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ANTIMICROBIAL POTENTIAL AND GC-MS ANALYSIS OF BRYOPHYTE *TARGIONIA HYPOPHYLA L.*

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ABSTRACT:

Targionia hypophylla L., a terricolous liverwort occurs in close vicinity of water identified with carnation of violet blue or black ventral sporophytes often seen during rainy seasons. Thalli light to dark green, in close clusters, overlapping, usually with apical end projecting outward and downward. Thallus thin, somewhat brittle, abovate to linear - oblong, simple or once, rarely dichotomously branched, 10-15 mm. long and 3-6 mm. wide. Present exploration carried out to trace the antimicrobial potential of the bryophytic division of plants having pioneer status during conquest of land. At first instance the preliminary phytochemical characteristics were analysed. The plant extract in different solvents like ethanol, methanol, chloroform, acetone and petroleum ether and water were tested against different gram negative and gram positive bacterial and fungal strains for antimicrobial sensitivity test. Further, using sophisticated techniques like GC-MS chemical analysis of plant carried out to trace the potential chemical content with respect to future elucidation of compounds for pharmacology.

Key words :- *Liverwort, Antimicrobial test, GC MS-Analysis*

INTRODUCTION:

Bryophytes are the most fascinating cryptogramic plants occurs in nature with close vicinity of water bodies which dwells first on the land. Bryophytes are more common in humid areas and during rainy seasons, but usually show a preference for microclimatic niches such as crevices of rocks and trees and the vicinity of small shady springs (Shaw and Renzaglia, 2004). Bryophytes make a significant contribution to the floral diversity of this "watery planet" and since its inception constitute an important component of the forest ecosystem being the first colonizers on variety of habitats. They are highly specific group of plants with about 25,000 species distributed the world over, making it the second largest group of land plants after angiosperms (Alam *et al.*, 2011). The occurrence of antimicrobial substances in thalli of bryophytes has been reported by Madsen and Pates (1952) for the first time in bryophyte *Sphagnum portoricense* and *Sphagnum strictum* against pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Wolter (1964) screened antifungal activity of 18 species of bryophytes belonging to *Pellia epiphylla* and *Diplophyllum albicans* and 16 other mosses. Banerjee and Sen (1979) by examining 52 species (40 genera) of the bryophyte where 29 species showed activity against at least one bacterium. The liverworts *Asterella sanguinea, Marchantia paleacea* and the moss *Brachythecium procumbens* showed the broadest spectrum of antibacterial activity. Latiff *et al.,* (1989) screened 14 moss species belonging to 10 families of Malaysia for antibacterial activity. The ethanolic extract of the mosses were tested against *E.coli, S. aureus and Bacillus subtilis. S. aureus* was the most susceptible bacterium which was inhibited by all the moss extract. Grammes *et al.*, (1994) carried out *in-vitro* culture of *Fossombronia pusilla* and isolated or analyzed terpenoids, as same produced in natural condition of plants. Mewari and Kumar (2008) used crude methanol and flavonoid extracts of *Marchantia polymorpha* L. against three bacterial strain viz., *E. coli, P. mirabilis, S. aureus* and four fungal strains viz., *A. flavus, A. niger, C. albicans and T. mentagrophytes* for antimicrobial screening. Sawant and Karagade, (2010) investigated *in vitro* antimicrobial activity extracts of three liverworts, *T. hypophylla, P. intermedium,* and *A. wallichiana* in various solvents have a broad spectrum of activity. Krishnan *et al.*, (2012) reported *in-vitro* microbicidal potentiality of *Targionia hypophylla* L. and *Bryum* species of bryophytes. Asakawa (2001) reported the chemical constituents from the bryophytes like acetogenins, monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids and bis (bibenzyl)s from Japanease, Taiwanese, New Zealand, Argentinean and European regions. Ücüncü *et al.,* (2010) analyzed the chemical constituents of mosses like *Torula, Homalothecium, Hypnum* and *Pohila* species using GC-MS techniques and recorded presence of many essential oil with antimicrobial activity. Russell (2010) reported that, the extracts of some bryophytes in South Western British Columbia, possess novel chemical compounds with antimicrobial activity.

MATERIAL & METHODS:

The thalli of *Targionia hypophyla* were collected from Melghat forest of Amravati District and cleaned carefully and washed under tap water followed by shade drying to use in powder form. Using Soxhlet apparatus, the powdered samples of plants were extracted in ethanol, methanol, petroleum ether, chloroform and acetone and different solvent and evaporated by rotary evaporator in controlled condition of temperature to get dried extracts were stored in labelled sterile wide mouthed screw capped bottles at 4^0C and used for further study (Parekh and Chanda, 2008). The standard pathogenic bacterial and fungal strain cultures were procured from Microbial Type Culture Collection and Gene Bank (IMTECH), Chandigarh, India. Disc diffusion method was used for the antibacterial sensitivity test by following the standard methods (NCCLS, 1990). Phytochemical analysis was done for probable phytoconstituents like alkaloids, flavonoids, tannins, phenolics, steroids, saponins and terpenoids regarding the nature of constituents present in crude drug (Sadashivam and Manickam, 2005). Methanol extract of the plant send for GC-MS analysis to Shivaji University, Kolhapur and results were obtained. GC-MS analysis of the sample was carried out using Shimadzu Make QP-2010 with non-polar 60 M RTX 5MS Column.

