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Study of diversity of mosses, analysis and ex situ conservation from Baramati Tehsil, Pune and Revdanda, Raigad-Maharashtra, India

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Abstract
The current research focus on the study of moss diversity and secondary metabolite analyses using FTIR and further research was undertaken on these bryophytes that were preserved by ex-situ method in the lab. Mosses collected from Baramati and Revadanda Borli ranging from wet shady to temperate to tropical climates. These five bryophytes species as *Fissidens crenulatus*, *Steeriophyllum anceps*, *Hyophila involuta*, *Riccia discolor and Targionia hyophylla* maintained by Ex-situ preservation and analysed. *Riccia discolor* > *Targionia hyophylla* > *Hyophila involuta* > *Steeriophylum anceps* > *Fissiden secrenulatus* are examples of secondary metabolites that demonstrate broad tendencies of decreasing halo compound concentration.

Keywords: Bryophytes, *Ex-situ*, FTIR investigation of secondary metabolites.

Introduction

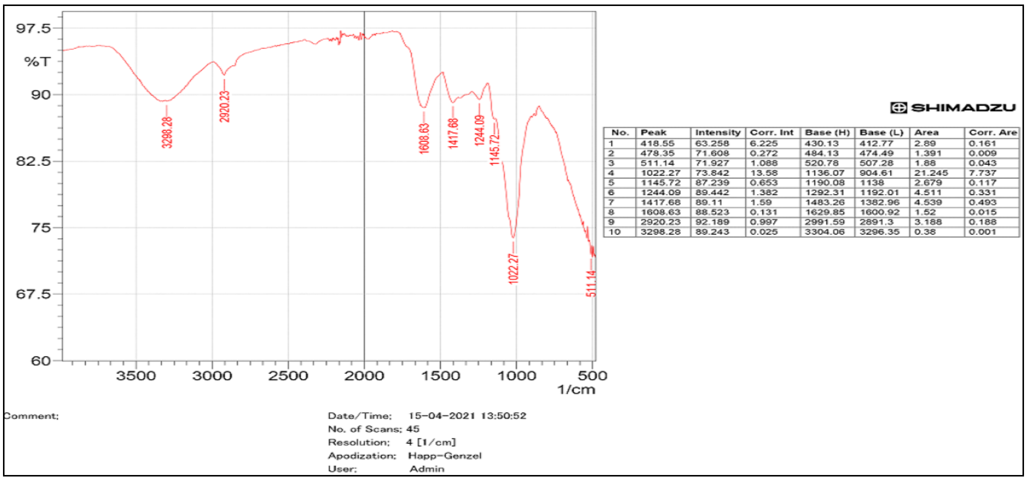
Mosses are members of the bryophytes, the second largest group in the plant kingdom. They are, nonetheless, among the most basic terrestrial plants (Vienna, 2013) [23]. Mosses have the most species followed by angiosperms. Mosses are a dominating category of bryophytes that occupy a unique position between vascular and non-vascular plants. Belonging to 11 orders are reported in Maharashtra's Western Ghats. There are 128 different species, 26 different families, and 59 different genera (Magdum and Colleagues, 2017) [15, 24]. Study on moss water-relations properties explaining 40-50 percent of the variation in morphology (Hedderson and Longton 1996) [8]. Mosses are a significant plant community at higher elevations in the Himalaya, growing in both humid and chilly conditions and accounting for 50 percent of active biomass. Moss chemistry was just discovered 30 years ago. Wadavkar *et al.* (2017) [24] in recent research on analytical techniques reveals proper overview about the chemical makeup of mosses.

The composition of mosses has attracted considerable attention in terms of applicability, with most research focusing on individual groups like as fatty acids, lipids, essential oils etc. Botan *et al*. (2013) [18, 19, 22, 23] investigated bryophyte composition to better understand their metabolism or metabolic activities. Secondary metabolite research aids in understanding the effects of stress reactions such as stress or moisture, pollution stress (for example, heavy metal impact), oxidative stress, and UV radiation impacts (Goffinet and Wang, 2012) [20]. Secondary metabolites in mosses are significant to research because they differ from those in higher plants (Shaw and Goffinet, 2008) [20]. According to Groombridge (1992) [6], *Sphagnum* moss is an ecologically and commercially significant category. Ex-situ conservation is a commonly used and successful approach for conserving fragile mosses and increasing the number of rare moss species (Halling and Hodegetts, 2000) [7]. Ex-situ conservation constitutes continuously sub-culture of moss material to keep the living collection alive and this material is tailored to growing in culture conditions (Lynch 2000) [14]. Secondary metabolite characterization was carried out by analyzing the presence of various chemicals and substances.

