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Screening for bioactivity of *Mutinus elegans* extracts

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Abstract. *Mutinus elegans* is a species of fungi that is commonly called as Elegant Stinkhorn. The aim of this study was to screen the crude extracts of the fungus for phytochemical analysis, antimicrobial activity, antioxidant assay and anticancer activity. Extraction of the fungal sample in Soxhlet apparatus was done with *n*-hexane and methanol as the solvent. Stock solutions of the crude methanol extract were prepared and used for microbiological assay. Thin layer chromatography was performed in order to determine the number of active components in *n*-hexane, and methanol solvent system for the fungus *Mutinus elegans.* Further, antioxidant assay was performed using DPPH radical scavenging assay. The fungal sample was then tested for cytotoxicity assay against MG63 osteosarcoma cell lines. The antimicrobial assay of *Mutinus elegans* extract exhibited activity against five pathogens. The zone of inhibition was measured with respect to standard antibiotics. Gas chromatography and Mass spectrometry (GC/MS analysis), revealed the presence of dibromo-tetradeca*n*-1-ol-acetate, 2-myristynoyl-glycinamide, fumaric acid, and cyclohexylmethyldecyl ester compounds were presented in methanol and *n*-hexane extract of *Mutinus elegans*. The present study concludes the presence of bioactive compound in the extract which exhibited antimicrobial and antioxidant activity in *Mutinus elegans.*

1. Introduction

The search for biologically active compounds with potential pharmaceutical activity from natural sources is crucial for drug discovery [1-4]. Scientists have scrutinized only a small percentage of fungal species so far (about 100,000) and an even smaller number explored for their metabolites with medicinal properties. However, secondary metabolites from fungus have been developed as drugs against various diseases and used also as agrochemical fungicides. Drugs such as statin which is used against cholesterol, antibiotics, fungicides and immunosuppressive agents are being obtained from fungus [3,5,6]. Mushroom forming fungi [3,7,8] are recognized to be prolific producers of bioactive compounds and traditionally believed as a remedy for many diseases [9-11]. Countries in Asia for centuries have been using mushrooms extensively for centuries in combating numerous ailments [2, 3, 8, 11, 12].

Mutinus elegans, also called as the devil's dipstick, and dog stinkhornit belongs to family Phallaceae. It is a saprobic fungal species, habitually grows in the soil on leaf litter or woody debris. The fungi commence its development in the form of an "egg". When *Mutinus elegans* approaches maturity, a pinkish orange thin stalk springs up from the ground and it tapers to an arrow like tip. Slimy, sticky green liquid is secreted on the stalk which consists of the spores. The slime emits a characteristic foul smell which attracts flies and other insects which feed on it and assist in dispersal. Coletto and Lelli, [13] reported bioactivity of 32 basidiomycete fungi. Among them, devil's dipstick is one of the potent fungi which demonstrated both antibacterial as well as antifungal action against different pathogens. The study revealed that the *Mutinus elegans* possess strong antibacterial activity. Not much work has been

conducted on secondary metabolite of *Mutinus elegans* and its bioactivity of the extracts. Therefore, this present study was undertaken to identify the secondary metabolites, using two different solvent (non-polar, medium polar and highly polar), phytochemical analysis, antimicrobial activity, antioxidant assay and anticancer activity as well.

2. Materials and Methodology

2.1. Sample collection

Mutinus elegans was collected from Thanjavur, Tamil Nadu, India. The sample was collected in sterile polyethylene bags and transported to the laboratory. Further, the fungal sample was sun dried for 3-4 days until it reached a constant weight and was stored in airtight containers.

2.2. Soxhlet extraction

The dried fungal sample of *Mutinus elegans* was ground to coarse powder was prepared using a mixer grinder. 30 g of the powdered fungal sample was serially extracted in *n*-hexane and methanol using a Soxhlet apparatus for 4-5 h followed by filtration method. Dried filtered extracts was used in this study.

