Research Paper

Identification and *In Silico* Analysis of Interaction of Active Compounds in *Pimpinella anisum* with *Trichophyton rubrum* Aspartate-B-Semialdehyde Dehydrogenase and Sialidase

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Trichophyton rubrum **is the major causative organism of dermatophytosis. The methanolic extracts of *Pimpinella anisum* seed has shown *in vitro* anti-dermatophytic activity. In the present study, the active compounds present in *Pimpinella anisum* seed-methanolic extracts was tentatively identified by liquid chromatography/electrospray ionization tandem mass spectrometry. Further molecular docking analysis of these active compounds with *Trichophyton rubrum* proteins aspartate-beta (β)-semialdehyde dehydrogenase and sialidase was studied. Liquid chromatography/electrospray ionization tandem mass spectrometry analysis revealed the presence of different class of compounds such as polyphenols, flavonoids, tannins, phenolic acids, phenolic acid derivative, fatty acids and lignan. *In silico* molecular docking analysis of these compounds with *Trichophyton rubrum* proteins aspartate-β-semialdehyde dehydrogenase revealed the top five compounds with lowest binding energy were phelligridin I (-10.1 kcal/mol), pectolinarin (-9.6 kcal/mol), fortunellin (-9.5 kcal/mol), epigallocatechin gallate (-9.4 kcal/mol) and kaempferol-3-O-glucuronide (-9.2 kcal/mol). In sialidase, the compounds phelligridin I (-10.6 kcal/mol), fortunellin (-9.5 kcal/mol), chicoric acid (-9.2 kcal/mol), epigallocatechin gallate (-9.1 kcal/mol) and kaempferol-3-O-glucuronide (-8.6 kcal/mol) bound with lowest binding energy. All these compounds were found to orient in the active site forming interactions with amino acids involved in catalysis of these proteins. The proteins aspartate-beta-semialdehyde dehydrogenase and sialidase are considered important drug target of *Trichophyton rubrum*. *In silico* analysis has shown promising results hence, these compounds identified in the present study might be further studied for its *in vitro* anti-dermatophytic activity. Also, the results from the present study clearly indicate that the active compounds present in *Pimpinella anisum* seed-methanolic extracts through its interaction with proteins present in *Trichophyton rubrum* might have shown *in vitro* anti-dermatophytic activity.**

Key words: *Pimpinella anisum*, *Trichophyton rubrum*, aspartate-β-semialdehyde dehydrogenase, sialidase

The superficial fungal infection called dermatophytosis is limited to the stratum corneum of the epidermis, or to the hair and nails. The infection caused by fungal dermatophytes involves coordinated process such as adhesion, penetration and colonization of keratinized tissues[1]. Dermatophytosis is an important public health problem worldwide particularly in developing countries[2]. Although the disease hardly causes death, it affects the quality of life and it is responsible for high morbidity[3,4]. Onychomycosis, a form of dermatophytosis, is a fungal infection of the fingernails or toenails. Onychomycosis is common in older people and it is related to peripheral vascular disease, immunologic disorders and diabetes mellitus. Onychomycosis is difficult to treat and might lead to cellulites and foot ulcer[5-7]. *Trichophyton rubrum* (*T. rubrum*)

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is the major dermatophyte fungus as well as the most prevalent pathogen that caused human dermatophytoses, which accounts for about 70 % of the total dermatophyte infections[8-10]. This organism may remain viable in the environment for over 6 mo, thus accounting for widespread of infections. Transmission of *T. rubrum* infection occurs often from person to person through either by shedding of infected skin cells and hair or by direct body contact[11]. Also, the widespread of infections may occur due to viability of *T. rubrum* in the environment for over 6 mo[12].