RESULT & DISCUSSION:

Antimicrobial sensitivity test of *Targionia hypophylla*

Plant extracts of liverwort *T. hypophylla* were obtained in different solvents were tested against test microorganism showed significant activity of antibiosis (Table-1). The aqueous extract of the plants were inhibitory against *E. coli, S. aureus* and fungus *A. niger*. However, the *petroleum ether* extracts showed no any response to the all microorganisms and reactions were nullified. The ethanol extracts were more effective with broad spectrum of antibiosis against most of the microorganisms except the *S. flexneri* and *R. oryzae*. The chloroform extracts of the plant showed positive result against six microorganisms i.e. *E. coli, K. pneumoniae, S. flexneri, S. typhimurium, A. niger* and *C. albicans*. However, the methanol extracts of the plant was found positive against five selected microorganisms like *E. coli, K. pneumoniae, S. flexneri, S. typhimurium, and C. albicans*. The acetone extracts showed very promising response of action in all the extracts except *K. pneumoniae* and *R. oryzae*. Among all the extracts, the petroleum ether extract was found non-reactive against the entire microorganism. However, slight induction was observed in some petri plates rarely but non-recordable. The aqueous extract is less reactive than the other extracts but ethanol, chloroform, methanol and acetone extracts were more responsive to most of the pathogens. The maximum responses to all the extracts were found in microorganism *E. coli, S. typhimurium, A. niger* and *C. albicans* and less in *K. pneumoniae, S. flexneri,* and *S. aureus* or very less in *P. vulgaris*. Most of the extracts bear light green, yellow green, dark green and blackish green colour in crude form (Fig-1).

Phytochemical GC-MS analysis of *T. hypophyla*

Most of the bryophytes are being used as medicinal plants in China, Europe, North America and rest of the world. Bryophytes have been applied as decoctions to cure various kinds of diseases. The present study emphasizes to elicit out probable phyto-constituents from the thalli of *Targionia hypophylla* experimental bryophytes which subjected for the phytochemical and GC-MS analysis. Preliminary phytochemical test showed the presence of alkaloids, flavonoids, sterols, tannins and terpenoids. Methanol extract was selected due to its antimicrobial results which were obtained previously. The liverwort *Targionia hypophylla* in GC MS analysis analysed on the basis of retention time, percent area of the peak and compound determination with molecular formula and molecular weight (Fig-2). On the basis of data received and GC-MS chromatogram, it showed the presence of different and diversified compounds like, Longifolene, Bicyclo [5.3.0] decane, 2-methylene-5-(1-methylvinyl)-8-methyl, Patchouli alcohol, n-Hexadecanoic acid and 9-Octadecenoic acid (Table -2).

Krishnan *et al.,* (2012) confirmed that, the bryophytes having alkaloids are pharmacologically active as they have physiological effects on human as well as other animals and serves as therapeutic and anti-malarial drugs. Petroleum ether extract of liverwort *Targionia hypophylla* were active against both gram positive and gram negative bacteria and fungi. *Targionia* were particularly active against bacteria *E. coli* and fungus *C. albicans* and *A. niger*. However, these species were less reactive to the bacteria *S. flexneri* and almost non-reactive to the fungus *R. oryzae*. These findings are in contrast with the findings of Banerjee and Sen (1979) that these liverworts are active against bacteria *P. aeruginosa*.

This may be due to the variations in chemical composition of particular species of plants, which can also vary according to the geographical origin and harvesting seasons (Burt, 2004). It also showed that specific antibacterial compounds,

effective against the selected bacterial or fungal species, tends to be isolated more effectively from liverworts using ethanol and methanol.

