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GC-MS, FTIR, and HPLC analysis of *Trichoderma sps, Rhizopus sps,* and *Aspergillus sps* of *Saraca asoca* for anticancer molecules

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ABSTRACT: Endophytic fungi combined with medicinal plants are reservoirs of therapeutic compounds. These endophytes are considered one of the major sources of bioactive molecules used in different aspects of health care. The present study attempted to assess the possibility of using the endophytic extract. The study showed a complete endophytic extract by using the GC–MS profile, indicating the presence of different volatile molecules in the endophytic extract. The HPLC fingerprint of S. asoca endophytic extract represents the characteristic markers of this herb. The endophytic extract's HPLC showed caffeine, a Ret Time [min]- 3.052, and a Width [min]- 0.1322. The FTIR analysis of the functional groups present in the Trichoderma sps are =C-H (Alkynes, Amides), O-H (Carboxylic acids), C=C(Alkynes), C=C (Aromatic compounds), C-F (Alkyl and Aryl Halides). These functional groups are used in the production of antibiotics and antifungal drugs. The GC-MS study of the endophytic extract showed the presence of 59 compounds, which possessed anticancer properties. The anticancer activity was studied further.

KEYWORDS: GC-MS, HPLC, FTIR, therapeutic compounds

1. INTRODUCTION

Saraca asoca Roxb. Wild. known as Ashoka, has many common names in different languages - Ashoka in Hindi, Kankeli in Sanskrit, Ashokadamara in Kannada, Ashokapatta in Telugu, Asokam in Malayalam, and Asogam in Tamil. Plants have been used for medicinal purposes long before the prehistoric period. Ayurveda, the traditional system of medicine, continues to be widely practiced on many accounts. Endophytes, *i.e.,* microorganisms (e.g. fungi, bacteria, etc.) asymptomatically residing inside the internal tissues of the plant hosts, are known to benefit the hosts (1) symbiotically. The plant *S. asoca* is a prime option for screening its endophytes for pharmaceutically relevant chemicals, including those first attributed to its herbal formulations, due to its well-known therapeutic efficacy. Although herbal formulations are known to exert their biological effects through the combination of many chemicals, this would theoretically require finding individual chemical entities from *S. asoca* extracts that might be held accountable for the extracts' biological effects. The chemical diversity of the endophytic population is largely shaped by the uniqueness of the niche they occupy. Hence, endophytic fungi residing in plants restricted to exotic habitats or those with ethnobotanical value are more likely to synthesize a unique, broad range of novel secondary metabolites.

Fig1. *S. asoca* Leaves

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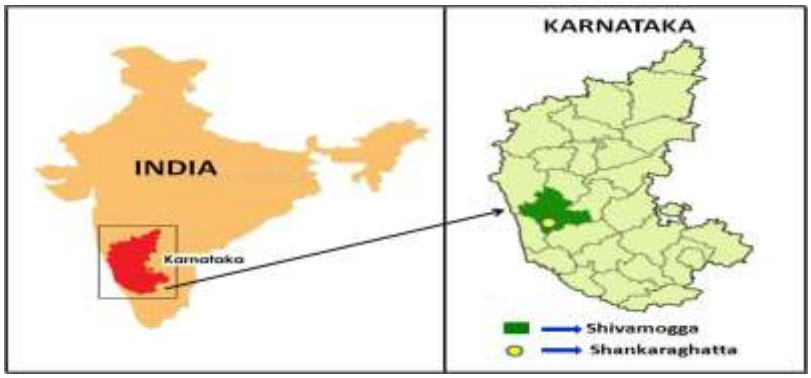
2. MATERIALS AND METHODS

2.1 Collection of plant materials:

The collection of plant material is situated at the Shanakarghatta region in Bhadravati taluk, Shivamogga district of Karnataka.