At present allylamines, polyenes, echinocandins, azoles and other agents, such as griseofulvin and 5-flucytosine are the antifungals used widely in clinical treatment[8,13,14]. Allyamine (terbinafine) interfere with ergosterol biosynthesis through inhibition of squalene epoxidase. Such lack in ergosterol would affect the fungal cell wall synthesis thereby its growth[15]. Azole (fluconazole) inhibits the cytochrome 450 sterol 14 a-demethylase and affects the conversion of lanosterol into ergosterol. This leads to the disruption of fungal membranes and increase the phospholipids accumulation within the cell[16]. Other than these, spinosins target β-1,3-glucan synthase enzyme of the fungus[17]. Although these antifungal agents are available, the cases of deep infection are increasing in immunosuppressed and immunocompromised patients[18,19]. Also, an increasing number of cases of resistant dermatophyte infections have been reported. In particular, *T. rubrum* is most persistently described in resistance to standard treatments[20]. Long-term and discontinuing treatments favors resistant acquisition in fungus. The biochemical mechanisms for such resistance to available drugs are point mutations, alteration in drug target sites and increased expression as well as activity of efflux transporter[8]. Also, cross-resistance has also been reported in *T. rubrum*, that is terbinafine-resistant isolate was found to be resistant to azoles due to overexpression of the multidrug efflux transporter[21]. Hence, identification of novel drugs against dermatophytes are ever increasing. Especially, the search for natural compounds is considered advantageous to overcome multidrug resistant with less side-effects[22].

Currently, there is a lot of activity in the quest for natural products with novel uses, particularly in relation to pest management. Plant extracts with antimicrobial characteristics and a range of secondary metabolites, such as alkaloids, quinones, flavonoids, glycosides, saponins, tannins and terpenoids have gained attention in the field of plant disease prevention. These extracts include aromatic and medicinal plants. *Pimpinella anisum* is the plant belonging to the Umbelliferae family and its seeds are reported for various biological activities such as antidiabetics, antioxidant, antispasmodic action and antimicrobial effect[22]. Among the many hundreds of soil-borne microorganisms that cause plant diseases, *Fusarium oxysporum*, *Sclerotium rolfsii*, and *Macrophomina phaseolina* are the most prevalent fungal infections.

Previous study has shown the anti-dermatophyte effect of *Pimpinella anisum* Seed (PAS) extract. Also, the effective treatment for recalcitrant infection of *T. rubrum* might be combinatorial therapy of available drugs with natural compounds. Accordingly, previous report has shown the synergistic effect of essential oil prepared from PAS along with terbinafine[23]. In addition, our previous study has shown the antifungal activity of PAS-Methanol Extract (PAS-ME) against *T. rubrum*. However, the active constituents present in PAS-ME and its target protein in *T. rubrum* has not been determined. Keeping these facts in view, in the present study PAS-ME was prepared and the active compounds present was tentatively identified using Liquid Chromatography/Electrospray Ionization tandem Mass Spectrometry (LC-ESI/MS/MS).

The ability to multiply numerous analytes within a single analytical run at a low incremental cost is a benefit of LC-MS tests. This might make laboratory setup simpler and provide additional valuable information, including metabolite profiles. Further, *in silico* molecular docking analysis was performed to understand the interaction of identified compounds present in *Pimpinella anisum* Methanolic Extract (PAME) with *T. rubrum* proteins, Aspartate-β-Semialdehyde Dehydrogenase (ASADH) and sialidase. Till date, drugs against various other *T. rubrum* proteins have been identified, however multidrug resistant organisms were developed due to point mutations in these proteins thereby decreasing the affinity of these drugs towards these proteins[8]. Hence, in the present study the potential compounds identified in PAME are studied against these new target proteins of *T. rubrum*.

MATERIALS AND METHODS

Materials:

Methanol was purchased from Merck, India.

Preparation of PAME:

Seeds of *Pimpinella anisum* were collected from Hosur and its associated Ghats region, Krishnagiri District, Tamil Nadu, India. The seeds were shade dried, powdered and stored in dry air-tight containers in dark place. About 10 g of powdered seed sample was extracted using methanol for 8 h in Soxhlet apparatus. The methanol was evaporated using rotary evaporator and PAME obtained was used for compound identification.

LC-ESI/MS/MS:

LC-ESI-MS/MS analysis was performed using the column XSelect CSHT C18 2.5 µm in Xevo TQ-S micro Triple Quadrupole Mass Spectrometry (Waters, USA). The gradient mobile phase consists of 2 solvents, 0.1 % Formic Acid (FA) in water and acetonitrile was used. The injection volume was 2 µl. Spectra was recorded in ESI positive mode between m/z 100 and 1000.