The bryophyte *Targionia* possess compound Bicyclo (5.3.0) decane, 2-methylene-5-(1-methylvinyl)-8-methyl. Chen *et al.,* (2013) found this compound as potent composition with potential of anticancer activities among essential oil obtained from *Myrrh* and *Frankincense.* 9-octadecenoic acid and 9, 12 octadecadienoic acid (z-2) methyl esters are commonly called Linoleic acid which is a polyunsaturated fatty acid commonly produced in plants. The bryophytes species *Targionia* showed occurrence of these compounds. Park *et al.,* (2013) reported antimicrobial activity of gamma linolenic acid from *Enteromorpha linza* against several bacteria like *Streptococcus mutans* and fungi *Candida albicans.* The liverwort *Targionia hypophylla* showed the presence of chemical constituent Longifolene i.e. a tricyclic sesquiterpenoids. It commonly occurs in plants like pines, angiosperms, small amount in fungi and bryophytes. Ücüncü *et al.*, (2010) reported antimicrobial activity of longifolene among various mosses like *Torula, Hyphum* and *Pohila* against bacteria *E. coli, P. aeruginosa, S. aureus* and fungi *Candida albicans*. This finding corresponds to the present investigations of antimicrobial activity of *Targionia* species. Hata *et al.* (1993) discovered an *"Acne-Vulgaris"* treatment comprising of longifolene, caryophyllene, centrene and thujopsene as a key ingredients and got U. S. patent no. US-S200429A for such a novel medicine. The compound Bicyclo (5.3.0) decane, 2-methylene-5-(1-methylvinyl)-8-methyl. Chen *et al.,* (2013) found this compound as potent composition with potential of anticancer activities among essential oil obtained from *Myrrh* and *Frankincense.*

Patchouli alcohol or patchouli is a terpene compound with typical patchouli scent and used in chemotherapy drugs. Yang *et al.,* (2013) evaluated the antimicrobial activity of patchouli alcohol and pogostone against various bacteria like *E. coli, P. aeruginosa, S .aureus* and *S. dysentyeriae*. The liverworts *Targionia*, *Asterella* and *Plagiochasma* spp. in present study found reactive to these bacterial pathogens with similar promising results.

9-octadecenoic acid and 9, 12 octadecadienoic acid (z-2) methyl esters are commonly called Linoleic acid which is a polyunsaturated fatty acid commonly produced in plants. The bryophytes *Targionia,* showed occurrence of these compounds. Park *et al.,* (2013) reported antimicrobial activity of gamma linolenic acid from *Enteromorpha linza* against several bacteria like *Streptococcus mutans* and fungi *Candida albicans.*

CONCLUSION:

Hence, in present investigation, many chemical compounds distributed variably and dominantly present among *Targionia* species showing co-relation between chemical compound analyzed and their probable antimicrobial sensitivity against microorganisms. Hence, all the solvent extracts possesses some novel chemical compounds with highly antimicrobial potential is confirmed in the present investigation. Future studies on character elucidation of compounds will be remarkable insight for drug designing and benefits of mankind.

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Table- 1 : Antimicrobial sensitivity test of *Targionia hypophylla*

Plant Herbal Preparation	Solvent Extract	Zone of Inhibition [mm]										AVERAGE
		EC	PV	KP	SF	SA	PA	ST	AN	CA	RO	
Targionia hypophyla	Aqueous	3	0	0	0	4	0	0	4	0	0	
	Petroleum Ether	0	0	0	0	0	0	0	0	0	0	
	Ethanol	07	04	06	0	08	09	09	07	09	0	
	Chloroform	05	0	08	03	0	0	06	07	06	0	
	Methanol	06	0	05	06	0	0	07	0	08	0	
	Acetone	05	09	0	04	06	07	03	08	03	0	
	Ampicillin	22	20	27	29	34	31	30	-	-	-	
	Nystatin	-	-	-	-	-	-	-	31	29	28	

***** Data represented in mean of three replicates.

***EC** = *Escherichia coli* [MTCC-729], **PV=** *Proteus vulgaris* [MTCC-744], **KP** = *Klebsiella pneumoniae* [MTCC-661], **SF** = *Shigella flexneri* [MTCC-1457], **SA=** *Staphylococcus aureus* [MTCC-96] **, PA=** *Pseudomonas aeruginosa* [MTCC-424], **ST** = *Salmonella typhimurium* [MTCC-98]**, AN** = *Aspergillus niger* [MTCC-281], **CA=** *Candida albicans* [MTCC-227], **RO=** *Rhizopus oryzae* [MTCC-554]

