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# 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, terpinoids 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 cultures[1]. Unlike many genera in ascomycetes *Xylaria* Hill ex Schrank, has received an attention on its secondary metabolites over past twenty-five years[2]. Previous studies revealed that the secondary metabolites possess a variety of C-skeletons, and most of them are having bioactive compounds like sesquiterpins and N- containing compounds which are primary chemical constituents of Xylaria. The same isolates showed diverse pharmacological properties, including cytotoxic, antimicrobial, antifungal, anthelmintic, antivirus and antimalarial activities[3]. 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 active[5]. The present investigation will limelight 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 *Xylia 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 fallowed for the identification[6].

***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 meter [7].

***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 800C 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 procedure[8].

***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 procedure[10].

***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 evaporation [11].

***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 method [12].

***Antimicrobial activity***

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

***Bacteria***

*Xanthomonas campestris* [MTCC-2286], *Pseudomonas syringae* [MTCC-1604], *Agrobacterium tumefaciens* [MTCC-431], *Klebsiella pneumonia* [MTTC- 7028], *Escherihia 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], *Crysosporium 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 Method[13].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±20c 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 around[14]. 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±sd.

***GC-MS analysis***

Ethanolic 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 × 4-5µm (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 mushrooms[5]. Preliminary biochemicals screening from crude extract of *X. carpophila* showed presence of alkaloids, tannins, flavonoids, sterols, glycosides, terpinoids 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, terpinoids and phenols. Pet-ether extract inhibited *Klebsiella pneumonia* at maximum (22.66±0.57mm) followed by *Agrobacterium tumefaciens* (16.66±0.57mm), *Escherihia coli* (14.33±0.57mm) and *Staphylococcus aureus* (9±1mm) 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, terpinoids and phenols. Chloroform extract inhibited bacterial pathogen *Pseudomonas aeruginosa* (19.33±1.15mm) at maximum, followed by *Pseudomonas syringae* (17.66±0.57mm), *Agrobacterium tumefaciens* (17.33±0.57mm), *Escherihia coli* (14.33±0.57mm), *Klebsiella pneumonia* (11.66±0.57mm) and *Xanthomonas campestris* (8±1mm) but *Staphylococcus aureus* showed resistance to the chloroform extract. Ethanolic extract of *X. carpophila* showed the presence of flavonoids, sterols, glycosides, terpinoids and phenols in qualitative biochemical analysis. Antibacterial activity of ethanolic extract revealed the positive effect on inhibiting *Pseudomonas aeruginosa* (27.66±0.57mm) at maximum, followed by *Escherihia coli* (19.66±0.57mm), *Salmonella typhi* (17.66±0.57mm), *Agrobacterium tumefaciens* (16±1mm), *Klebsiella pneumonia* (14.33±0.57mm), *Pseudomonas syringae* (14±0mm), *Xanthomonas campestris* (7.66±1.52mm) and *Staphylococcus aureus* (8±1mm). 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 low[15]. 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. carphophilla* 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-Epoxypentenal (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 | + | + | + |
| Terpinoids | Salkowaski's test | + | + | + |
| Phenols | Ferric chloride test | + | + | + |

**Table 3**
***Antibacterial activity of X. carpophila.***

| Sl No. | Organism | Zone of inhibition in mm | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Petroleum ether | | | Chloroform | | | Ethanol | | | Standard |
| | | 100 | 50 | 25 | 100 | 50 | 25 | 100 | 50 | 25 | |
| 1 | *Klebsiella pneumonia* | 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 | *Salmonellatyphi* | 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 | *Staphylococcus aureus* | 9±1 | 6.66±0.57 | 0 | 0 | 0 | 0 | 8±1 | 0 | 0 | 27±1.73 |
| 4 | *Pseudomonas aeruginosa* | 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 | *Pseudomonas syringae* | 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 | *Xanthomonas campestris* | 0 | 0 | 0 | 8±1 | 0 | 0 | 7.66±1.52 | 5.66±0. 57 | 5±1 | 24.33±2.08 |
| 7 | *Escherihia coli* | 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 | *Agrobacterium tumefaciens* | 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.***

| Sl 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-Epoxypentenal | 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 | Citronellolepoxid (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.***