Molecular docking analysis:

The structures of compounds were retrieved from PubChem. The three-dimensional crystal structure of *T. rubrum* proteins, ASADH (PDB ID:4ZHS) and sialidases (PDB ID: 7P1U) were retrieved from protein data bank[24, 25]. The ligand and proteins were prepared using AutoDock tools 1.5.6[26]. Docking study was carried out by AutoDock Vina version 1.1.2[27]. The PyMol software and Discovery Studio were used to analyze the intermolecular interactions between the ligand and protein.

RESULTS AND DISCUSSION

The compounds identified in PAS-ME at different retention time using LC-ESI-MS/MS analysis were given in Table 1. The results showed the presence of compounds from various groups such as polyphenols (ligstroside), flavonoids (kaempferol-3-O-neohesperidoside, kaempferol-3-O-galactoside, kaempferol-3-O-glucoside, kaempferol-3-O-glucuronide (KGR), kaempferol-3-O-rhamnoside, isorhamnetin-3-O-arabinose, luteolin-7,3-dimethyl ether, kaempferol, naringenin, isoschaftoside, myricetin, fortunellin, pectolinarin and swertisin), tannins (Epigallocatechin Gallate (EGCG) and 5-galloylquinic acid), phenolic acids (rosmarinic acid, chlorogenic acid and feruloyl glucose), phenolic acid derivative (1,3-O-dicoumaroylglycerol and 1-O-p-coumaroyl-3-O-caffeoylglycerol), fatty acid (1,4-epoxy-1-methoxy-8,13-diacetoxy-10-hydroxygermacra-5(E),7(11)-dien-6,12-olide and linoleic acid), lignan (chrysoeriol-7-O-neohesperidoside and secoisolariciresinol) and other compounds (esculin and phelligridin I).

The binding energy of compounds present in PAS-ME with *T. rubrum* ASADH is given in Table 2. The top five compounds with lowest binding energy were found to be phelligridin I (-10.1 kcal/mol), pectolinarin (-9.6 kcal/mol), fortunellin (-9.5 kcal/mol), EGCG (9.4 kcal/mol) and KGR (-9.2 kcal/mol).

The connolly surface figure of *T. rubrum* ASADH that shows the orientation of top five compounds with lowest binding energy is shown in fig. 1A. The interaction of the compounds with amino acids of *T. rubrum* ASADH is shown in fig. 1B-fig. 1F. Detail of amino acids that forms hydrogen bonding, hydrophobic interaction and electrostatic interactions with the compounds are given in Table 3.

The compounds formed hydrogen bonding with amino acids such as Thr15, Ala17, Ser40, Asp88, Asn109, Lys111, Arg114, Asn153, Gly186, Ala187, Gly188, Pro190, Gly191, Asp196, Tyr204, Pro206, Lys211 and Gly334. Hydrophobic interactions were formed with the amino acids Ala17, Val18, Lys55, Cys154, Val184, Gly188, Tyr189, Val192, Pro206 and Ala345. The compounds formed electrostatic interaction with amino acids Lys55, Asp88 and Lys211.

The binding energy of compounds present in PAS-ME with *T. rubrum* sialidase is given in Table 2. The top 5 compounds with lowest binding energy were found to be phelligridin I (-10.6 kcal/mol), fortunellin (-9.5 kcal/mol), chicoric acid (-9.2 kcal/mol), EGCG (-9.1 kcal/mol) and KGR (-8.6 kcal/mol). The orientation of top 5 compounds with lowest binding energy in the *T. rubrum* sialidase is shown in fig. 2A. The detail of interaction between the compounds and amino acids of *T. rubrum* sialidase is shown in fig. 2B-fig. 2F. Detail of interacting amino acids of *T. rubrum* sialidase through hydrogen bonding, hydrophobic interaction and electrostatic interactions are given in Table 4.