2.2 Sample preparation:

The plant material was washed in running tap water to remove unwanted debris, and finally, it was washed with distilled water and mercuric chloride. Plant materials were studied by using aseptic procedures, and inner tissues were excised by using sterile scissors. Plant materials were cut into small pieces of 1cm long and 3-4mm broad in size. All the work was performed in a Laminar airflow. Cultures on PDA media were identified according to their morphology, colony appearance, mycelium color, conidia, and conidiophores. which were observed for magnification using the compound microscope with 10X and 40X objective lenses. Identified fungal species were cultured on PDB broth for large-scale cultivation. The inoculated flasks were incubated at room temperature (26°C) for 7-21 days and allowed to grow the fungal mats. Mats are used as endophytic extracts for their study. The endophytic extract was stored in an airtight poly-tube and sent to further tests for GC-MS analysis to identify the compounds present in the endophytic extract.

A B C

The figure above. A. *Trichoderma sps*. mat culture. B. The mat on the pestle and mortar was separated.

C. *Trichoderma sps.* crude extract

2.3 Gas Chromatography-Mass Spectroscopy Analysis: GC-MS analysis was conducted to investigate anticancer properties in the endophytic extract of *Trichoderma sps, Rhizopus sps,* and *Aspergillus sps*. Out of 3 sps, 2 sps showed more anticancer properties, which are Trichoderma sps and Rhizopus sps. But I have taken the best sps for further tests, that is *Trichoderma sps*. GC-MS analysis is a combined technique that is used to identify different substances within the sample. It works on the separation of individual compounds by Gas Chromatography according to their Retention time. separated compounds are further analyzed at a molecular level by Mass Spectroscopy (2). The GC-MS analysis of our endophytic extract of *Trichoderma sps* revealed the presence of 59 anticancer properties.

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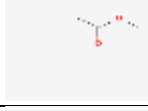
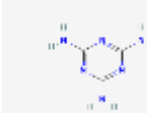
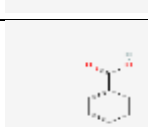
2.4 High-Performance Liquid Chromatography: HPLC analysis of crude extract was carried out in line with methods (3), where significant peaks in the extract were identified using an analytical HPLC Dionex P580 HPLC system coupled to a photodiode array detector A mobile phase consisting of the sample was used for separation with 12:85:3 in an isocratic mode with an injection volume of 20 µL. The flow rate was 0.7 mL/min, and the detection wavelength of the diode array detector (DAD) was set to 280 nm with an 18-minute run time for both the standard and the sample.

2.5 FTIR analysis of *Trichoderma sps*: Currently, the functional groups of the endophytic extract of Trichoderma sps are being studied using nuclear magnetic resonance (NMR). The functional groups present in this extract are =C-H (Alkynes, Amides), O-H (Carboxylic acids), C=C(Alkynes), C=C (Aromatic compounds), C-F (Alkyl and Aryl Halides). This functional group may also be used in the preparation of drugs like antibiotics and antifungal drugs (4).

3. RESULT AND DISCUSSION

Compound analysis, GC-MS analysis (for investigation of the compounds present) of the methanol extract. The GC-MS of the endophytic extract of *Trichoderma sps* shows more anticancer properties than the others, and also these compounds are used in cancer treatment. This technique is used to identify different substances within the sample, and these separated compounds are further analyzed at a molecular level by Mass Spectroscopy.

SI. No	RT	Ligands	Formula	M.W	Structure	Bioactive uses
1	2.180	methyl ester	$C_3H_6O_2$	74		HeLa cervical Cancer cells
2	2.272	Propanoic acid	$C_4H_6O_3$	102		Anticancer properties
3	2.626	Pyrazine	$C_5H_6N_2$	94		Lung Cancer(A549)
4	3.539	2-Hydroxy-gamma-Butyrolactone	$C_4H_6O_2$	86		Anticancer properties
5	5.410	1,3,5-Triazine-2,4,6-triamine $$ Melamine $$ S-Triazinetriamine $$ Cyanuramide $$ Cyanurotriamide $$ Cyanurotriamine $$ Hicophor PR $$ I	$C_3H_6N_6$	126		Anticancer properties
6	6.292	Benzoic acid $$ Benzenecarboxylic acid $$ Benzeneformic acid $$ Benzenemethanoic acid $$ Benzoesaeure GK $$ Benzoesaeure	$C_7H_6O_2$	122		Lung, breast, bladder, and colorectal cancer

