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Virtual screening of Compounds from Microcolonial Fungal Strain TD-062 Obtained from the Thar Desert of India

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Abstract

Computational tools facilitate screening of small molecules for high throughput screening (hits). Virtual screening has huge demand in pharmaceutical industry due to time, cost and resource effectiveness and shortening the lead time. ADMET represents Absorption, Distribution, Metabolism, Excretion and Toxicity. The forecast of the ADMET properties assumes a significant part in light of the fact that these properties represent the failure of about 60% of all drugs in the clinical phases. On the basis of different parameters, ADMET score study can help in prediction of drug activity, identification of compounds or modification of lead compounds for drug designing. A dark coloured yeast-like micro colonial fungi, TD-062, was obtained from red soil of Thar Desert. GC-MS analysis of extracts from this isolate showed the presence of compounds. Application of ADMET scores showed that one of the compounds, Octadecane, Docosane and 1-H-beta-pregna, is promising with reference to its toxicity profile, and can be selected for further drug designing.

Keywords Pharmaceutical, micro colonial fungi, toxicity and drug activity.

Introduction

The rise of multi-drug resistant pathogens has necessitated the need for novel antimicrobial drugs. Natural products, explicitly those from microorganisms, have served as the skeleton for antimicrobial molecules for long [1]. Under-investigated territories harbor novel biodiversity and chemo diversity. Based on this hypothesis, during a screening program for antimicrobial molecules, a yeast-like, dark microcolonial fungi, TD-062, was acquired from the red rocky soil of the Thar Desert, India, an arid desert with a mean annual rainfall to mean annual evaporation (MAR/MAE) ratio of 0.7.

Microcolonial fungi (MCF) or black yeast is considered to be a very stress–resistant organism [2], mainly found on bare rock surface in cold and hot deserts of different regions of the world. Most of these species have similar phylogeny and show less morphogenetic complexity [3]. These fungi are subjected to extreme environments from high humidity to complete dryness and varying temperatures. Their morphology depends on oligotrophic conditions, radioactivity and solar radiation. Microcolonial fungi have different mechanisms to survive while growing on rock surface, such as compact tissue like colony organization, presence of several UV absorbing compounds with antioxidant and antimicrobial activities [4]. *Aureobasidium*, *Kabatella, Hortaea, Exophiala, Knufia* and *Fonsecaea* have been described under this category [5]. ADMET characteristics of compounds based on the Lipinski "Rule of five" plays a crucial role in screening of drug with advance drug profile. According to these rule, molecules that violate three or more rules are not suitable for drug development [6].The present study had the following objectives (i) to characterise by GC-MS and analyse antimicrobial compounds from the MCF isolate TD-062. (ii) to understand the in-silico toxicity profile of antimicrobial compound for drug development

Materials and methods

Growth and maintenance

Isolate TD-062 was acquired from red rocky soil of the Thar Desert, India and cultured on Potato Dextrose Agar for five days at 37 °C. The isolate was stained using lactophenol cotton blue stain, and visualised under microscope.

Antimicrobial activity

The isolate TD-062 was inoculated in 100 ml of potato dextrose both and incubated for 5 days at 37 °C and 185 rpm, subsequently centrifuged at 3000 rpm for 15 min and the supernatant was used as the aqueous extract. In parallel experiment set ups, the supernatant was mixed with equal volume of different organic solvents such as petroleum ether (PE), butanol (BT) and ethyl acetate (EA), individually, vortexed for 2 hour, the organic extract layer evaporated and dissolved in methanol to obtain a final concentration of 1 mg ml^{-1}.

Further, the aqueous and organic solvent extracts (PE, EA, BT) of TD-062 were checked for antimicrobial activities against Gram positive, Gram negative bacteria, fungi, plant pathogens and pathogenic clinical–*Micrococus luteus* (MTCC-106), *Pseudomonas fluoresences* (MTCC-2421), *Saccharomyces cerevisiae* (MTCC-1874) and *Bipolaris maydis, yeast, Xanthomonas oryzea, X. citri, X. campestris, X. anopodis, X. curcubiteae* and *Escherchia coli* [Clinical isolates *E. coli* 26437, 26418 from urine sample and *E. coli* 26484, 26460 from throat swab in collaboration with Ujjawal hospital pyragraj Uttar Pradesh] on Mueller Hinton agar and incubated at 37 °C for 24 hours for positive control tetracycline (10 µg ml^{-1}) was used [7] . Subsequently the zone of inhibition was measured in cm.

