



ANTIMICROBIAL PROPERTY AND GC-MS ANALYSIS OF *XYLARIA CARPOPHILA* (Pers.) Fr.

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ABSTRACT

The sporocarps of *Xylaria carpophila* were collected from fruits of *Xylia xylocarpa*. Physicochemical were analysed and results revealed highest percentage of water soluble extractives followed by ash content. Alcohol soluble extractive were 10.76%, total moisture content (5.9%) and foreign matter (0.6 %). Extraction was done by Soxhlet apparatus using petroleum ether, chloroform and ethanol, the extract were subjected for qualitative phytochemicals analysis revealed the presence of alkaloids, tannins, flavonoids, sterols, glycosides, terpenoids and phenols. GC-MS analysis of ethanoic extract showed many bioactive compounds. Anti-microbial potentials were studied against pathogenic bacteria and fungal strains. Results revealed the extracts were effective against bacteria but failed to show inhibitory activity against the tested fungal strains.

KEYWORDS: *Xylariaceae, Physicochemical analysis, Phytochemicals, Extraction, Secondary metabolites.*



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INTRODUCTION

Xylariaceae members are the promising source of secondary metabolites and having unique pigments in their stromata and in cultures¹. Unlike many genera in ascomycetes *Xylaria* Hill ex Schrank, has received an attention on its secondary metabolites over past twenty-five years². Previous studies revealed that the secondary metabolites possess a variety of C-skeletons, and most of them are having bioactive compounds like sesquiterpines and N- containing compounds which are primary chemical constituents of *Xylaria*. The same isolates showed diverse pharmacological properties, including cytotoxic, antimicrobial, antifungal, anthelmintic, antiviral and antimalarial activities³. In the recent investigation on the cultures of *Xylaria carpophila* (Pers.) Fr.⁴ resulted in the isolation of xylocarpins A-E and five new sesquiterpenes. Secondary metabolites extracted of *Xylaria* are biologically very active⁵. The present investigation will highlight on the antimicrobial activity of *Xylaria carpophila* against pathogenic bacteria and fungi with the confirmation of bio-constituents in different extracts along with the physicochemical parameters of sporocarp and GC-MS analysis of ethanolic extract.

MATERIAL AND METHODS

Sporocarp of *Xylaria carpophila* were collected from fruits of *Xylaria xylocarpa* from Jambekoppa of Sagara taluk, Shivamogga district, Karnataka between August to October 2016. Collected samples were studied for their morphological and anatomical characters. Classical taxonomy was followed for the identification⁶.

Determination of Foreign Matter

One gram of sample was weighed and foreign matter was carefully separated. The matter differing in colour and texture were considered as foreign. The separated matter was weighed and subtracted from one gram and percentage was calculated.

Determination of Moisture Content

One gram of powder was weighed and dried at 80°C for 24 hours in hot air oven. After 24 hours the powder was weighed again and the difference in the weight was determined. The percentage of moisture was calculated.

Determination of pH

The 5% (w/v) (5 g in 100 ml of water) powdered *X. carpophila* was kept on shaker for 5 hours with 140 rpm and filtered. The filtrate was analysed for the pH using pH meter⁷.

Determination of Water Soluble and Alcohol Soluble Extractive

Five grams of powder was taken in a 100 ml conical flask. Twenty-five ml of distilled water was added to it and kept on a rotatory shaker (140 rpm) for 24 hours. After 24 hours it was filtered and dried in hot air oven at 80°C for 24 hours and weighed again. The difference in weight was determined and percentage of water soluble extractive were calculated. Alcohol soluble extractives were estimated as per the modified procedure⁸.

Determination of Total Ash Content

A clean and dried silica crucible was weighed. Ten gram of powder was taken and kept in muffle furnace and heated up to 300°C for 3-4 h until the whole powder turns into ash. The crucible was cooled and weighed again. The difference in the weight was calculated^{8,9}.

Determination of Water Soluble Ash and Acid Insoluble Ash.

One gram powder was added to a clean conical flask containing 10 ml of distilled water. The mixture was kept on a shaker with 140 rpm for 8h and filtered through ash less filter paper. The residue remained in the paper was kept in a crucible (silica) and subjected to muffle furnace for 3-4hour. The weight of ash obtained was noted and percent of water soluble ash was determined. Acid insoluble ash was determined by using the standard procedure¹⁰.

