed*. 46 : 1531-1536.

Ma, T., Teng, Y., Christie, P. and Luo, Y. 2015. Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. *Front. Environ. Sci. Eng*. 9 : 259-268.

Marsh, C.M. and Hiekane, C.J. 1991. Quantitative analysis of bitumen by conventional high performance thin-layer chromatography. *J. Planar Chromatogr. Modern TLC*. 4 : 293-98.

Martinez-Razo, L.D., Martinez-Ibarra, A., Vazquez-Martinez, E.R. andCerbon, M. 2021. The impact of Di-(2-ethylhexyl) phthalate and Mono(2-ethylhexyl) phthalate in placental development, function, and pathophysiology. *Environ. Int*. 146 : 106228.

Mcfarlane, J.E., and Henneberry, G.O. 1965. Inhibition of the growth of an insect by fatty acids. *Journal of Insect Physiology*. 11(9) : 1247-1252.

Mullens, B.A., Reifenrath, W.G. and Butler, S.M. 2009. Laboratory trials of fatty acids as repellents or antifeedants against houseflies, horn flies and stable flies (Diptera: Muscidae). *Pest Manag Sci*. 65: 1360-1366.

Ortiz, A. and Sansinenea, E. 2018. Di-2-ethylhexylphthalate may be a natural product, rather than a pollutant. *Journal of Chemistry*. 2018: 1-7.

Rajamanikyam, M., Vadlapudi, V., Parvathaneni, S.P., Koude, D., Sripadi, P., Misra, S., Amanchy, R. and Upadhyayula, S.M. 2017. Isolation and characterization of phthalates from *Brevibacterium mcbrellneri* that cause cytotoxicity and cell cycle arrest. *EXCLI J*. 16 : 375-387.

Shao, H., Huang, X., Wei, X. and Zhang, C. 2012. Phytotoxic effects and a phytotoxin from the invasive plant *Xanthium italicum* Moretti. *Molecules*. 17: 4037-4046.

Shaw, A.J., Szovenyi, P. and Shaw, B. 2011. Bryophyte diversity and evolution: windows into the early evolution of land plants. *Am. J. Bot*. 98 : 352-369.

Siegler, E.H. 1925. Some insecticidal properties of the fatty acid series. *Journal of Agricultural Research*. XXIX (5): 259-261.

Veeresham, C. 2012. Natural products derived from plants as a source of drugs. *J Adv Pharm Technol Res*. 3(4) : 200-201.

Xin, Y., Liu, C., Hou, D. and Li, T. 2004. Analysis of the chemical constituents of essential oil of flesh from Nanguoli Pear. *Food Sci*. 25 : 223-225.

Yawjen, D. and Beichen, H. 2018. Hexanoic acid alleviated cotton aphid (*Aphis gossypii* Glover) infestations in cucumber. *Journal of Taiwan Agricultural Research*. 67(2) : 224-228.

Zhu, X.Z., Guo, J., Shao, H. and Yang, G.Q. 2014. Effects of allelochemicals from *Ageratinaadenophora* (Spreng.) on its own autotoxicity. *Allelopath. J*. 34: 253-264.