Materials and Method

Mosses were collected from locations such as Baramati Tehsil and Revadanda Borli in India, ranging from wet shady to temperate to tropical climates, with the overall collected species including *Fissidens crenulatus*, *Steeriophyllum anceps, Hyophila involuta*, *Riccia discolor* and *Targionia hyophylla.* Moss fragments and thalloids were preserved in a disposable polythene box. They were then kept under surveillance for an investigation of their growth improvement. Moss conservation was carried out separately. Before estimation, mosses were thoroughly cleaned with water and dried at room temperature for 24 hours. This dried moss sample was then used for chemical analysis of secondary metabolites. Fine moss material weighing up to 5 gm was obtained for FTIR investigation of secondary metabolites. The molecular vibration is investigated using Fourier Transform Infrared (FTIR) spectroscopy.

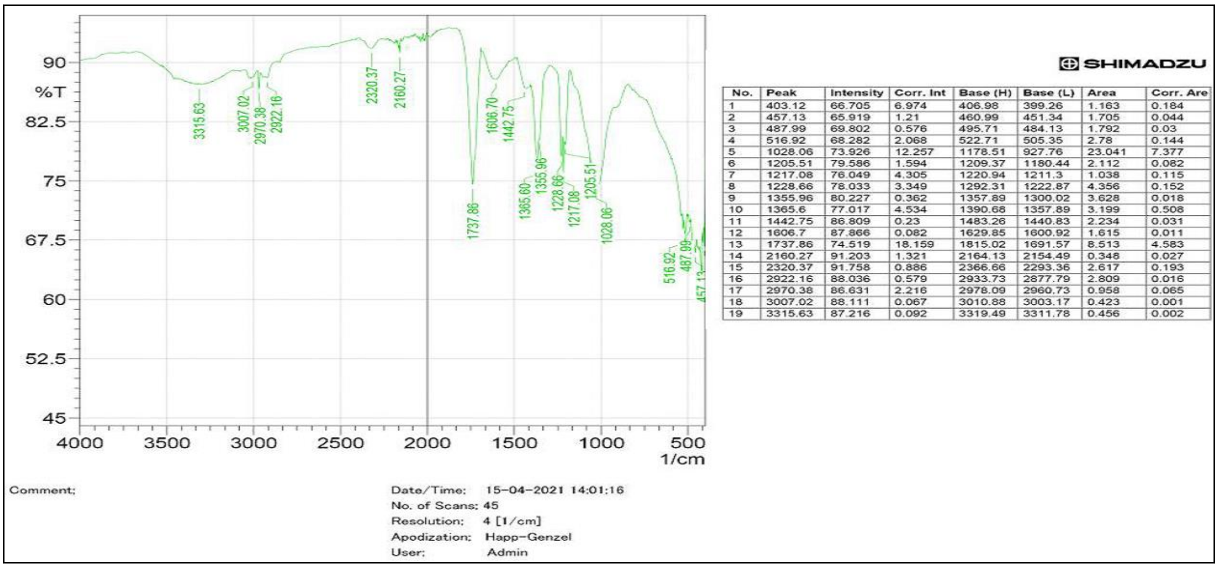
FTIR analysis was used to examine samples as small as 10 micron. Reflectance techniques were applied, and no harm was done to sample material that was thin enough to fit under the microscope when attenuated total reflectance attachment to the microscope was used. The spectrometer directs beams or infrared light towards the sample and measures the beam and the frequency of absorbed infrared light by infrared light. This approach determined the molecular identities of *Fissidens crenulatus*, *Steeriophyllum anceps*, *Hyophila involuta*, *Riccia discolor* and *Targionia hyophylla.*



Graph 1: Fissidense crenulatus Frequency and compound classes.

Table 1: Fissidense crenulatus Frequency and compound classes.