Preparation of extracts

The sporocarp of *Xylaria carpophila* were shade dried to remove moisture content, dried samples were grinded manually to make coarse powder. Three hundred grams of material was subjected to Soxhlet extraction for 24 hours for each solvent. Three solvents (petroleum ether, chloroform and ethanol) were used for extraction of metabolites(solvents were used based on their polarity) The dissolved extracts were concentrated under reduced pressure in a rotatory evaporator before being transferred to petri dishes for complete evaporation¹¹.

Qualitative Phytochemical Screening

A preliminary qualitative phytochemical screening was done for secondary metabolites like alkaloids, flavonoids, glycosides, phenolic, steroids, tannins, triterpenes and saponins according to the Herborne method¹².

Antimicrobial activity

The extracts were tested against pathogenic bacteria and fungi. The test organisms were collected from the microbial type culture collection (MTCC). Chandigarh. India.

Bacteria

Xanthomonas campestris [MTCC-2286], *Pseudomonas syringae* [MTCC-1604], *Agrobacterium tumefaciens* [MTCC-431], *Klebsiella pneumonia* [MTCC- 7028], *Escherichia coli* [MTCC-1599], *Salmonella typhi* [MTCC-734], *Pseudomonas aeruginosa* [MTCC-1934], *Staphylococcus aureus* [MTCC-4734].

Fungi

Candida albicans (C.P. Robin) Berkhout, [MTCC- 1637], *Chrysosporium merdarium* (Link) J.W. Carmich., [MTCC-4608], *Trichophyton rubrum* (Castell.) Sabour., [MTCC-3272], *Cryosporium keratinophilum* D.Frey ex J.W.Car [MTCC-1367], *Fusarium solani* (Mart.) Sacc., [MTCC- 1040], *Penicillium chrysogenum* Thom, [MTCC-947].

Agar Well diffusion method

Antibacterial and antifungal activity of the sporocarp extracts were tested using Agar well Diffusion Method¹³.The prepared culture plates were inoculated

with test bacteria and fungi. Wells were made using 6 mm cork borer. The extracts were dissolved in dimethyl sulfoxide (DMSO) of different concentration (100%, 50%, and 25%) and wells were loaded with extracts using micro pipette. Ciprofloxacin and Terbinafine were used as standard for bacteria and fungi respectively. DMSO was used as control for test microorganisms. The plates were incubated at $37\pm 20^\circ\text{C}$ for 24 hours for bacterial activity and 78 hours for fungal activity. The zone formation was observed in plates around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around¹⁴. The reading also includes the well diameter.

STATISTICAL ANALYSIS

The readings were taken in 4 replicates and the average values were tabulated and the results were expressed in mean \pm sd.

GC-MS analysis

Ethanol extract was subjected to gas Chromatography and mass spectroscopy (GC Model: Thermo Trace GC Ultra, MS Model: Thermo DSQ II, at Vittal mallya scientific research foundation, Bangalore). The total run time was 33 minutes. The oven temperature was raised from 60°C to 250°C with the raise rate of 80°C per min. The identification of compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds in their library and the name, molecular weight and structure were determined.

RESULTS AND DISCUSSION

Xylaria carpophila was morphologically characterised by erect Stroma, simple, 5-8 cm long, 0.3 cm broad, gregarious or in clusters, black, minute hairs at base, rough surface, fertile portion is bulged containing ascospores, apex rounded, perithecia globose, ascospores brown with obtuse ends, $10-14.5 \times 4-5\mu\text{m}$ (Figure-1). Physicochemical analysis (Table-1) revealed that sample was found to contain high percentage of water soluble extractives (15.68%) followed by 11.2% total ash content, in total ash content water soluble ash was 92% and 18% of acid insoluble ash. Alcohol soluble extractive was 10.76%, total moisture content (5.9%) and foreign matter (0.6%). pH of 5 % w/v solution of aqueous extract was 7.29. Many bioactive compounds with potent health enhancing properties have been isolated from medicinal mushrooms⁵. Preliminary biochemicals screening from crude extract of *X. carpophila* showed presence of alkaloids, tannins, flavonoids, sterols, glycosides, terpenoids and phenols. Saponins were completely absent in all the solvent extracts and alkaloids and tannins are absent in the ethanolic extract (Table-2). All the extracts were