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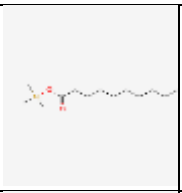
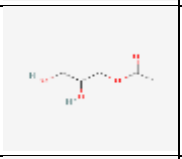
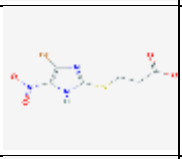
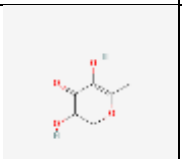
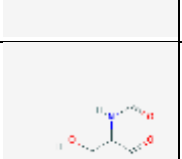
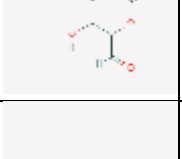
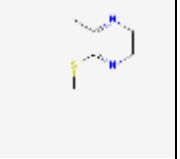
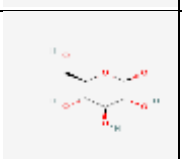
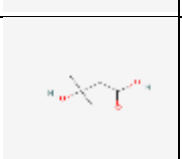
7	7.020	11-Bromoundecanoic acid, TMS derivative $$ Undecanoic acid, 11-bromo-, trimethylsilyl ester $$ Trimethylsilyl 11-bromoundecanoate #	$C_{14}H_{29}BrO_2Si$	336		Breast cancer
8	7.057	1,2,3-Propanetriol, 1-acetate $$ Acetin, 1-mono- $$ alpha-Monoacetin $$ Glycerol alpha-monoacetate $$ 1-Monoacetin $$	$C_5H_{10}O_4$	134		Prostate cancer
9	7.120	Imidazole-2-[3-thiopropionic acid], 5-bromo-4-nitro- $$ 3-[(4-Bromo-5-nitro-1H-imidazol-2-yl)sulfanyl]propanoic acid #	$C_6H_6BrN_3O_4S$	295		Lung, breast, melanoma cancer
10	7.330	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- $$ 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one $$ 2,3-dihydro-3,5-dihydroxy-	$C_6H_8O_4$	144		Anticancer properties
11	7.850	2-Acetamido-2-deoxy-d-mannolactone	$C_8H_{11}NO_6$	217		Anticancer properties
12	8.371	Pyrazine, 2-methyl-3-(methylthio)- $$ 2-Methyl-3-(methylthio) pyrazine $$ 2-Methyl-3-methylmercaptopyrazine	$C_6H_8N_2S$	140		Anticancer properties
13	10.165	alpha-D-Glucopyranoside, methyl $$ Glucopyranoside, methyl, alpha-D- $$ alpha-Methylglucoside $$ Methyl alpha-D-glucopyranoside	$C_7H_{14}O_6$	194		Lungs, Pancreas, and Prostate Cancer45
14	10.240	Butanoic acid, 3-hydroxy-3-methyl- $$ Butyric acid, 3-hydroxy-3-methyl- $$ beta-Hydroxy-isovaleric acid $$ 3-Hydroxyisovaleric acid	$C_5H_{10}O_3$	118		Colorectal cancer
15	10.285	D-Mannoheptulose $$ D-manno-2-Heptulose $$ (+)-Mannoheptulose $$ Mannoheptulose, D- $$ Hept-2-ulose #	$C_7H_{14}O_7$	210		Breast cancer