TABLE 1: TENTATIVE IDENTIFICATION OF COMPOUNDS PRESENTS IN PAS-ME BY LC-MS/MS ANALYSIS

Retention time	Predicted compound	Molecular weight	m/z [M]/[M+H]
5.2	Ligstroside	524	523.92
5.529	Kaempferol-3-O-neohesperidoside	594	595.1
6.009	Rosmarinic acid	360	361.22
6.303	Phelligridin I	624	625.13
6.54	Chlorogenic acid	354	355.5
6.798	Chrysoeriol-7-O-neohesperidoside	608	609.1
6.927	Kaempferol-3-O-galactoside	448	449.23
7.049	Kaempferol-3-O-glucoside	448	449
7.443	KGR	462	463.44
7.6	Kaempferol-3-O-rhamnoside	432	434.17
8.21	Isorhamnetin -3-O-arabinose	448	449.02
8.647	Luteolin-7,3′-dimethyl ether	314	315.21
9.12	Chicoric acid	474	475.55
9.313	Kaempferol	286	288.25
10.395	Naringenin	272	272.21
11.686	Isoschaftoside	564	565.29
11.886	Myricetin	318	319.14
12.481	Naringenin	272	274.24
12.811	Kaempferol	286	288.25
12.976	Fortunellin	592	593.21
13.291	Fortunellin	592	593
14.309	Pectolinarin	622	623.31
14.588	Pectolinarin	622	623.31
15.176	Swertisin	446	447.34
15.706	Esculin	340	341.19
16.695	5-Galloylquinic acid	344	344.28
16.881	Chlorogenic acid	354	355.27
18.078	Linoleic acid	281	282.23
18.286	1,3-O-Dicoumaroylglycerol	384	385.38
18.566	1-O-p-Coumaroyl-3-O-caffeoylglycerol	400	401.34
19.168	EGCG	458	458.05
20.701	Feruloyl glucose	356	357.16
21.905	1,4-epoxy-1-methoxy-8,13-diacetoxy-10-hydroxygermacra-5(E),7(11)-dien-6,12-olide	410	411.49
22.579	Secoisolariciresinol	362	363.39

TABLE 2: DOCK SCORE OBTAINED FOR VARIOUS COMPOUNDS IDENTIFIED IN PAS-ME BY MOLECULAR DOCKING.

Name of the ligands	Dock scores (kcal/mol)	
	ASADH (PDB ID: 4ZHS)	Sialidase (PDB ID: 7P1U)
Ligstroside	-8.2	-8
Kaempferol-3-O-neohesperidoside	-7.4	-8.5
Rosmarinic acid	-7.9	-7.9
Phelligridin I	-10.1	-10.6
Chlorogenic acid	-7.9	-8.5
Chrysoeriol-7-O-neohesperidoside	-9	-8.4
Kaempferol-3-O-galactoside	-7.4	-7.1
Kaempferol-3-O-glucoside	-8.3	-7.5
KGR	-9.2	-8.6
Kaempferol-3-O-rhamnoside	-7.8	-7.3
Isorhamantin-3-O-arabinoside	-7.6	-8
Luteolin-7,3′-dimethyl ether	-7.9	-7.4
Chicoric acid	-8.4	-9.2
Kaempferol	-7.9	-7.5
Naringenin	-7.8	-7.4
Isoschaftoside	-7.8	-8.3
Myricetin	-8	-8.1
Fortunellin	-9.5	-9.5
Pectolinarin	-9.6	-8.5
Swertisin	-8.8	-7.4
Esculin	-8.2	-7.8
5-Galloylquinic acid	-8.1	-6.8
Linoleic acid	-5.4	-5
1-O-p-Coumaroyl-3-O-caffeoylglycerol	-8.2	-7.8
1,3-O-dicoumaroylglycerol	-8.1	-8.1
EGCG	-9.4	-9.1
Feruloyl glucose	-8	-7.3
1,4-epoxy-1-methoxy-8,13-diacetoxy-10-hydroxygermacra-5(E),7(11)-dien-6,12-olide	-7.1	-6.8
Secoisolariciresinol	-7.2	-7.2