Metabolic profiling

Further purification studies were carried using EA extract since it exhibited best antimicrobial activity. To detect suitable mobile phase, the EA extract was first subjected to thin layer silica gel chromatography (TLC) (Silica gel 60_{F254}, 20 cm × 20 cm, Merck). ethyl acetate, petroleum ether and butanol (organic solvents) and water individually and in combinations were used to optimise the separation of antimicrobial compounds. Well separated compounds were clearly observed in ethyl acetate and Hexane [2:1] as evaluated by UV visualization. Hence, mobile phase (ethyl acetate and Hexane [2:1]) was selected for the column chromatography. Subsequently, the ethyl acetate extract was partially purified by column chromatography with silica gel G (mesh size 60-120) slurry packed in the column. The fractions collected were subjected to assess antimicrobial activity against the same panel of target organisms. The active fractions of EA extract, showing better antimicrobial activity in comparison to other extracts were pooled and analyzed by GC-MS. 1 µl of active

pooled fractions was injected into the machine [GC-MS-QP2010Ultra, SHIMADZU]. Compounds were separated using a RTX-5MS capillary column with dimensions 25.0m (length) x 0.25mm (diameter) x 0.25µm (film thickness) [7]. The obtained mass spectrum data (collated retention time (RT), similarity index (SI), mass ion spectra (MS), and retention indices (RI-observed and calculated), obtained after GC-MS analysis was further interpreted. RI was calculated using the Kovats linear index [8]. Obtained data was compared with WILEY8 libraries, National Institute of Standards and Technology (NIST11) and PubChem database.The ADMET properties of the molecules describe their pharmacodynamics and pharmacokinetics properties; which includes acute oral toxicity (AO), P-glycoprotein inhibitor (Pgpi), (Caco-2)permeability (Caco-2), human intestinal absorption (HIA), human ether-a-go-go-related gene inhibition (hERG), carcinogenicity (CARC), substrate and different inhibitors (CYP1A2,CYP2C19, CYP3A4, CYP2D6 and CYP2CP), these properties predict about the toxicity and viability of the compounds. For analysis of ADMET properties, the smile sequence was recieved from PubChem database, (https://pubchem.ncbi.nlm.nih.gov/). Input file of compound obtained from GC-MS analyses were fed into the software admetSAR http://lmmd.ecust.edu.cn/admetsar2/).

3. Result and Discussion

Antimicrobial activity of aqueous and organic solvents BT, EA, PE extracts showed antimicrobial activity against target organisms. EA showed antimicrobial activity against *X. oryzea, X. campestris, P.fluoresence, E. coli* (26437, 26418) from urine sample and *E. coli* (26484, 26460) from throat swab sample (Table 1). Hence EA extract was further used for metabolic profiling. The EA extract has been showed to have better activity in some or other studies [8].

Metabolic profiling

The partially purified fractions of EA extract of isolate TD-062 were used for metabolic profiling by GC-MS [11]. Sixteen compounds obtained by GC-MS analysis, some reported earlier for antimicrobial properties were analysed based on Similarity Index (SI), calculated and reported retention index (Table.2). These compounds were categorised in three categories carboxylic acid esters, alkane and hydrocarbon. Among the tentatively identified compounds, Docosane and Octadecane belongs to alkane group, 14- beta-h-pregna belonged to hydrocarbons and 1-H indole, 5methyl belongs to carboxylic acid group. The obtained data analysed was started with SI followed by Kovats Retention Index. Maximum SI value (92%) and (90%) was obtained for 14- beta-h-pregna and octadecane. Reported RI and calculated RI values for 14- beta-h-pregna were 0 and 1235.86 and these values for octadecane were 55.156 and 2191.667 respectively. We found marked differences among retention, index similarity index from calculated retention index and database, for 1-H indole, calculated and observed retention index (4.035 and 900) and for docosance (55.156 and 1452.849). Hence the compounds could be potentially novel.

Analysis of ADMET and physicochemical properties

As seen from table 3 the GC-MS data of four compounds (Docosane, Octadecane, 14-beta-h pregna and 1H-indole, 5methyl) were earlier reported for antimicrobial activity. Docosane is waxy compound in nature, mainly found in the high concentration within lemon balms. It is a plant metabolite; with antimicrobial activity [12]. Octadecane is hydrocarbon lipid molecule; it is a plant and bacterial metabolite, with antimicrobial activity [13]. 14-H-β-pregna is naturally occurring compound, partially or completely hydrogenated in nature, it acts as adrenergic receptor, derived from steroid hormone; with antimicrobial and anti-inflammatory properties [14]. 1-H-indole, 5methyl occurs from anoxic metabolism of L-tryptophan in the mammalian metabolite and has antimicrobial activity [15]. ADMET properties of these compounds indicates the permeate Caco-2 Permeability, Blood-Brain Barrier (BBB), Human Intestinal Absorption (HIA) which indicate that these compounds have better rate of absorption, cytochrome P450 (CYP450) and its other compounds enzymes indicates the role of drug metabolism. The prediction of these compounds reveals that they were not inhibited by the components of CYP50 except CYP450 1A2 and CYP4502C19 inhibitor of 1-H indole, 5methyl. Overall it shows low rate of CYP inhibitor which indicates that these molecules have no hindrance in drug metabolism. Except 1-H indole, 5methyl none of the molecule shows any carcinogenicity, AMES toxicity and acute oral toxicity effect. From admetSAR results it can be predicted that Octadecane, Docosane and 14-H-β-pregna could be further used for drug development.