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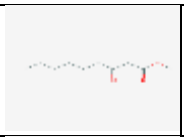
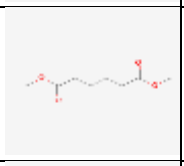
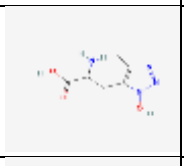
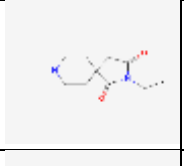
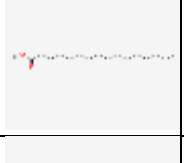
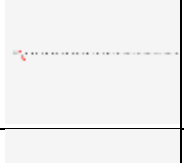
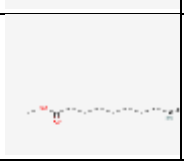
16	10.465	Nonanoic acid, 3-oxo-, methyl ester $$ Methyl 3-oxononanoate #	$C_{10}H_{18}O_3$	186		Lung cancer
17	11.100	Hexanedioic acid, dimethyl ester $$ Adipic acid, dimethyl ester $$ Dimethyl adipate $$ Dimethyl hexanedioate $$ Methyl adipate $$	$C_8H_{14}O_4$	174		Pancreatic cancer
18	11.440	1,2,5-Oxadiazole-3-carboxamide, 4-amino-N-(2-methoxyethyl)- $$ 4-Amino-N-(2-methoxyethyl)-1,2,5-oxadiazole-3-carboxamide #	$C_6H_{10}N_4O_3$	186		Anticancer properties
19	11.478	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- $$ 3-Isobutylhexahydropyrrolo[1,2-a] pyrazine-1,4-dione	$C_{11}H_{18}N_2O_2$	210		Anticancer properties
20	11.522	n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid	$C_{16}H_{32}O_2$	256		Colon cancer
21	11.673	Tetradecanoic acid, 12-methyl-, methyl ester $$ Methyl 12-methyltetradecanoate $$ Methyl tetradecanoate, 12-methyl	$C_{16}H_{32}O_2$	256		Breast cancer cells
22	11.720	Triacontanoic acid, methyl ester $$ Methyl melissate $$ Methyl melissicate $$ Methyl triacontanoate	$C_{31}H_{62}O_2$	466		Cervical cancer
23	11.856	Hexadecanoic acid, 15-methyl-, methyl ester $$ Methyl isoheptadecanoate $$ Methyl 15-methylhexadecanoate	$C_{18}H_{36}O_2$	284		Breast, colon, oral Squamous cell carcinoma, prostate cancer
24	12.196	9,11-Octadecadienoic acid, methyl ester, (E, E)- $$ Methyl trans-9, trans-11-octadecadienoate $$ Methyl trans, trans-9,11-octadecadienoate	$C_{19}H_{34}O_2$	294		Cervical, Breast, leukemia cancer
25	12.222	9-Octadecenoic acid, methyl ester, (E)- $$ Elaidic acid, methyl ester $$ Methyl elaidate $$ Methyl trans-9-octadecenoate	$C_{19}H_{36}O_2$	296		Cervical, Breast, leukemia cancer