potential against the tested pathogenic bacteria (Table-3, Figure-3). Petroleum ether extract showed the positive results for alkaloids, tannins, flavonoids, steroids, glycosides, terpenoids and phenols. Pet-ether extract inhibited *Klebsiella pneumonia* at maximum ($22.66\pm 0.57\text{mm}$) followed by *Agrobacterium tumefaciens* ($16.66\pm 0.57\text{mm}$), *Escherihia coli* ($14.33\pm 0.57\text{mm}$) and *Staphylococcus aureus* ($9\pm 1\text{mm}$) but, *Pseudomonas syringae*, *Salmonella typhi* and *Xanthomonas campestris* did not show any effect. Biochemical analysis of chloroform extract showed positive results for alkaloids, tannins, flavonoids, sterols, glycosides, terpenoids and phenols. Chloroform extract inhibited bacterial pathogen *Pseudomonas aeruginosa* ($19.33\pm 1.15\text{mm}$) at maximum, followed by *Pseudomonas syringae* ($17.66\pm 0.57\text{mm}$), *Agrobacterium tumefaciens* ($17.33\pm 0.57\text{mm}$), *Escherihia coli* ($14.33\pm 0.57\text{mm}$), *Klebsiella pneumonia* ($11.66\pm 0.57\text{mm}$) and *Xanthomonas campestris* ($8\pm 1\text{mm}$) but *Staphylococcus aureus* showed resistance to the chloroform extract. Ethanolic extract of *X. carpophila* showed the presence of flavonoids, sterols, glycosides, terpenoids and phenols in qualitative biochemical analysis. Antibacterial activity of ethanolic extract revealed the positive effect on inhibiting *Pseudomonas aeruginosa* ($27.66\pm 0.57\text{mm}$) at maximum, followed by *Escherihia coli* ($19.66\pm 0.57\text{mm}$), *Salmonella typhi* ($17.66\pm 0.57\text{mm}$), *Agrobacterium tumefaciens* ($16\pm 1\text{mm}$), *Klebsiella pneumonia* ($14.33\pm 0.57\text{mm}$), *Pseudomonas syringae* ($14\pm 0\text{mm}$), *Xanthomonas campestris* ($7.66\pm 1.52\text{mm}$) and *Staphylococcus aureus* ($8\pm 1\text{mm}$). All the extracts showed concentration dependent zone of inhibition against tested pathogenic microbes, due to the presence of bioactive compounds present in it. Antimicrobial activity of *Ganoderma* spp. showed positive effect inhibiting *Pseudomonas aeruginosa*¹⁴ hence *X. carpophila* also explored as an antibacterial agent by pharmaceutical industry in extracting effective compound. The antifungal activities of crude mushroom extracts were generally low¹⁵. All the extracts were failed to inhibit the tested fungal organisms, which is similar to the findings of antimicrobial activity of *Microporus xanthopus*¹⁶. Ethanolic extract of *X. carpophila* was subjected to GC-MS analysis and chromatograms clearly showed 44 peaks and each peak indicates the bioactive compound (Figure-2). The bioactive compounds recognised through GC-MS analysis showed many biological activity are listed in (Table-4). The major compound includes Ergosterol, Octadeca-9, 12-Dienoic acid methyl ester, Hexadecanoic acid, N, N Dimethyl acetoacetamide and 4, 5-Epoxy pentenal (Table-5). Similar compounds were reported from the fruiting bodies of *Xylaria Polymorpha*¹⁷.

Table 1
Physicochemical analysis of *X. carpophila*.

Parameter	Percentage results
Foreign matter	0.6
Moisture content	5.9
Water soluble extractive	15.68
Alcohol soluble extractive	10.76
pH of 5 % w/v solution of aqueous extract	7.29

Total ash content	11.2
Water soluble ash	92
Acid-insoluble ash	18

Table 2
Phytochemical constituents of *X. carpophila*.