2.3. Phytochemical Screening of crude fungal extract

The phytochemical components of the fungus, *Mutinus elegans* was screened using the standard methods explained by Kamba and Hassan [15]. The phytochemical components analyzed were saponins, steroids, cardiac glycosides, quinones, tannins, flavanoids, alkaloids, phenols, terpenoids, carbohydrates and proteins.

2.4. Antibacterial assay

2.4.1. Agar well diffusion method. The antibacterial activity of fungal crude extracts of *Mutinus elegans* against selected clinical clinical pathogens was assessed [16].The antibacterial present in the fungal extract was allowed to diffuse through the Mueller-Hinton agar and interact with freshly inoculated pathogens. Briefly, on each bacterial inoculum was swab streaked with pathogens *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter* sp., *Pseudomonas aeruginosa* and *Proteus mirabilis* from nutrient broth with overnight incubation,which were obtained from Microbial Biotechnology Lab, VIT University. The wells were cut onto agar plates and different concentrations of fungal extract were introduced in the wells, i.e. 25, 50, 75, 100 µL (25 mg mL^{-1}) and kept for 24 h at 37 °C. The clear zone of inhibition was determined in mm. Clear zone of the fungal extracts and comparison with negative control was recorded [17].

2.5. Antioxidant analysis

The antioxidant *analysis* of the fungal extracts was calculated by DPPH radical scavenging method. Fungal extracts of *Mutinus elegans* with concentration 25 mg mL^{-1} was pipetted into 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution concentration in the ratio (1:1). The *analysis* was performed in dark at 517 nm. The percentage of the DPPH radicals scavenging was calculated using standard formula [18].

2.6. Anticancer Activity

MG63 cell lines were used to determine the cytotoxicity of *Mutinus elegans* using MTT assay. The anticancer activity was performed by the method described by Daniel et al. [19]

2.7. Analytical methods

2.7.1. Thin Layer Chromatography. Using pre-coated TLC plates, the crude extracts were fractionated using four different solvent systems. The first solvent was prepared by adding hexane, ethyl acetate and methanol in the ratio of 4:2:2. The second solvent was prepared by mixing hexane, formic acid and glacial acetic acid in 4:2:2 ratio. Third solvent system was prepared using hexane, acetone and ethyl acetate mixed in the ratio of 4:2:2 while the last solvent was prepared by mixing chloroform with methanol in 8:2 ratio. Solvent front was drawn on the pre-coated TLC sheet, with a pencil marked a line across the plate at 10 mm (=1 cm) from one end. The four freshly prepared solvents were added into glass beaker, labelled respectively and was closed with aluminium foil. A capillary tube was used to make a spot of the sample extract on the sheet and placed inside the beaker upright. Care was taken that the spot was above the solvent. As the solvent reached the marked solvent front line, the TLC sheet was removed and the end points of the visible pigments were marked respectively for each prepared solvents. The TLC sheet was then air dried and visualized under UV light source, using UV Trans-illuminator by fluorescence quenching less than 254 nm.

2.8. GC/MS

Fungal extracts of *Mutinus elegans* were determined by GC/MS. The analysis condition for GC/MS was carried out by standard method described by Gajendiran et al. [20].The secondary metabolite was identified based on the mass spectral database by using MS scan (m/z; 55-400).

3. Results and Discussion

3.1. *Phytochemical Analysis.*

The fungal extracts of *Mutinus elegans* (Figure 1) obtained after Soxhlet extraction by *n*-hexane and methanol were dried completely for further analysis. The crude extracts of *Mutinus elegans* were subjected to phytochemical studies to determine the presence of different bioactive compounds this was followed by GC/MS analysis.

Figure 1. Fungus *Mutinuselegans*.

Mutinuselegans methanol extract possess flavanoids, quinones, tannins, saponin and carbohydrates whereas *n*-hexane possess flavanoids, quinones, steroids, alkaloids, terpenoids. The presence of phytochemicals compounds are known to have pharmaceutical and therapeutic properties. Saponins and flavonoids were present in all the extracts. Saponins are mild detergent with therapeutic effects such as anticancer, hypercholestrolemic, antioxidant activity [21].