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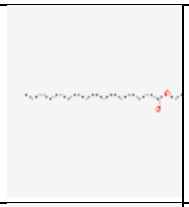
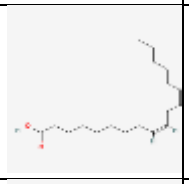
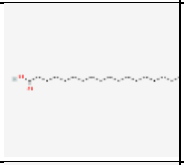
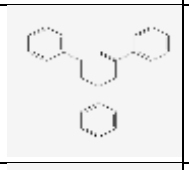
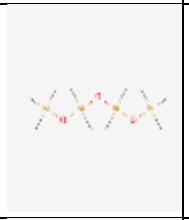
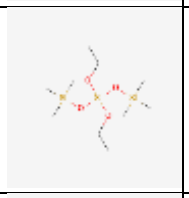
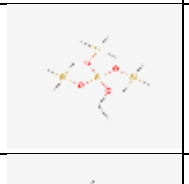
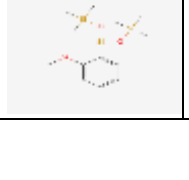
26	12.330	Methyl stearate $$ Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester $$ n-Octadecanoic acid, methyl ester $$ Kemester 9718	$C_{19}H_{38}O_2$	298		Breast cancer
27	12.365	10E,12Z-Octadecadienoic acid	$C_{18}H_{32}O_2$	280		Breast cancer
28	12.485	Octadecanoic acid $$ Stearic acid $$ n-Octadecanoic acid $$ Humko Industrene R $$ Hydrofol Acid 150 $$ Hystrene S-97	$C_{18}H_{36}O_2$	284		Breast cancer
29	13.854	Cyclohexane, 1,3,5-triphenyl	$C_{24}H_{24}$	312		Lung cancer
30	15.011	1,2-Bis(trimethylsilyl)benzene $$ Trimethyl[2-(trimethylsilyl)phenyl] silane #	$C_{12}H_{22}Si_2$	222		Breast, Prostate, Colorectal cancer
31	15.610	Tetrasiloxane, decamethyl- $$ Decamethyl tetrasiloxane $$	$C_{10}H_{30}O_3Si_4$	310		Anticancer properties
32	18.144	Silicic acid, diethyl bis(trimethylsilyl) ester $$ 3,3-Diethoxy-1,1,1,5,5,5-hexamethyltrisiloxane $$ Diethyl bis(trimethylsilyl) orthosilicate #	$C_{10}H_{28}O_4Si_3$	296		Anticancer properties
33	18.192	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane $$ 3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsilyloxy)trisiloxane	$C_{11}H_{32}O_4Si_4$	340		Lung cancer
34	18.370	Ethoxy(phenyl)silanediol, 2TMS $$ 3-ethoxy-1,1,1,5,5,5-hexamethyl-3-phenyltrisiloxane	$C_{14}H_{28}O_3Si_3$	328		Lung cancer

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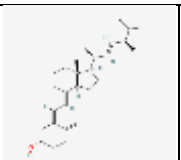
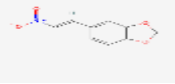
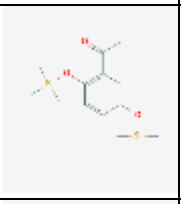
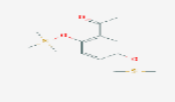
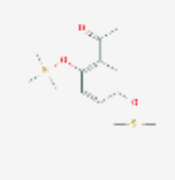
35	18.548	Ergosterol $$ Ergosta-5,7,22-trien-3-ol, (3.beta.,22E)- $$ Ergosterin $$ Provitamin D $$ Provitamin D2 $$ (3.beta.)-	$C_{28}H_{44}O$	396		Breast cancer
36	18.370	Ethoxy(phenyl)silanediol, 2TMS $$ 3-ethoxy-1,1,1,5,5,5-hexamethyl-3-phenyltrisiloxane	$C_{14}H_{28}O_3Si_3$	328		Lung cancer
37	18.548	Ergosterol $$ Ergosta-5,7,22-trien-3-ol, (3.beta.,22E)- $$ Ergosterin $$ Provitamin D $$ Provitamin D2 $$ (3.beta.)-	$C_{28}H_{44}O$	396		Breast cancer
38	18.370	Ethoxy(phenyl)silanediol, 2TMS $$ 3-ethoxy-1,1,1,5,5,5-hexamethyl-3-phenyltrisiloxane	$C_{14}H_{28}O_3Si_3$	328		Lung cancer
39	18.548	Ergosterol $$ Ergosta-5,7,22-trien-3-ol, (3.beta.,22E)- $$ Ergosterin $$ Provitamin D $$ Provitamin D2 $$ (3.beta.)-	$C_{28}H_{44}O$	396		Breast cancer
40	18.370	Ethoxy(phenyl)silanediol, 2TMS $$ 3-ethoxy-1,1,1,5,5,5-hexamethyl-3-phenyltrisiloxane	$C_{14}H_{28}O_3Si_3$	328		Lung cancer
41	18.548	Ergosterol $$ Ergosta-5,7,22-trien-3-ol, (3.beta.,22E)- $$ Ergosterin $$ Provitamin D $$ Provitamin D2 $$ (3.beta.)-	$C_{28}H_{44}O$	396		Breast cancer
42	23.116	benzoic acid, 2-[(acetylamino)carbonyl]- $$ 2-(acetylcarbamoyl)benzoic acid	$C_{10}H_9NO_4$	207		Lung, breast, bladder, colorectal cancer
43	23.145	benzoic acid, 4-[[(trimethylsilyl)oxy] methyl]-, trimethylsilyl ester $$ trimethylsilyl 4-{[(trimethylsilyl)oxy] methyl} benzoate	$C_{14}H_{24}O_3Si_2$	296		Lung, breast, bladder, colorectal cancer
44	23.370	3-Hydroxy-2-methylbenzoic acid, 2TMS $$ Benzoic acid, 3-hydroxy-2-methyl-, 2TMS	$C_{14}H_{24}O_3Si_2$	296		Colon, lung cancer
45	23.415	5-Methylsalicylic acid, 2TMS derivative $$ Benzoic acid, 5-methyl-2-trimethylsilyloxy-, trimethylsilyl ester $$ Trimethylsilyl 5-methyl	$C_{14}H_{24}O_3Si_2$	296		Colon, lung cancer