Constituents	Test name	Inference		
		Petroleum ether	Chloroform	Ethyl alcohol
Alkaloids	Mayer's test	+	+	-
Saponins	Foam test	-	-	-
Tanins	Gelatin test	+	+	-
Flavonoids	Lead acetate solution test	+	+	+
Steroids	Salkowaski's test	+	+	+
Glycosides	Keller-Killiani's test	+	+	+
Terpenoids	Salkowaski's test	+	+	+
Phenols	Ferric chloride test	+	+	+

Table 3
Antibacterial activity of *X. carpophila*.

SI No.	Organism	Zone of inhibition in mm									Standard
		Petroleum ether			Chloroform			Ethanol			
		100	50	25	100	50	25	100	50	25	
1	<i>Klebsiella pneumonia</i>	22.66±0.57	18.66±0.57	14±1	11.66±0.57	11.33±0.57	9±1	14.33±0.57	12±1	10±1	26.66±1.5
2	<i>Salmonellatyphi</i>	0	0	0	7.66±0.57	4.66±0.57	0	17.66±0.57	14±1	13.33±1.52	23.66±1.15
3	<i>Staphylococcus aureus</i>	9±1	6.66±0.57	0	0	0	0	8±1	0	0	27±1.73
4	<i>Pseudomonas aeruginosa</i>	9±1	8.33±0.57	8±1	19.33±1.15	11±1	9.33±0.57	27.66±0.57	14.66±0.57	12.66±1.52	27.33±0.57
5	<i>Pseudomonas syringae</i>	0	0	0	17.66±0.57	15.66±0.57	13.66±1.52	14±0	13±1	12.33±1.52	24±1
6	<i>Xanthomonas campestris</i>	0	0	0	8±1	0	0	7.66±1.52	5.66±0.57	5±1	24.33±2.08
7	<i>Escherihia coli</i>	14.33±0.57	13.33±1.15	11.66±0.57	14.33±0.57	13±1	12.33±0.57	19.66±0.57	11±1	9.66±1.52	21.33±0.57
8	<i>Agrobacterium tumefaciens</i>	16.66±0.57	14.33±0.57	7.66±0.57	17.33±0.57	14±1	13.66±1.15	16±1	14.33±0.57	11.66±1.52	30.33±2.51

Table 4
Bioactive compounds identified in ethanol extract and their biological activity.

SI No	Name of the compound	Percentage composition	biological activity reported
1	2(3H)-Furanone, dihydro- (CAS) Butyrolactone	0.50	It is used as a recreational intoxicant with effects similar to alcohol ¹⁸ .
2	Propane, 1,1-diethoxy-2-methyl-	0.42	No Activity reported.
3	2-methyloxazole	0.94	No Activity reported.
4	2H-Pyran-2-one, 5,6-dihydro-	0.31	No Activity reported.
5	5,6-Dihydro-2H-pyran-2-one	3.11	Anti-tumorous agents ¹⁹ .
6	4,5-Epoxy-pentenal	4.00	No Activity reported.
7	1,3,5-Triazine-2,4,6-triamine (CAS) 2,4,6-Triamino-s-triazine	1.10	Used as a biomarker ²⁰ .
8	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (CAS) 3,5-dihydroxy-2-methyl-5,6-D	1.77	Antimicrobial ²¹ .
9	2-Hexenoic acid, 5-hydroxy	2.72	Antioxidant ²² .
10	Butanal, 3-hydroxy-	0.78	No Activity reported.
11	N,N- Dimethyl acetoacetamide	5.84	No Activity reported.
12	5-oxo-pyrrolidine-2-carboxylic acid methyl ester	7.25	Anti-inflammatory, antiarthritis ²³ .
13	2-Pyrrolidinecarboxylic acid-5-oxo-, ethyl ester	0.57	No Activity reported.
14	D-Glucitol, 1,4-anhydro-	2.00	No Activity reported.
15	4-Methyl-3-(2-methylpropyl)-6-isopropyl-2,5-dioxomorpholine	0.59	No Activity reported.
16	methyl ester of N-isovaleryl-L-leucine	0.32	Dietary source ²⁴ .
17	Tetradecanoic acid	0.74	Antimicrobial and antioxidant activity ²⁵ .
18	2a,3,4,5-Tetrahydrobenz(cd)indol-2(1H)-one	1.03	No Activity reported.
19	Pentadecanoic acid.	1.23	No Activity reported.
20	Pentadecanoic acid, 14-methyl-, methyl ester (CAS) methyl 14-methyl-pentadecanoate	0.54	Insect repellent ²⁶ .
21	Citronellol-epoxid (R oder S)	0.60	No Activity reported.
22	Hexadecanoic acid (CAS) Palmitic acid	10.46	No Activity reported.