3.2. Thin Layer Chromatography

Only one spot was observed under the UV light for methanol extract and *n*-hexane extract of *Mutinus elegans* with $R_f$value of 0.90 and 0.67 respectively. Separate spots on the sheets confirm the presence of compound which was further determined by GC/MS.

3.3. Antibacterial assay

Antibacterial assay was carried out by measuring the zone of inhibition for the extracts of *Mutinus elegans* which revealed that maximum activity was found in methanol extract than *n*-hexane extract against nine clinical pathogenic strains. *Mutinus elegans* extracts was able to inhibit six pathogenic strains which include *Klebsiella pneumoniae*, *E. coli*, *Enterobacter*sp., *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Shigella dysenteriae* showed zone of inhibition range of 9-12 mm in diameter. There was a maximum zone of inhibition of 12 mm against *Proteus mirabilis* suggests potential antimicrobial effect. Different concentrations of the extract showed different level of inhibition with a highest of 12 mm diameter. The result with all the zones and concentrations for both the extracts against nine clinical pathogenic strains is listed in **table 1**.

Table 1 Antibacterial assay of fungus *Mutinus elegans*

Clinical Pathogens	**Zone of inhibition (mm)**			
	25 mg/ml	**50 mg/ml**	**75 mg/ml**	**100 mg/ml**
Staphylococcus aureus	-	-	-	-
Eschericha coli	8	9	-	-
Salmonella sp.	-	-	-	-
Shigella dysenteriae	-	6	3	-
Klebsiella pneumonia	-	4	7	11
Serratia marcescens	-	-	-	-
Enterobacter sp.	9	9	4	-
Pseudomonas aeruginosa	6	6	-	-
Proteus mirabilis	-	9	9	12

3.4. Antioxidant Activity (%) Assay

The total antioxidant property of the extracts refers to the free radical scavenging capability of the components present in the extracts. DPPH gives purple coloration to the reaction mixture but if antioxidants are present, there is a color change from purple to yellow showing decolorization. Maximum scavenging was observed in the fungal crude extracts as shown in **Table 2**.

Table 2 Antioxidant activity of *Mutinuselegans*.

Mutinuselegans	**OD at 517 nm**	**% Scavenging Activity**
Standard (10 mg mL^{-1})	1.810	_
n-hexane extract	0.300	24.85
Methanol extract	1.613	64.20

The reducing characteristicspredominantlycorrelate with the presence of reducing agents with antioxidant reaction, by splitting the free radical chain through donating a hydrogen atom. Tannin is a group of

polyphenolic phytochemicals, acts as a dietary astringent. A number of studies have shown the correlation of the antioxidant activity of extracts with the phenolic content of the extracts. The antioxidant property of the phenolics is mainly because of their redox potential acts as a good reducing agent, hydrogen donor and single oxygen quenchers.

3.5. Gas Chromatography Mass Spectrometry (GC/MS)

The chromatogram of methanol and *n*-hexane extracts of *Mutinuselegans* contains different bioactive compounds. The peaks procured from the mass spectrometric unit were compared with the NIST library data to obtain the best match and structure of the compounds which could be the reason of the antimicrobial activity of the extracts. The GC/MS analysis of methanol, and *n*-hexane extracts were depicts in Figure2, and Figure3 respectively. The analysis of GC/MS, revealed the presence of dibromo-tetradeca*n*-1-ol-acetate, 2-myristynoyl-glycinamide, fumaric acid, and cyclohexylmethyldecyl ester compounds in methanol and *n*-hexane extract of *Mutinus elegans*.