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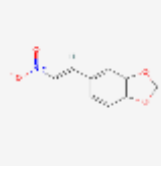
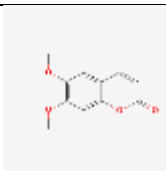
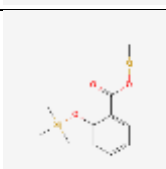
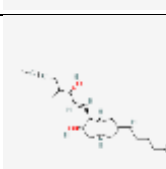
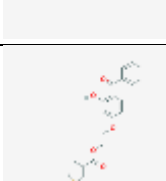
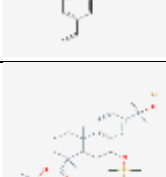
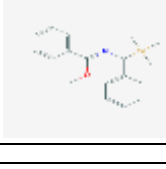
46	23.495	benzoic acid, 2-[(acetylamino)carbonyl]- $$ 2-(acetylcarbamoyl)benzoic acid	$C_{10}H_9NO_4$	207		Colon, lung cancer
47	23.525	2-(Allyloxycarbonyl)benzoic acid $$ Phthalic acid, monoallyl ester $$ Allyl hydrogen phthalate $$ Monoallyl phthalate	$C_{11}H_{10}O_4$	206		Colon, lung cancer
48	23.666	4-Hydroxybenzoic acid, 2TMS derivative $$ Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester $$ Benzoic acid, p-(trimethylsiloxy)	$C_{13}H_{22}O_3Si_2$	282		Colon, lung cancer
49	23.740	Sulochrin, 3TMS derivative $$ m-Anisic acid, 5-hydroxy-2-(4-methyl-gamma-resorcyloyl)-, methyl ester, O, O, O-tris-TMS	$C_{26}H_{40}O_7Si_3$	548		Colon, lung cancer
50	23.770	Isophthalic acid, allyl undecyl ester	$C_{22}H_{32}O_4$	360		Anticancer properties
51	24.032	Isosulochrin, 3TMS derivative $$ Benzoic acid, 2-(2,6-dihydroxy-4-methylbenzoyl)-3-hydroxy-5-methoxy-, methyl ester, O, O, O-tris-TMS	$C_{26}H_{40}O_7Si_3$	548		Colon, lung cancer
52	24.110	Ethyl homovanillate, TMS derivative $$ Benzeneacetic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-,	$C_{14}H_{22}O_4Si$	282		Colon, lung cancer
53	24.342	7,15-Dihydroxydehydroabietic acid, tris(trimethylsilyl)deriv. $$ Trimethylsilyl 7,15-bis[(trimethylsilyl) oxy] abieta-9	$C_{29}H_{52}O_4Si_3$	548		Anticancer properties
54	24.375	Benzeneacetic acid, 2-acetyl-3-methoxy- $$ (2-Acetyl-3-methoxyphenyl) acetic acid #	$C_{11}H_{12}O_4$	208		Anticancer properties
55	24.402	N-(2,6-Dimethylphenyl) benzamide, TMS derivative	$C_{18}H_{23}NOSi$	297		Colon, lung cancer