Fig-2: Analysis of antimicrobial sensitivity of *Targionia hypophylla*

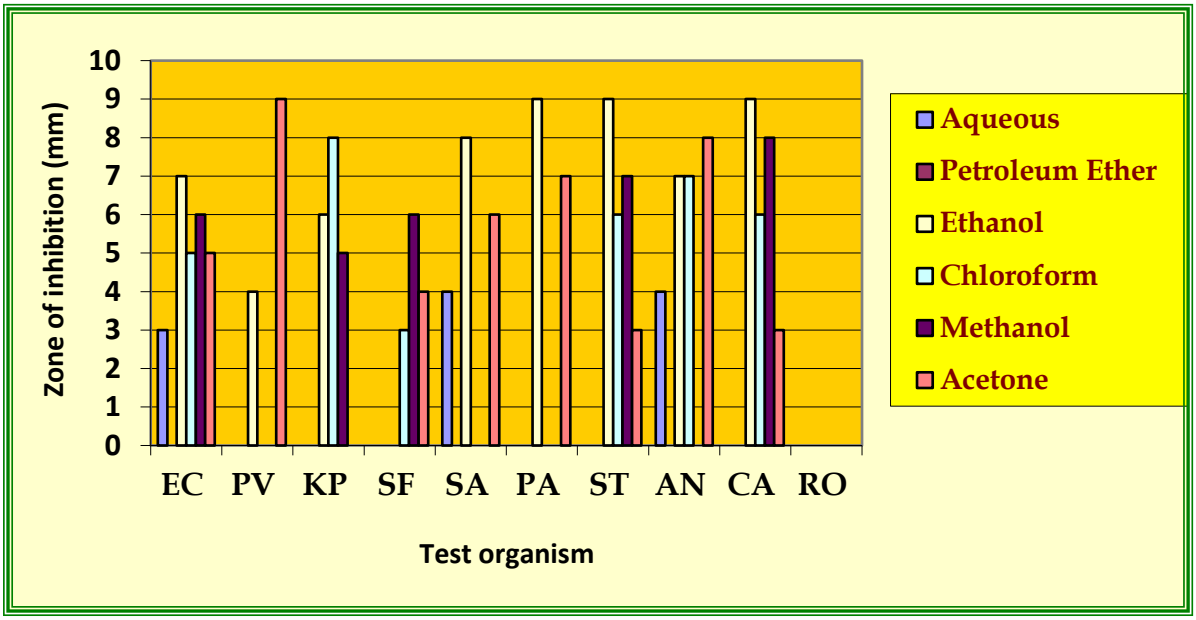

Fig 2: GC-MS chromatogram of *Targionia hypophylla*

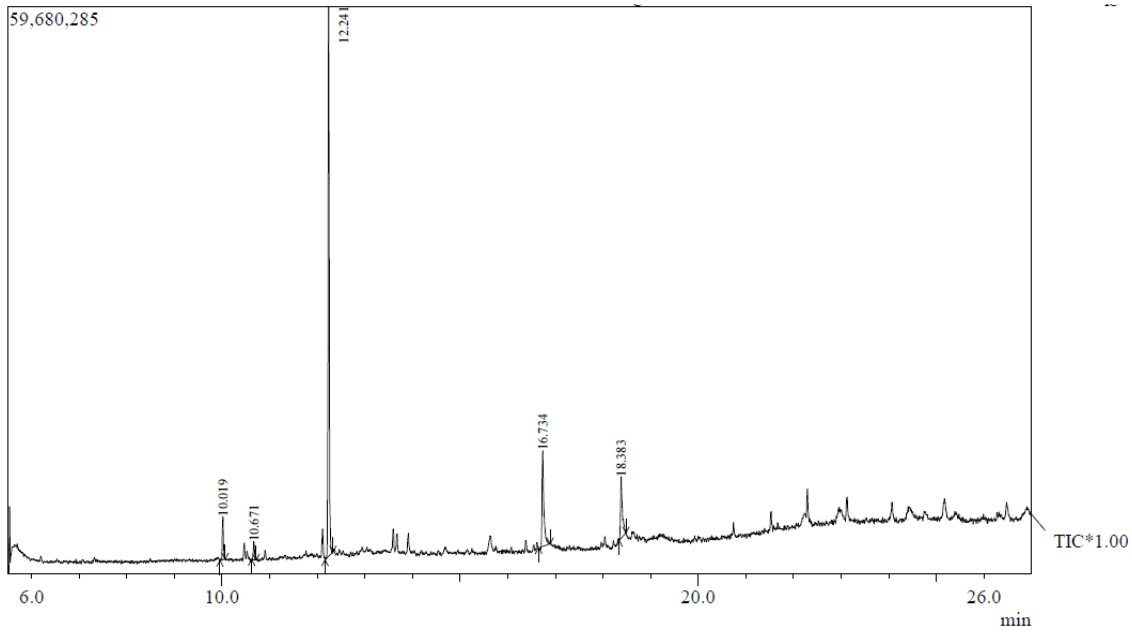

Table : 2 GC-MS analysis of *Targionia hypophylla*

Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Activity reported
1	10.01	4.95	Longifolene	$C_{15}H_{24}$	204	Sesquiterpene	Acne vulgaris treatment, Antibacterial and Antifungal
2	10.66	2.17	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl	$C_{15}H_{24}$	204	Alkene	Anticancerous
3	12.24	65.25	Patchouli alcohol	$C_{15}H_{26}O$	222	Terpene	Antibacterial
4	16.73	16.37	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide
5	18.38	11.26	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	Oleic acid	Antitumor and antibacterial activity.

(*Activity as per Data of Duke's library)