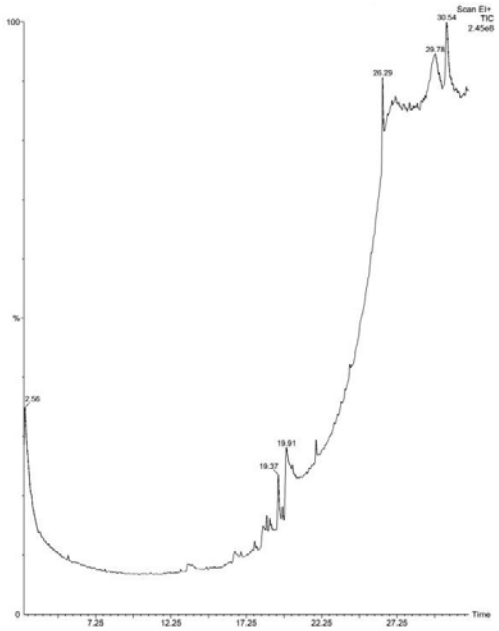

Figure 2 Chromatogram of methanolic extract of *Mutinus elegans*.

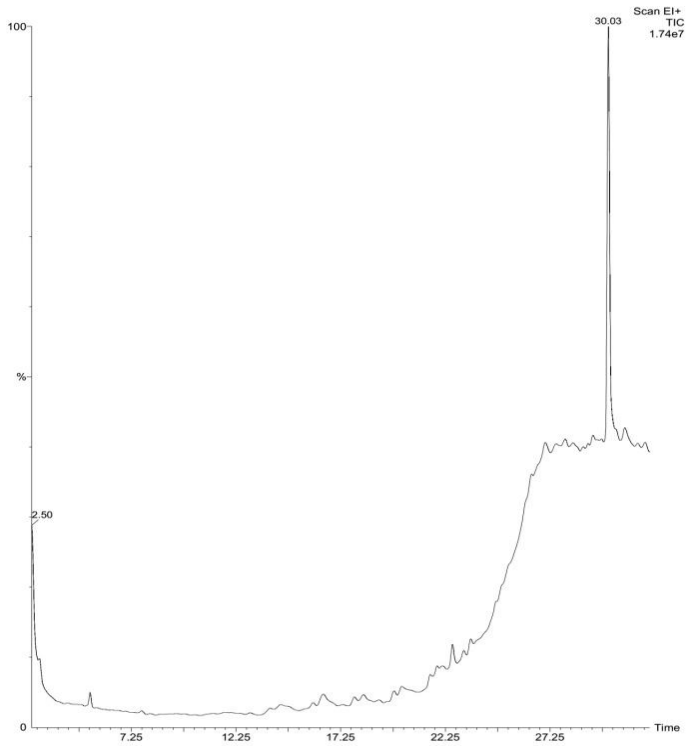

Figure 3. Chromatogram of *n*-hexane extract of *Mutinus elegans*.

3.6. Cytotoxicity studies

Since the extracts showed promising antioxidant activity they were further processed for anticancer activity against MG63 osteosarcoma cell lines. Cytotoxicity studies were done to determine the morphological changes in the cell after being treated with *Mutinus elegans* extracts. Based on the anticancer screening using MTT assay illustrated in Figure 4, with an increase in the concentration of the extract, there was inhibition of the cancer cell death. There was a moderate activity against the osteosarcoma cell lines, confirmed with the MTT assay. There have been no reports on the activity of *Mutinus elegans* extracts against MG63 cell lines, thus the present work was initiated to check its activity against MG63 cell lines.