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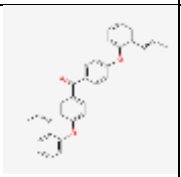
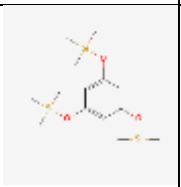
56	24.531	Bis(2-hydroxyethyl) phthalate $$ 1,2-Benzenedicarboxylic acid, 1,2-bis(2-hydroxyethyl) ester	$C_{12}H_{14}O_6$	254		Anticancer properties
57	24.795	Spiro [9,9'] difluorene, 2,2'-(2,5,8-trioxanonane-1,9-diyl)	$C_{31}H_{26}O_3$	446		Anticancer properties
58	24.905	1H-Pyrrole-3-propanoic acid, 4-acetyl-2-ethoxycarbonyl-5-methyl-	$C_{15}H_{21}NO_5$	295		Anticancer properties
59	25.512	1,3,5-Benzetriol, 3TMS derivative $$ 1,3,5-Tris(trimethylsiloxy)benzene $$ Silane, [1,3,5-benzenetriyltris(oxy)] tris [trimethyl-	$C_{15}H_{30}O_3Si_3$	342		Colon, lung cancer

HPLC analysis of endophytic extract: HPLC analysis of the endophytic extract showed the presence of caffeine, and it's a Ret Time [min]- 3.052, and a Width [min]- 0.1322. The anticancer effects of caffeine have been reported in various human cells and tissues, and similar results were noted in MGC-803 and SGC-7901 GC cells in the present study.

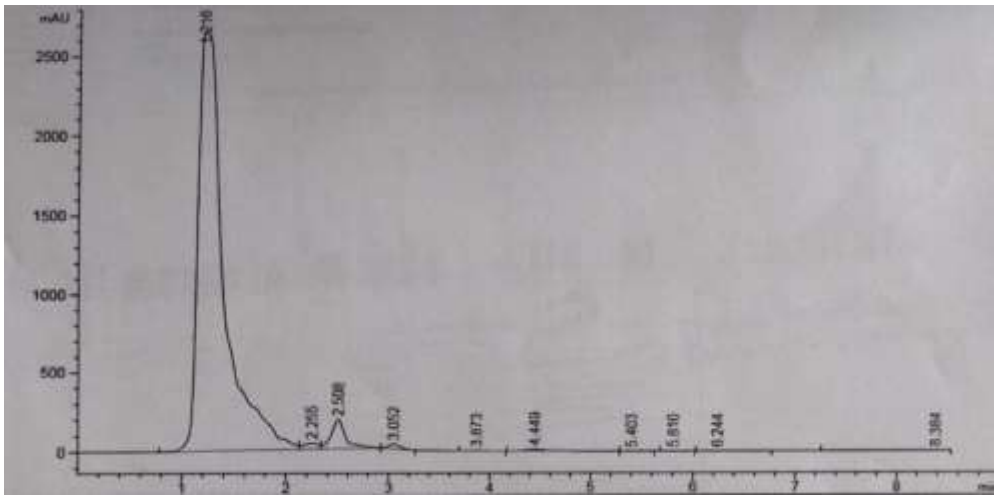


Figure 1. Shows the HPLC results of *Trichoderma sps*

FTIR analysis of endophytic extract: The FTIR analysis of the endophytic extract of *Trichoderma sps* by using Agilent Technologies the frequency of 3272 its bond =C-H stretch, and its functional groups are Alkynes, Amides, and the frequency of 2922 its bond shows O-H and its functional groups are Carboxylic acids, and the frequency of 1523 shows the bond C=C, and its functional group are aromatic compounds, the frequency of 1031 shows the bond C-F, and its functional groups are Alkyl and Aryl Halides which are all used in antibiotics, antifungal drugs(5).