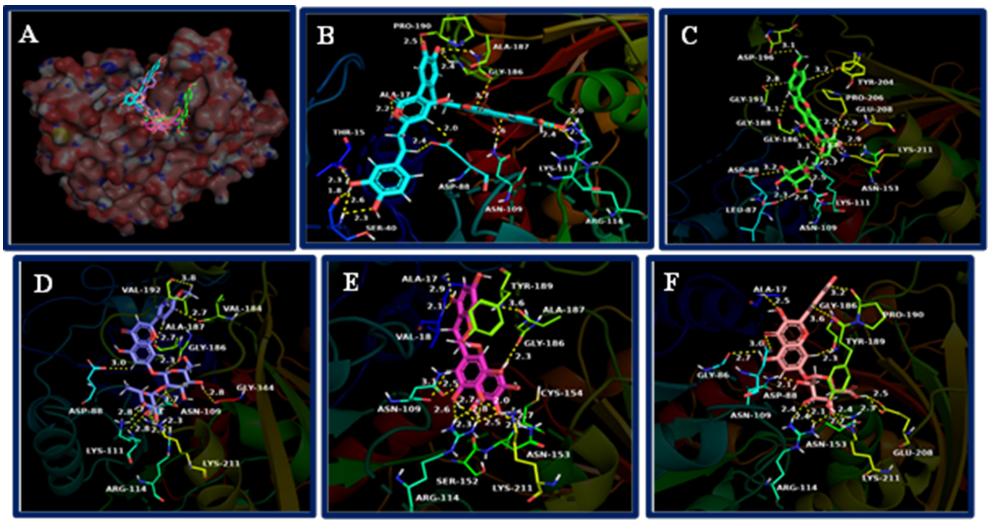

Fig. 1: Interaction of top five compounds present in PAS-ME with lowest binding energy in the active site of *T. rubrum* ASADH. (A) Connolly surface figure of *T. rubrum* ASADH that shows the orientation of five compounds in the active site. Detail of interacting amino acids of *T. rubrum* ASADH with (B) Phelligridin I; (C) Pectolinarin; (D) Fortunellin; (E) EGCG and (F) KGR

TABLE 3: DETAILS OF INTERACTION BETWEEN COMPOUNDS AND AMINO ACIDS PRESENT IN *T. rubrum* ASADH

Ligand	Interacting amino acid residues (Sialidase; PDB ID: 7P1U)		
	Hydrogen bond	Hydrophobic interaction	Electrostatic interaction
Phelligridin	Arg62, Gly79, Asn85, Arg86, Asn127, Gln150, Arg173, Trp204, Arg267, Arg390	Trp204, Ala206	Asp87, Glu251, Arg267
Fortunellin	Arg62, Asp87, Asn127, Asn154, Trp204, Arg267, Pro268, Gly269	Arg86	Arg86, Asp87
Chicoric acid	Arg62, Asp87, Asn127, Arg324, Arg390	-	Asp87, Glu251
EG	Arg62, Asp87, Asn127, Gln150, Tyr360	Trp204, Ala206, Arg267	Asp87, Glu251
KGR	Arg62, Asp87, Trp204, Arg267, Arg324, Arg390	-	Asp87, Glu251, Arg267

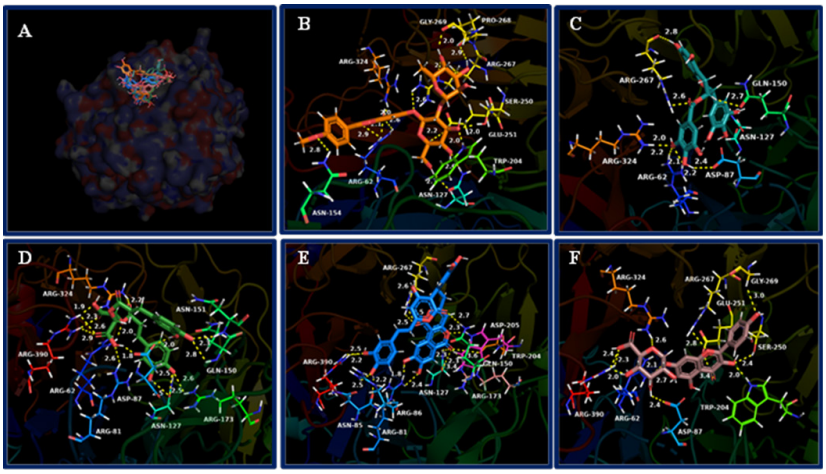

Fig. 2: Interaction of top five compounds present in PAS-ME with lowest binding energy in the active site of *T. rubrum* sialidase, (A) Connolly surface figure of *T. rubrum* sialidase that shows the orientation of five compounds in the active site. Detail of interacting amino acids of *T. rubrum* sialidase with (B) Phelligridin I; (C) Fortunellin; (D) Chicoric acid; (E) EGCG and (F) KGR