23	allo inositols	1.38	No Activity reported
24	Heptadecanoic acid (CAS) Margaric acid	0.54	No Activity reported
25	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS) Methyl linoleate	1.06	Flavouring ²⁷ .
26	octadeca-9,12-dienoic acid methyl ester	12.70	No Activity reported
27	9-Octadecenoic acid (Z)- (CAS) Oleic acid	1.77	No Activity reported
28	5-methyl-3-(1-methylvinyl)-1,4-hexadiene	0.69	No Activity reported
29	Santalol, cis-, alpha.-	0.32	Perfumes ²⁸ .
30	bicyclo[2.2.1]heptane, 2-ethylidene-1,7,7-trimethyl-	1.07	No Activity reported.
31	alpha.-Santalol	0.20	Chemo preventive agent ²⁹ .
32	Cyclohexaneethanamine, N-.alpha.-dimethyl-(CAS) Cyclohexaneethanamine, N,.alpha.-dimethyl	1.01	No Activity reported.
33	Santolina triene	1.11	Antimicrobial ³⁰ .
34	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2.17	Hemolytic, pesticide, flavour, antioxidant ³¹ .
35	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate	1.04	No Activity reported.
36	Cholesta-6,22,24-triene, 4,4-dimethyl-	0.80	Antibacterial, Trypanocidal activity ³² .
37	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester (CAS) 1-Monolinolein	6.64	No Activity reported.
38	9(11)-Dehydroergosteryl benzoate	1.53	No Activity reported.
39	1-Eicosanol (CAS) n-Eicosanol	0.65	No Activity reported.
40	Dehydroergosterol 3,5-dinitrobenzoate	1.06	No Activity reported.
41	Ergosterol	14.20	Antimicrobial ³³ .
42	Ergosta-5, 8-dien-3-ol, (3.beta.)-	0.93	No Activity reported.
43	Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) Clionasterol	1.58	Used to treat Hyperlipidemias ³⁴ .
44	Ergosta-4,6,8(14),22-tetraen-3-one	0.74	Cytotoxic ³⁵ .

Table 5
Major compound found in *X. carpophila* of ethanolic extract.

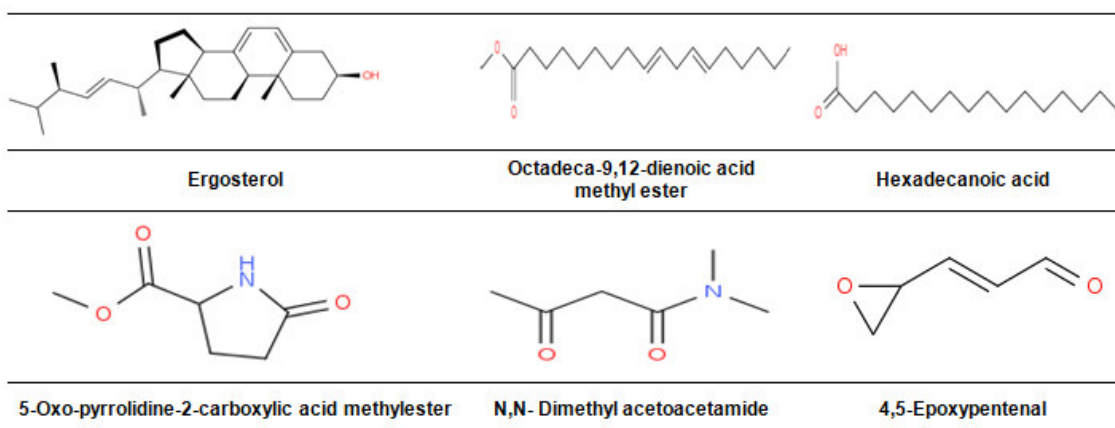


Figure 1
Sporocarp of *X. carpophila* on substratum.