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Table 1: Shows the FTIR results of *Trichoderma sps*

Frequency	Bond	Functional groups
3272	=C-H	Alkynes, Amides
2922	O-H	Carboxylic acids
2188	C=C	Alkynes
1618	C=C	Aromatic compounds
1492	C=C	Aromatic compounds
1523	C=C	Aromatic compounds
1031	C-F	Alkyl and Aryl Halides

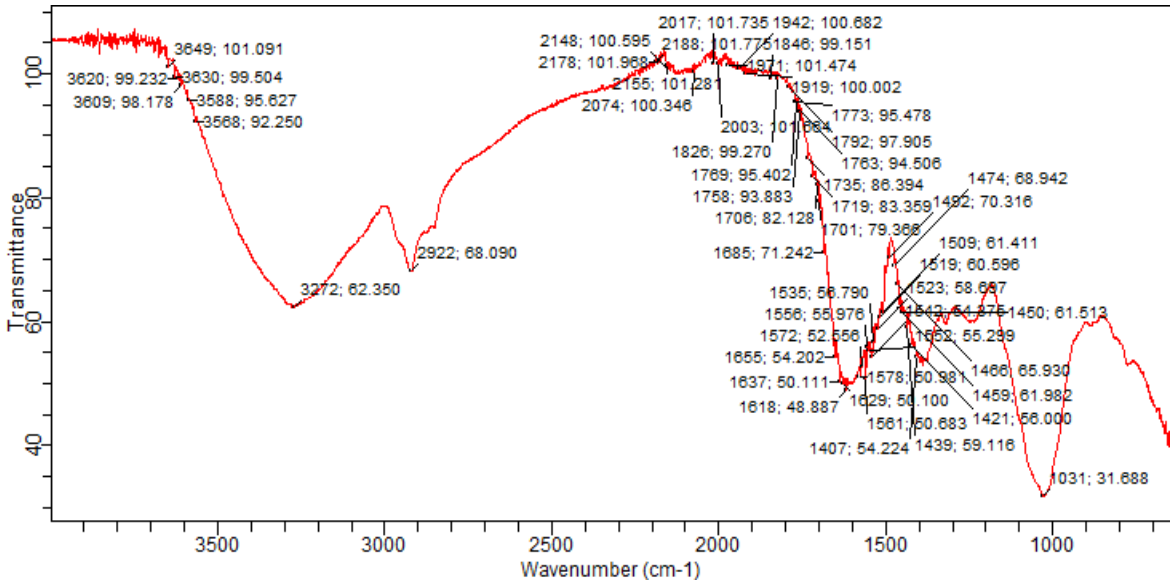

Figure 2: Shows the FTIR results of *Trichoderma sps*

CONCLUSION

Endophytic microbes are microorganisms that colonize the intracellular spaces within the plant tissues without exerting any adverse or pathological effects. Fungal endophytes reside in the internal tissues of plants and occur in almost every plant on Earth, from the Arctic to the tropics. The endophyte has a host relationship that is described as a symbiotic relationship, like mutualism, commensalism, or parasitism. The study showed a complete endophytic extract using the GC–MS, indicating the presence of different volatile molecules in the endophytic extract. The HPLC of the endophytic extract showed the presence of caffeine. The FTIR analysis of the different types of functional groups present in the *Trichoderma sps*. These functional groups are used in the manufacturing of antibiotics and antifungal drugs. The GC-MS of the endophytic extract showed the presence of 59 compounds, which contain anticancer properties. These compounds are used as an anticancer activity for further study.

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