Sr.No	Frequency	Appearance	Group	Compound Class
1.	418.55cm-1	Strong	C-I Stretching	Halo compound.
2.	478.35cm-1	Strong	C-I stretching	Halo Compound.
3.	511.14cm-1	Strong	C-I Stretching	Halo compound.
4.	1022.27cm-1	Sotrong	C=c bending	Alkene
5.	1145.72cm-1	Strong	C-O Stretching	Aliphatic ether
6.	12.44.09cm-1	Medium	C-N Strecthing	Amine
7.	1417.68cm-1	Strong	S=O Strecthing	Sulfate
8.	1608.63cm-1	Strong	C=C Stretching	Unsaturated Ketone
9.	2920.23cm-1	Medium	C-H Stretching	Alkene

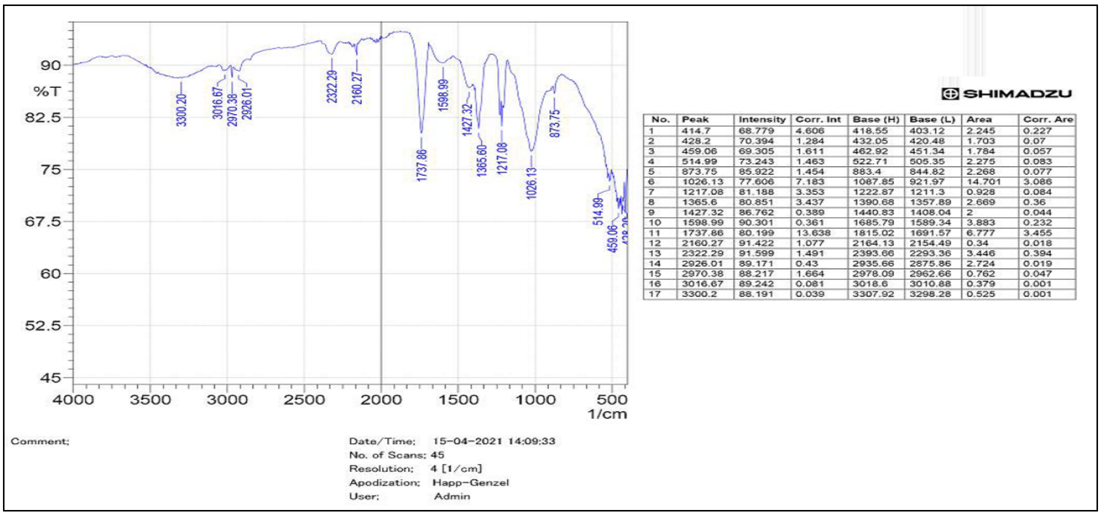


Graph 2: Steeriophyllum anceps

Table 2: *Steeriophyllum anceps* Frequency and compound classes.

Sr. No	Frequency	Appearance	Group	Compound Class
1.	403.12cm-1	Strong	C- I Stretching	Halo Compound
2.	457.13cm-1	Strong	C- I Stretching	Halo Comp
3.	487.99cm-1	Strong	C- I Stretching	Halo Compound
4.	516.92cm-1	Strong	C- I Stretching	Halo Compound
5.	1028.06cm-1	Strong	C= C bending	Alkene
6.	1205.51cm-1	Strong	C- O Stretching	Tertiary Alco
7.	1217.08cm-1	Strong	C- O Stretching	Vinyl ether
8.	1228.66cm-1	Medium	C- N Stretching	Amine
9.	1355.96cm-1	Strong	S=O Stretching	Sulfonic Acid
10.	1365.6cm-1	Strong	S=O Stretching	Sulfonamide
11.	1442.75cm-1	Medium	O-H Bending	Carboxylic Acid
12.	1606.7cm-1	Strong	C=C Stretching	Unsaturated Ketone

13.	1737.86^{cm-1}	Strong	C=O Stretching	Aldehyde
14.	2160.27^{cm-1}	Strong	N=N=N Stretching	Azide
15.	2320.37^{cm-1}	Strong	O=C=O Stretching	Carbon Dioxide
16.	2922.16^{cm-1}	Medium	C-H Stretching	Alkane
17.	2970.38^{cm-1}	Medium	C-H Stretching	Alkane
18.	3007.02^{cm-1}	Strong broad	N-H Stretching	Amine Salt
19.	3315.63^{cm-1}	Strong Sharp	C-H Stretching	Alkyne