TABLE 4: DETAILS OF INTERACTION BETWEEN COMPOUNDS AND AMINO ACIDS PRESENT IN *T. rubrum* SIALIDASE

Ligand	Interacting amino acid residues (ASADH; PDB ID: 4ZHS)		
	Hydrogen bond	Hydrophobic interaction	Electrostatic interaction
Phelligridin	Thr15, Ala17, Ser40, Asp88, Asn109, Lys111, Arg114, Gly186, Ala187, Pro190	Ala17, Val18, Lys55, Cys154, Val184, Ala345	Lys55, Asp88
Pectolinarin	Leu87, Asp88, Asn109, Lys111, Asn153, Gly186, Gly188, Gly191, Asp196, Tyr204, Pro206, Lys211	Ala17, Val18, Tyr189, Val192, Pro206	-
Fortunellin	Asp88, Asn109, Lys111, Arg114, Val184, Ala187, Gly186, Val192, Lys211, Gly334	Ala17, Val18, Pro89, Val184, Tyr189	-
EG	Ala17, Val18, Asn109, Lys111, Arg114, Ser152, Asn153, Cys154, Gly186, Ala187, Tyr189	Pro89, Lys111, Cys154	Lys211
KGR	Ala17, Gly86, Asp88, Asn109, Arg114, Asn153, Gly186, Thr189, Pro190, Glu208, Lys211	Ala17, Gly188	Asp88

The compounds formed hydrogen bonding with amino acids such as Arg62, Gly79, Asn85, Arg86, Asn127, Gln150, Arg173, Trp204, Arg267, Pro268, Gly269, Arg324, Tyr360 and Arg390. Hydrophobic interactions were formed with the amino acids Arg86, Trp204, Ala206 and Arg267. The compounds formed electrostatic interaction with amino acids Arg86, Asp87, Glu251 and Arg267.

To understand the molecular mechanism of antifungal activity of PAS-ME against *T. rubrum*, the active molecules in extract were identified and interaction of these identified compounds with proteins of *T. rubrum* was studied. Till date, the proteins such as squalene epoxidase, cytochrome 450 sterol 14 a-demethylase and β-1,3-glucan synthase of fungus are targeted by the antifungal drugs[8,13-17]. However, point mutations in these proteins as well as increased expression of drug efflux proteins has increased the incidence of fungal infection[8]. Also, the side-effects of available drugs are making it difficult to treat the dermatophyte and leads to deep infection[28]. In addition, fungi are eukaryotic organism like mammals, hence it is necessary to target the structure unique to fungi[29]. Hence, two new protein targets such as ASADH and sialidase have been targeted in the present study. Also, till date, the crystal structure of these proteins of *T. rubrum* are only available in protein data bank. Hence interaction of the compounds in PAS-ME with these proteins might give a definitive understanding of antifungal activity of these compounds against *T. rubrum*.

The fungal aspartate pathway is required for the biosynthesis of amino acids such as threonine, isoleucine and methionine. These are essential for fungal viability and is found to be indispensable[24]. Also, the aspartate pathway is involved in cell-wall biosynthesis, the protective dormancy process and virulence factor production by providing the source for these processes[24,30-32]. ASADH catalyze, the second reaction in aspartate pathway and does not have homologous in mammalian cells[29,33]. It has been found that ASADH expression is increased upon exposure of *T. rubrum* to human skin, suggesting its role in virulence of the fungus[34]. Inhibitors are targeting ASADH could act as potent antifungal agent against *T. rubrum*[24]. The amino acids Cys154 and His251 is considered catalytically important amino acids in *T. rubrum* ASADH. Further, the open loop structure is required for the proper binding of the substrate and catalytic activity of *T. rubrum* ASADH. The amino acids such as Gly188, Tyr189, Pro190 and Gly191 are important residues present in loop structure. The first five compounds with lowest binding energy in docking such as phelligridin-I, pectolinarin, fortunellin, EGCG and KGR oriented spanning the entire loop structure into the active site. Among these compounds, EGCG showed both hydrogen bonding and hydrophobic interaction with catalytic amino acid Cys154. As well as phelligridin formed hydrophobic interaction with Cys154. All the compounds interacted with amino acids present in loop structure either through hydrogen bonding and hydrophobic interaction or through both interactions. Such interaction with these compounds would hinder the binding the positioning of substrate for the catalytic function of the *T. rubrum* ASADH. In addition, it might also disturb the binding of coenzyme, Nicotinamide Adenine Dinucleotide Phosphate (NADP) to the enzyme thereby affecting its function.