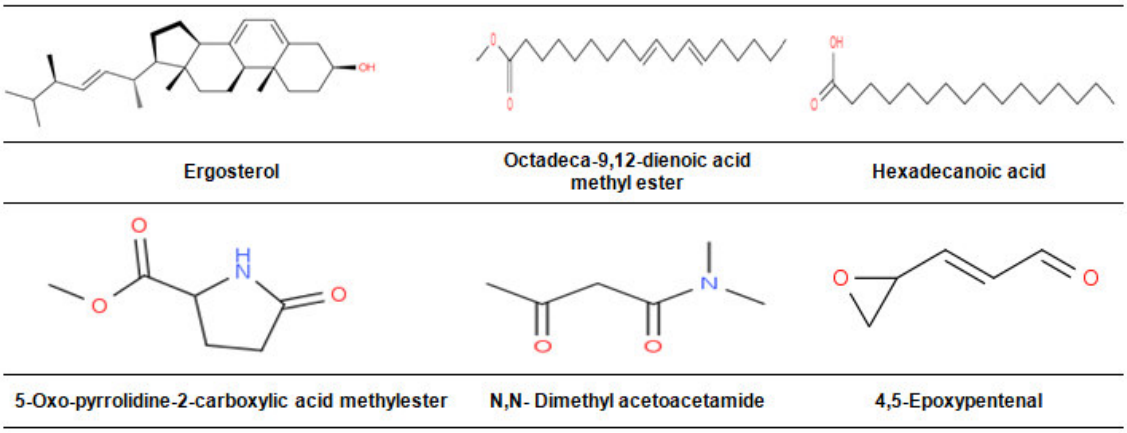


**Figure 1**
**Sporocarp of *X. carpophila* on substratum.**

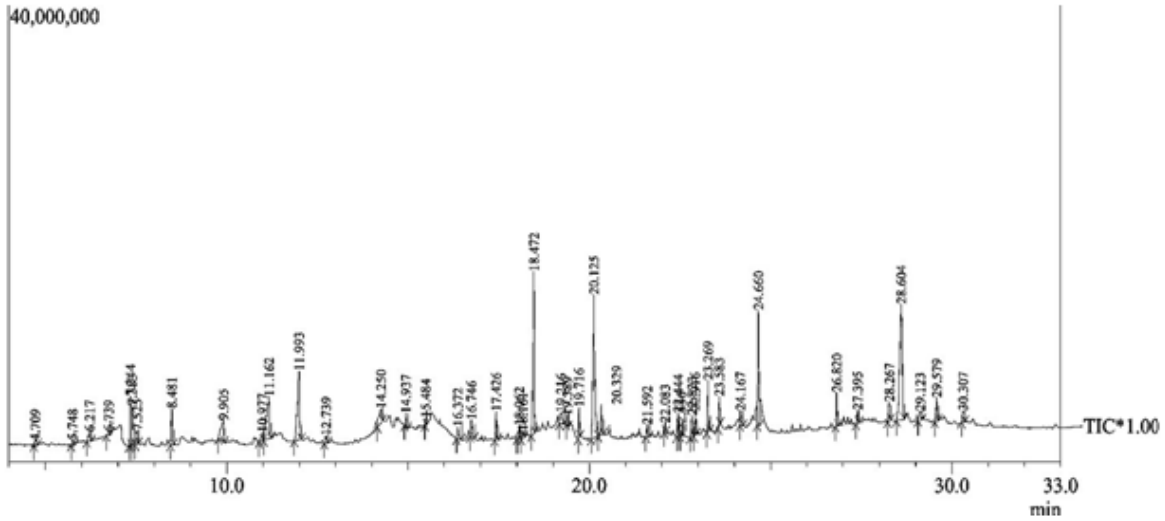


**Figure 2**
***Chromatogram showing the compounds present in ethanol extract of X. carpophila.***

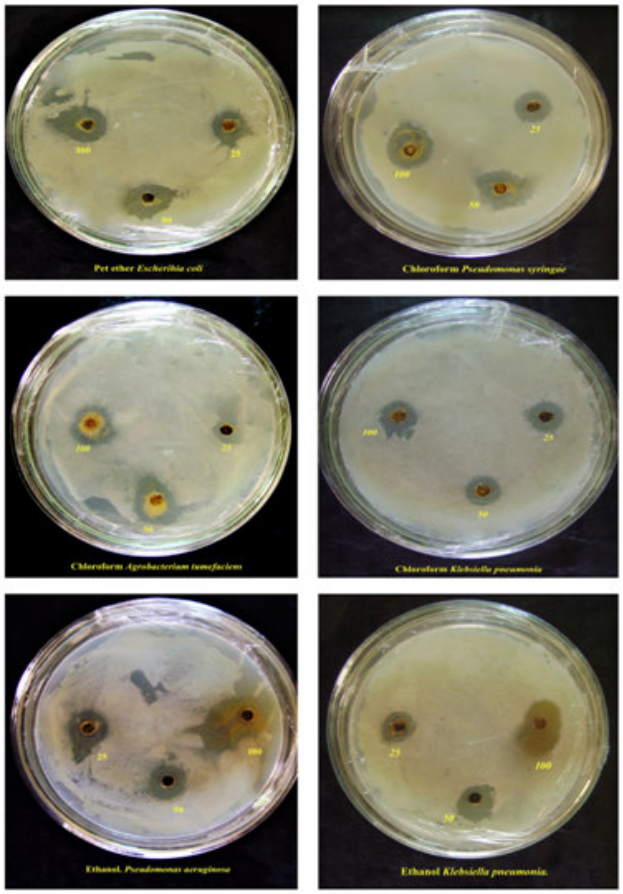

**Figure-3**
***Antibacterial activity showed by Pet ether, Chloroform and Ethanolic extracts.***

## 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.

## REFERENCES


1. Stadler M, Yu-Ming JU and Rogers JD. Chemotaxonomy of *Entonaema, Rhopalostroma* and other Xylariaceae. Mycol. Res. 2004;108:239–256.
2. Whalley AJS, Edwards RL. The Xylariaceae: A case study in biological and chemical diversity. Pure Appl. Chem. 1998;70:1-11.
3. Song F, Wu SH, Zhai YZ, Xuan QC, Wang T. Secondary metabolites from the genus *Xylaria* and Their Bioactivities. Chemistry & Biodiversity. 2014;11:673-694.
4. Yin X, Feng T, LI ZH, Jsu J, Ll Y, Tan N H. Chemical investigation on the cultures of the fungus *Xylaria carpophila*. Nat. Prod. Bioprospect.2011; 1:75–80.
5. Ramesh V, Karunakaran C, Rajendran A. Evaluation of synergistic and antibacterial activity of Xylaria curta against drug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, Mycology.2012; 3(4): 252-257.
6. Pande A. Ascomycetes of peninsular India. Scientific publisher (India). 2008.
7. Iqbal D, Raju K, Pawar, Rajeev KR, Sharma. Physico-chemical standardization of *Butea monosperma* (Lam.) Kuntze (Palasha): An ayurvedic drug. Int journal of pharma quality assurance. 2010; 2:49-51.
8. Gupta AK. Quality standards of Indian medicinal plants. Indian council of medicinal research, India. 2003.
9. Indrayan AK, Sharma S. Durgapal D. Kumar N, Kumar M. Valued plants from Uttaranchal. Current Sci. 2005; 89:1252-1255.
10. Ahmad RV, Sharma RK. Proceedings of WHO training cum-workshop, Evaluation of drug for standardization. Pharmaceutical lab for Indian medicine, Ministry of health and family welfare, Govt. of India, Ghaziabad. 2001.
11. Jayashree KK, Krishnappa M. Screening of *Entoloma speculum* for antimicrobial properties. Int J Pharm Bio Sci. 2017;8:289-293.
12. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Int Pharma Sci. 2011;1(1):98-106.
13. Das K, Tiwari RKS, Shrivastava. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of med Plants Research. 2009;4:104-111.
14. Jonathan SG, Awotona FE. Studies on Antimicrobial potentials of three *Ganoderma* species. Afr. J. Biomed. Res. 2010;13:133-139.
15. Jonathan S. Gbolagade. Ishola O, Fasidi. Antimicrobial activity of some selected Nigerian mushrooms. Afr J. Biomed Res. 2005;8:83-87.