Graph 3: Hyophila involuta

Table 3: *Hyophila involuta* Frequency and compound classes

Sr. No	Frequency	Appearance	Group	Compound Class
1.	414.7^{cm-1}	Strong	C- I Stretching	Halo Compound
2.	428.2^{cm-1}	Strong	C- I Stretching	Halo Compound
3.	459.06^{cm-1}	Strong	C- I Stretching	Halo Compound
4.	514.99^{cm-1}	Strong	C- I Stretching	Halo Compound
5.	873.75^{cm-1}	Strong	C= C Bending	Alkene
6.	1026.13^{cm-1}	Strong	C= C Bending	Alkene
7.	1217.08^{cm-1}	Strong	C= C Bending	Alkene
8.	1365.6^{cm-1}	Strong	S= O Stretching	Sulfonamide
9.	1427.32^{cm-1}	Mediun	O-H Bending	Alcohol
10.	1598.99^{cm-1}	Strong	C=C Stretching	-
11.	1737.86^{cm-1}	Strong	C=O Stretching	Aldehyde
12.	2160.27^{cm-1}	Strong	N=N=N Stretching	Azide
13.	2322.29^{cm-1}	Strong	O=C=O Stretching	Carbon Dioxide
14.	2926.01^{cm-1}	Medium	C-H Stretching	Alkane
15.	2970.38^{cm-1}	Medium	C-H Stretching	Alkane
16.	3016.64^{cm-1}	Strong Broad	N-H Stretching	Amine Salt
17.	1300.2^{cm-1}	Strong G	C-O Streching	Aromatic Ester

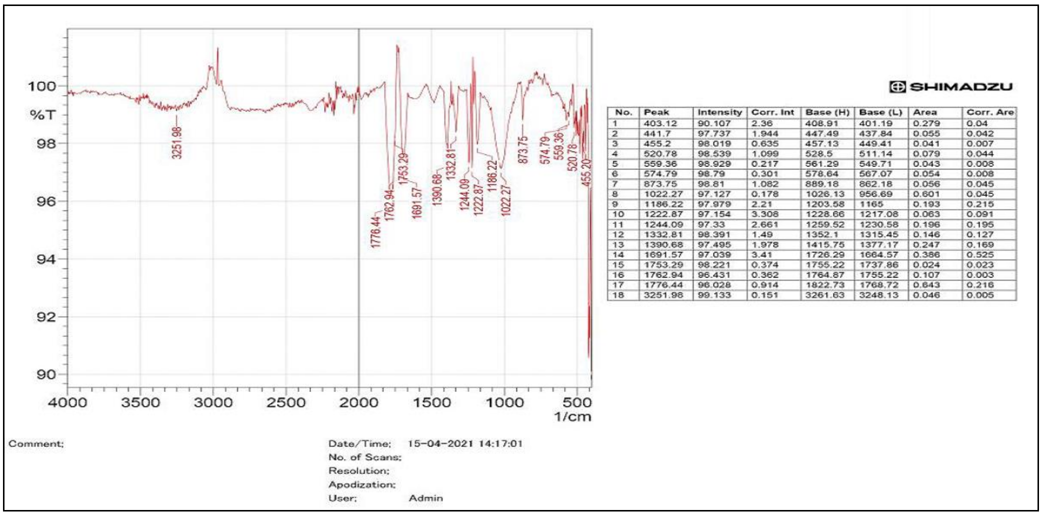
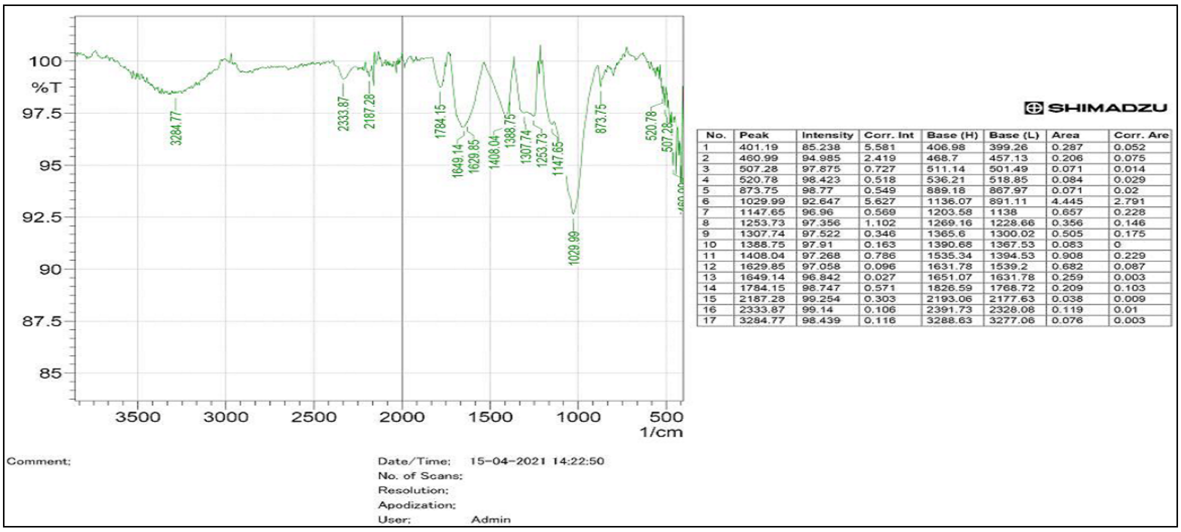