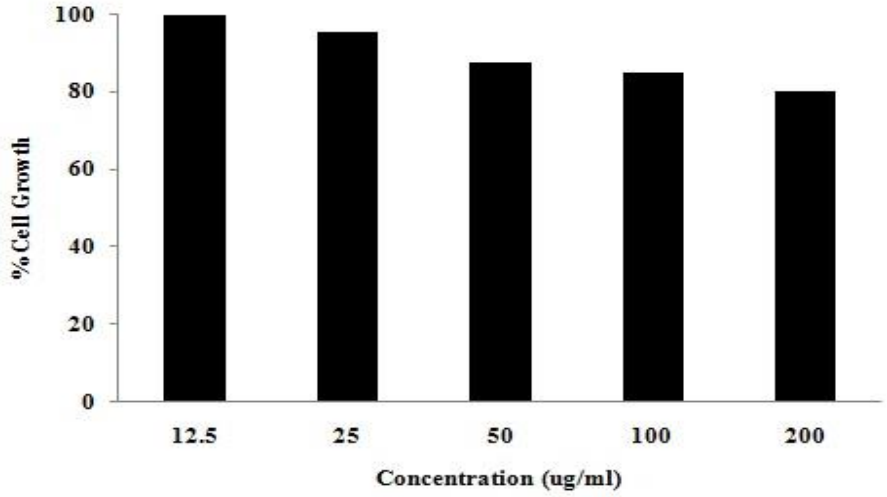

Figure 4. Anticancer activity of fungus *Mutinuselegans.*

4. Conclusion

The study provides information on the diversity of the fungus, *Mutinus elegans*. The fungus, *Mutinus elegans* as a source of naturally occurring bioactive compounds have shown remarkable potential in pharmaceutical and therapeutic area. They are a great source of secondary metabolites with enormous biological prospective. The methanolic extract showed good phenolic content and radical scavenging activity. It can be concluded that the presence of bioactive compound in the extract of *Mutinus elegans* showed antibacterial, antioxidant, and anticancer activity.

References

[1]. Pan SY, Pan S, Yu ZL, Ma DL, Chen SB, Fong WF, Han YF and Ko KM 2010 *J. Pharm. Pharm. Sci.***3** 450-71.
[2]. Xu K, Liang X, Gao F, Zhong J and Liu J 2010 *Process Biochem* **8** 1261-7.
[3]. Gordon MH 1990 Elsevier Applied Science, London, United Kingdom 1-18.
[4]. Debbab A, Aly AH and Proksch P 2012 *Fungal Diversity* **57(1)** 45-83.
[5]. Hasan SS, Ahmed SI, Bukhari NI and Loon WCW 2009 *Complementary Therapies in Clinical Practice*, ***15*(3)** 152-157.
[6]. Kozlovskii AG, Zhelifonova VP and Antipova TV 2013 *Appl. Biochem. Microbiol* **49(1).**
[7]. Sullivan R, Smith JE and Rowan NJ 2006 *Perspect Biol. Med*, ***49*(2)** 159-170.
[8]. Chauhan R and Abraham J 2013 *Iran J Basic Med Sci* ***16*(7)** 882-86.
[9]. Kawagishi H 2010 *ChemInform*, **41(38)**
[10]. De Silva DD, Rapior S, Fons F, Bahkali AH and Hyde KD 2012 *Fungal Diversity****55*** **(1)** 1-35.
[11]. Wasser SP 2011 *ApplMicrobiol Biotechnol* **89(5)** 1323-1332.
[12]. CFR Ferreira I, A Vaz J, Vasconcelos MH and Martins A 2010 Anti-Cancer Agents Med Chem**10(5)** 424-36.13.
[13]. BiancoColetto MA, Lelli P *1999* Antibacterial and antifungal activity of 32 new strains *Allionia (Turin).* ***36*** 89–92.
[14]. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A and Gray-Goodrich M 1991. *J Natl Cancer Inst*, ***83*(11)** 757-766.
[15]. Kamba AS and Hassan LG 2010 *Afr J Pharm Pharmacol* **4(9)** 645-652.
[16]. Rice-Evans C, Miller N and Paganga G 1997 *Trends Plant Sci* 2(4) 152-159.
[17]. Tunjung-Sari AB, Wahyudi T, Mufida DC, Tamarizki M, Perwitahati D, Syamsuddin IK and Jati M 2016. *Pelita Perkebunan***32(1)** 34-42.
[18]. Mosmann T 1983 *J Immunol Methods* **65(1-2)** 55-63.
[19]. Daniel UN, Ohalete CN and Nnoli MC 2012 *WorldJ. Pharm Pharm. Sci* ***1*(4)** 1181-1188.
[20]. Gajendiran A, Thangaraman V, Thangamani S, Ravi D and Abraham J 2016 *Bull Pharm Res* **6(3)** 114-9.
[21]. Chauhan R and Abraham J 2015 *Int J Pharm Sci Res* **6(2)** 757.