The sialidase from *T. rubrum* prefer the sialic acid substrate, 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN); hence it is a sialidase (Kdnase)[25]. Although sialic acid is absent in *T. rubrum*, the C8 monosaccharide 3-deoxy-d-manno-oct-2-ulosonic acid (Kdo) was found in *T. rubrum* samples in a recent study[25]. Although the role of Kdo in *T. rubrum* virulence has yet to be determined, Kdo is a component of endotoxin lipopolysaccharide in bacteria[35]. In addition, it is crucial for growth and survival of microorganism. Other than *T. rubrum*, sialidase from *Aspergillus fumigatus* is also a Kdnase and its activity was not inhibited by the classical sialidase inhibitor, 2-deoxy-2,3-didehydro-N-acetylneuraminic acid. Also, Kdnase from *A. fumigatus* was found to be important for fungal cell wall integrity and virulence[36]. In addition, Kdnase is not present in the host hence it may be potential target for the development of novel antifungal agents. In accordance, identifying compounds that could target sialidase from *T. rubrum* would inhibit its survival. The top five compounds with lowest binding energy with *T. rubrum* sialidase such as phelligridin I, fortunellin, chicoric acid, EGCG and KGR bound in the substrate, KDN binding site. In general, the fungal sialidase consists of key active site residues an arginine triad that interacts with the carboxylic acid group of KDN, a nucleophilic

tyrosine, its associated general acid (Glu), and an acid/base (Asp). The compounds from PAS-ME formed hydrogen bonding, hydrophobic interaction or electrostatic interactions with amino acids such as Arg62, Asp87, Gln150, Glu250 and Arg267, which play significant role in the orientation of substrate KDN in the active site as well as in its catalysis[25,37]. Hence, the interaction of these compounds would inhibit the catalytic activity of *T. rubrum* Kdnase. Such inhibition would disturb the cell wall thereby the virulence of *T. rubrum*.

The top scored compound phelligridin that interacted with both ASADH and sialidase has previously been found to inhibit neuraminidase (also known as sialidase) present in influenza viruses[38]. In addition, phelligridin were reported for anticancer, inhibition of oxidative stress, and anti-inflammatory activity (through inhibiting amyloid beta aggregation)[39-41]. Pectolinarin that interacted with *T. rubrum* ASADH was reported for beneficial effects such as antidepressant antidiabetic, antitumor, antiviral, anti-rheumatoid arthritis, analgesic, anti-inflammatory, hepatoprotective and neuroprotective activity[42]. Fortunellin, EGCG and KGR interacted with both ASADH and sialidase with lowest binding score was found to have various other biological activities such as anticancer, antidiabetic, antioxidant, antiviral, hepatoprotection and antiinflammation[43-46]. Chicoric acid that interacted with *T. rubrum* Kdnase is known for hepatoprotective, nephroprotective, neuroprotective, antioxidative and anti-inflammatory activity[47-49]. Other than these compounds, most of the compounds identified in PAS-ME interacted in the active site of both *T. rubrum* ASADH and Kdnase with low binding score. Hence, synergistically, these compounds might have shown toxicity against *T. rubrum* and may be further studied for potential anti-dermatophyte agents.

In conclusion, the active compounds present in PAS-ME were identified by LC-ESI-MS/MS analysis. The compounds identified belonged to various groups such as polyphenols, flavonoids, tannins, phenolic acids, phenolic acid derivatives, fatty acids and lignan. *In silico* molecular docking analysis of these compounds with *T. rubrum* proteins ASADH and KDNase revealed the compounds phelligridin I, pectolinarin, fortunellin, chicoric acid, EGCG and KGR to interact with these proteins with low binding energy. These compounds oriented in the active site forming interactions with amino acids involved in catalysis of these proteins. Since, the proteins ASADH and Kdnase is an important drug target to identify drug against *T. rubrum*, the compounds identified in the present study interacting with these proteins might be further studied for its *in vitro* anti-dermatophytic activity. Further, the molecular mechanism of PAS-ME for its anti-dermatophyte activity might be due the interaction of these active compounds present in the extract with these proteins.

Conflict of interests:

The authors declared no conflict of interests.

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