Fig 4: Riccia discolor

Table 4: *Riccia discolor* Frequency and compound classes

Sr. No.	Frequency	Appearance	Group	Compound Class
1.	403.12^{cm-1}	Strong	C-I Stretching	Halo Compound
2.	4041.7^{cm-1}	Strong	C-I stretching	Halo Compound
3.	4041.7^{cm-1}	Strong	C-I stretching	Halo Compound
4.	455.2^{cm-1}	Strong	C-I Stretching	Halo Compound
5.	520.78^{cm-1}	Strong	C-I Stretching	Halo Compound
6.	559.36^{cm-1}	Strong	C-I Stretching	Halo Compound
7.	574.79^{cm-1}	Strong	C-I Stretching	Halo Compound
8.	873.75^{cm-1}	Strong	C-I Stretching	Halo Compound
9.	1022.27^{cm-1}	Strong	C-I Stretching	Halo Compound
10.	1186.22^{cm-1}	Strong	C-O Stretching	Tertiary Alcohol
11.	1222.87^{cm-1}	Strong	C-O Stretching	Vinyl Ether
12.	1244.09^{cm-1}	Medium	C-N Stretching	Amine
13.	1332.81^{cm-1}	Strong	C-N Stretching	Aromatic Amine
14.	1390.68^{cm-1}	Medium	O-H Bending	Phenol
15.	1691.56^{cm-1}	Strong	C=O Strethcing	Primary Amide
16.	1753.29^{cm-1}	Strong	C=O Stretching	Esters
17.	1762.94^{cm-1}	Strong	C=O Stretching	Carboxylic Acid
18.	1776.44^{cm-1}	Strong	C=O Stretching	Vinyl I Phenyl Ester
19.	3251.98^{cm-1}	Weak Broad	O-H Stretching	Alcohol



No.	Peak	Intensity	Corr. Int	Base (H)	Base (L)	Area	Corr. Are
1	401.19	85.238	5.581	406.98	399.26	0.287	0.052
2	460.99	94.985	2.419	468.7	457.13	0.206	0.075
3	507.28	97.875	0.727	511.14	501.49	0.071	0.014
4	520.78	98.423	0.518	536.21	518.85	0.084	0.029
5	873.75	98.77	0.549	889.18	867.97	0.071	0.02
6	1029.99	92.647	5.627	1136.07	891.11	4.445	2.791
7	1147.65	96.96	0.569	1203.58	1138	0.657	0.228
8	1253.73	97.356	1.102	1269.16	1228.66	0.356	0.146
9	1307.74	97.522	0.346	1365.6	1300.02	0.505	0.175
10	1388.75	97.91	0.163	1390.68	1367.53	0.083	0
11	1408.04	97.268	0.786	1535.34	1394.53	0.908	0.229
12	1629.85	97.058	0.096	1631.78	1539.2	0.682	0.087
13	1649.14	96.842	0.027	1651.07	1631.78	0.259	0.003
14	1784.15	98.747	0.571	1826.59	1768.72	0.209	0.103
15	2187.28	99.254	0.303	2193.06	2177.63	0.038	0.009
16	2333.87	99.14	0.106	2391.73	2328.08	0.119	0.01
17	3284.77	98.439	0.116	3288.63	3277.06	0.076	0.003