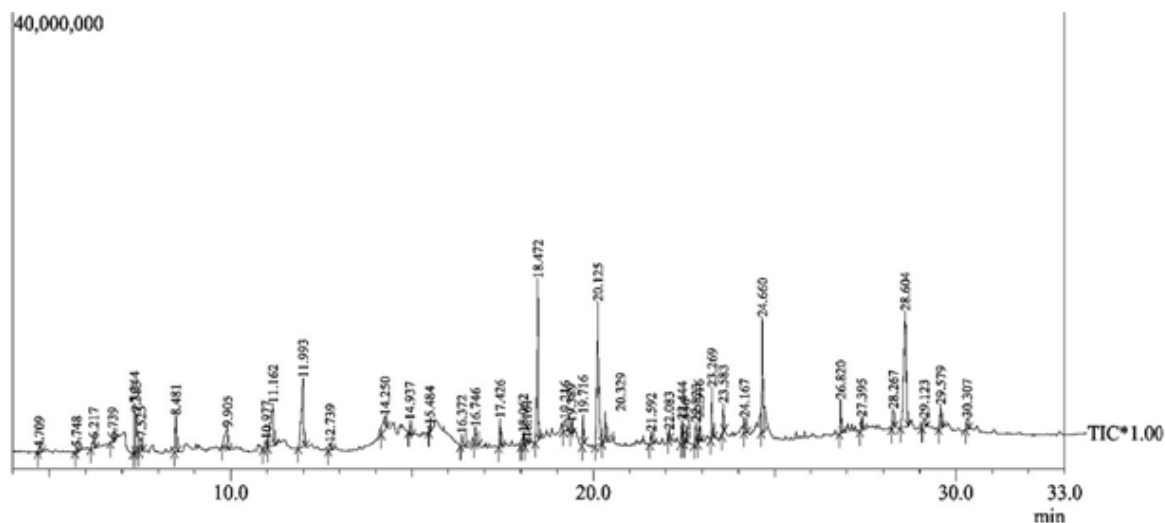


Figure 2
Chromatogram showing the compounds present in ethanol extract of *X. carphophila*.

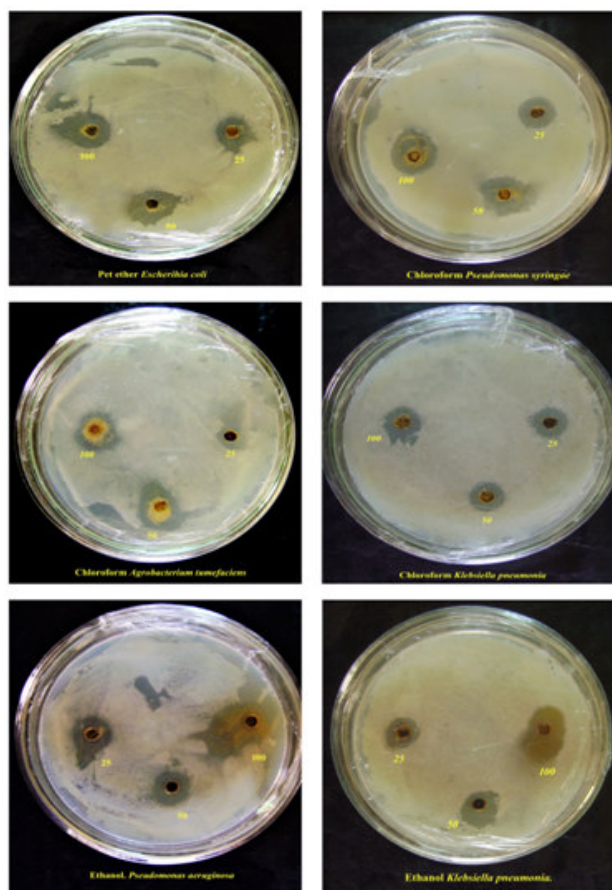


Figure-3
Antibacterial activity showed by Pet ether, Chloroform and Ethanolic extracts.

CONCLUSION

Xylariaceae members contains various bioactive constituents. In the present investigation, phytochemical analysis and GC-MS evaluation revealed *X. carphophilla* contains good number of secondary metabolites. All the extracts were effective against tested bacteria and fungal growth did show any effect. Isolation of pure

compounds and pharmacological activity work is in progress.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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