4. Conclusion

Analysis of antimicrobial activity showed that EA extract TD-062 has antimicrobial activity. Metabolite profiling of active fractions of EA extract showed the presence of four compounds belonging

Table.1 Antimicrobial activity of aqueous and organic solvent extract of TD-062

	Zones of inhibition (cm) Mean ± SD									
Extracts	**Target Organisms**									
	X. oryzea	***X. campestris***	***B. maydis***	***M. luteus (MTCC-106)***	***P.flurosence (MTCC-2421)***	***S.cerevisiae (MTCC-1874)***	***E. coli* (26484)**	***E. coli* (26460)**	***E. coli* (26418)**	***E. coli* (26437)**
Aqueous	**1.76±0.25**	**1.06±0.53**	-	-	-	-	**0.933±0.14**	**1.16±0.11**	**0.875±0.11**	**1.23±0.25**
EA	**1.73±1.64**	**1.87±0.56**	-	-	**1.23±0.76**	-	**1.17±0.14**	**1.23±1.32**	**1.03±0.5**	**1.73±1.5**
BT	**1.4±0.35**	**1.3±0.3**	-	-	-	-	**1.33±0.15**	**1.22±0.10**	**0.96±0.05**	**0.866±0.40**
PE	**0.66±0.20**	-	-	-	-	-	**1.06±0.11**	**1.13±0.15**	**1.1±0.1**	**1.1±0.17**

to carboxylic acid esters, alkane and hydrocarbon. Subsequently ADMET properties analyses of these compounds; predict that docosane, Octadecane and 1-H-beta-Pregna are compounds which can be a further lead for drug designing.

Conflict Of Interest

The authors declare no conflict of interest, financial or otherwise.

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Table.2 Compounds/metabolites and their characteristics, obtained by GC-MS from ethyl acetate extract of TD-062

Chemical group	Name of compound	Retention time (RT)	Chemical abstracts service (CAS) number	Retention indices from databases (RI*)	Calculated retention indices (RI $)	Similarity index(SI) %
Carboxylic acid Ester	1-H indole, 5methyl	24.27	614-96-0	4.035	900	87
Alkane	Docosane Octadecane	9.52 17.591	629-97-0 593-45-3	55.156 55.156	1452.849 2191.667	79 90
Hydrocarbon	14–beta-h-pregna	21.742	67462-34-4	0	1235.86	92

Table .3 Analysis of ADMET properties of ethyl acetate extract of TD-062 obtained by GC-MS of antimicrobial compounds.

ADMET Properties	Docosane	Octadecane	1-H-Indole, 5Methyl	14β-H-pregna
Molecular weight	310.61 g/mol	254.5 g/mol	131.17g/mol	288.5g/mol
Molecular formula	C22H46	C18H38	C9H9N	C25H36
AlogP	8.83	7.27	2.48	7.32
H bond donor	0	0	0	0
H bond acceptor	0	0	1	0
Absorption (logS)	-5.178	-5.178	-2.451	-5.272
Caco-2 Permeability	+	+	+	+
Human Intestinal Absorption	+	+	+	+
Blood-Brain Barrier	+	+	+	+
P-glycoprotein inhibitor	-	-	-	-
P-glycoprotein substrate	-	-	-	-

Subcellular organization	Lysosomes	Lysosomes	Lysosomes	Lysosomes
CYP4503A4 Substrate	-	-	-	-
CYP4502D6 Substrate	-	-	-	-
CYP4502C9 Substrate	-	-	-	-
CYP450 2C9 Inhibitor	-	-	-	-
CYP450 1A2 Inhibitor	-	-	+	-
CYP450 2D6 Inhibitor	-	-	-	+
CYP450 3A4 Inhibitor	-	-	-	-
CYP4502C19 Inhibitor	-	-	+	-
CYP Inhibitory Promiscuity	-	-	-	-
AMES toxicity	-	-	+	-
Carcinogens (Binary)	-	+	-	-
Carcinogenicity (Tertiary)	-	-	-	-
Fish Toxicity	+	+	-	+
***Tetrahymena pyriformis* Toxicity (µg/L)**	3.002	2.876	1.004	2.768
Honey Bee Toxicity	+	+	+	+
Biodegradation	+	+	-	+
AcuteOralToxicity (kg/mol)	0.944	0.968	1.605	1.683
HumanOral bioavailability	+	+	+	+

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