Graph 5: Targionia hyophylla

Table 5: *Targionia hyophylla* Frequency and compound classes

Sr. No	Frequency	Appearance	Group	Compound Class
1.	401.19^{cm-1}	strong	C-I stretching	Halo Compound
2.	460.99^{cm-1}	Strong	C-I Stretching	Halo Compound
3.	507.28^{cm-1}	Strong	C-I Stretching	Halo Compound
4.	520.78^{cm-1}	Strong	C-I Stretching	Halo Compound
5.	873.75^{cm-1}	Strong	C-I Stretching	Halo Compound
6.	1029.99^{cm-1}	Strong	C-I Stretching	Alkene
7.	1147.45^{cm-1}	Strong	C-O stretching	Ester
8.	1253.73^{cm-1}	Strong	C-N stretching	Aromatic Amine
9.	1307.74^{cm-1}	Strong	C-O Stretching	Aromatic Ester
10.	1388.75^{cm-1}	Strong	S=O stretching	Sulfonate
11.	1408.04^{cm-1}	Strong	C-F stretching	Fluoro Compound
12.	1649.14^{cm-1}	Strong	C-C stretching	Alkene
13.	1784.15^{cm-1}	Strong	C=O stretching	Acid Halide
14.	2187.28^{cm-1}	Weak	C=C Stretching	Alkyne
15.	2333.87^{cm-1}	Strong	O=C=O Stretching	Carbon Dioxide
16.	3284.77^{cm-1}	Weak broad	O-H Stretching	Alcohol

Results and Discussion

Based on their chemical structure, secondary metabolites are categorised into various kinds. *Fissidens ecrenulatus* (Table 1), a moss species obtained from Revadanda, Borli, had a greater concentration of halo chemicals. This halo molecule gives herbicide and pesticide tolerance. Another alkene compound is found in moderate amounts in all tree species. *Hyophila involuta* contains a lot of alkene, aliphatic ether, amine, sulphate, unsaturated ketone, carboxylic acid, alkine, aromatic ester, aromatic amine, phenol, and fluro compounds (Table 3). The anti-inflammatory and anti-hepatotoxic effects of phenolic compounds (O-H) are highly valued. Simple phenolic compounds exhibit antimicrobial action. The moss species gathered from Baramati Tehsil, *Riccia discolor* and *Targionia hyophylla* have a greater content of halo chemicals. Vinyl ether, amine, aromatic amine, phenol, primary amide, alkene, sulfonate, fluro compound, alkyne, and aromatic ester are also present (Table4 and Table5).

Secondary moss metabolites found belong to a class of compounds known as phenolic compounds, flavonoids, and terpenoids, all of which have anti-oxidant, anti-inflammatory, anti-tumor, and anti-microbial activities. Amino acids are vital in shielding the photosynthetic machinery from the effects of light in dry circumstances. The presence of a wide range of various compounds is revealed by the analysis of moss powder. Overall, moss extracts were the most potent, with the strongest resistance to herbicides and insecticides. Secondary metabolites, biological activities, and products and the quality of moss demonstrate a diverse range of moss components. All five tables contain information on secondary metabolites found in moss species. It can be seen that halo chemicals are abundant in *Riccia discolor, Targionia hyophylla*, *Hyophila involuta*, *Fissidense crenulatus*, and *Steeriophyllum anceps*.

The presence of halo chemicals was typically associated with unfavorable environmental circumstances such as insect predilection, pathogen attack, and UV harm (Xie and Lou,2009; Whitehead *et al.*, 2018) [26], . Secondary metabolites studied show broad tendencies of decreasing halo compound concentration, i.e., *Riccia discolor* > *Targionia hyophylla* > *Hyophila involuta* > *Steeriophylum anceps* > *Fissidense crenulatus.* Secondary metabolites, particularly terpenoids, may play a key role in moss environmental interaction (Asakawa *et al.,* 2013) [18]. Several phytotoxic compounds isolated from moss species were found to impair germination and growth of vascular plants. This toxicity has an impact on the search for other useful chemicals with antifungal, antibacterial, anti-inflammatory, and insect repellent properties (Asakawa *et al.*, 2013) [18].

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