16. Nataraja S, Meghalatha R , Krishnappa M. Preliminary Phytochemical Screening, Physicochemical Studies, Antimicrobial Activities of a Wild Fungus, *Microporus xanthopus* (Beav: Fr.) Kuntzee. Inventi Rapid: Planta Activa.1-4, Nat. Prod. Bioprospect. 2014; 2:1-4.
17. Jang YW, Lee I, Kim Y, Lee S, Lee HJ, Yu SH. Xylarinic acids A and B, new antifungal polypropionates from the Fruiting Body of *Xylaria polymorpha*. J. Antibiot. 2007; 60: 696–699.
18. Schrock A, Hari Y, Konig S, Auwarter V, Schurch S, Weinmann W. Pharmacokinetics of GHB and detection window in serum and urine after single uptake of a low dose of GBL - an experiment with two volunteers. Drug Test Anal, 2013;1-4.
19. Kralove H, Monosubstituted 5, 6-dihydro-2H-pyran-2-ones. Natural occurrence, biological activity and synthetic approaches. Univerzita Karlova v praze farmaceuticka fakulta v hradci kralove. Rigorous thesis.2009.
20. Ronald E. Baynes, Smith G, Sharon E. Mason, Barrett E, Beth M. Barlow, Jim E. Riviere. Pharmacokinetics of melamine in pigs following intravenous administration. Food and Chemical Toxicology, 2008;46:1196–1200.
21. Gopalakrishnan K, Udayakumar R. GC-MS Analysis of Phytocompounds of Leaf and Stem of *Marsilea quadrifolia* (L.). Int Journal of Biochem Res& review 2014; 4(6):517-526.
22. Ahmed G. Hegazi, Faten K, El-Hady A. Influence of Honey on the Suppression of Human Low Density Lipoprotein (LDL) Peroxidation (In vitro). ecam 2009;6(1):113–121.
23. Mathur M, Kamal R. Studies on trigonelline from *Moringa oleifera* and its in vitro regulation by feeding precursor in cell cultures. Brazilian Journal of Pharmacognosy.2012; 22(5):994-1001.
24. Feugang J, Konarski P, Zou D , Stintzing F, Zou C. Nutritional and medicinal use of *Cactus pear* (Opuntia spp.) cladodes and fruits. Frontiers in Bioscience. 2006;11: 2574-2589.
25. Preethi R, Devanathan V, Loganathan M. Antimicrobial and antioxidant Efficacy of Some Medicinal Plants against Food Borne Pathogens. Adv in Bio Research. 2010;4 (2):122-125.
26. Sunday E. Atawodi, Joy C, Atawodi. *Azadirachta indica* (neem): a plant of multiple biological and pharmacological activities. Phytochem Rev.2009;8:601–620.
27. https://pubchem.ncbi.nlm.nih.gov/compound/methyl_linoleate
28. https://pubchem.ncbi.nlm.nih.gov/compound/5281531#section=Food-Additives-and-Ingredients
29. Kaur M, Agarwal C, Singh R , Guan X , Dwivedi C, Agarwal R. Skin cancer chemopreventive agent, a-santalol, induces apoptotic death of human epidermoid carcinoma A431 cells via caspase activation together with dissipation of mitochondrial membrane potential and

cytochrome c release. Carcinogenesis. 2005; 26(2):369-380.
30. Shazly A. Hafez S, Wink M. Comparitive study of the essential oils and extracts of *Achillea fragrantissima*(Forssk.)Sch.Bip. and *Achillea santolina* L. (Asteraceae) from Egypt. Pharmazie. 2004; 59:226-230.
31. Tyagi T and Agarwal M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. Journal of Pharmacognosy and Phytochemistry. 2017; 6(1): 195-206.
32. Dandekar R, Fegade B, Bhaskar VH. GC-MS analysis of phytoconstituents in alcohol extract of *Epiphyllum oxypetalum* leaves. Journal of Pharmacognosy and Phytochemistry. 2015;4(1):149-154.
33. https://en.wikipedia.org/wiki/Ergosterol
34. https://pubchem.ncbi.nlm.nih.gov/compound/Clionasterol#section=Pharmacology-and-Biochemistry
35. Lee W, Park Y, Ahn J, Park S, Lee H. Cytotoxic Activity of Ergosta-4, 6, 8(14), 22-tetraen-3-one from the Sclerotia of *Polyporus umbellatus*. Bull. Korean Chem. Soc. 2005; 26(9):1464-1466.

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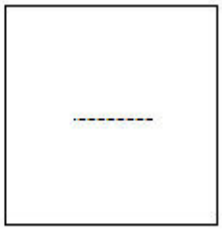


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