***In Vitro* and *In Silico* Antioxidant, Anti-Diabetic, Anti-HIV and Anti-Alzheimer Activity of Endophytic Fungi, *Cladosporium uredinicola* Phytochemicals**

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Keywords: *Cladosporium uredinicola*; anti-diabetes; anti-oxidant; anti-HIV; anti-cholinesterase

Abstract. The present work was aimed to identify phytochemicals in *C. uredinicola* methanol extract from qualitative, TLC and GC-MS method and evaluated for antioxidant, anti-HIV, anti-diabetes, anti-cholinesterase activity *in vitro* and *in silico*. The *C. uredinicola* extract showed flavonoids, tannins, alkaloids, glycosides, phenols, terpenoids, and coumarins presence in qualitative method. From GC-MS analysis, identified seven different phytochemicals and out of seven, four (coumarin, coumarilic acid, hymecromone, alloisioimperatorin) are coumarins. The *C. uredinicola* extract has shown significant antioxidant activity in DPPH (73) and FRAP (1359) method. The HIV-1 RT (83.81±2.14), gp 120 (80.24±2.31), integrase (79.43±3.14) and protease (77.63±2.14), DPPIV, β -glucosidase and acetyl cholinesterase activity was significantly reduced by the extract. The 2-diphenylmethyleneamino methyl ester had shown significant interaction with oxidant and HIV-1 proteins whereas alloisioimperatorin have interacted with diabetes and cholinesterase proteins followed by hymecromone with high binding energy. These three phytochemicals are non-carcinogens, non-toxic, readily degradable and have drug likeliness properties. The *C. uredinicola* phytochemicals are responsible for management of diabetes, HIV-1 and Alzheimer. Further *in vivo* work is needed to justify our research.

Introduction

Plant drugs constitute 25% of the total drugs and have no or minimal side effects. If we use these plant-based drugs continuously we need plants in large quantity and they may vanish on the earth in the future. So, to save plants, exploiting the endophytes to obtain plant-based drugs is practicing nowadays. Endophytes are endosymbionts resides in plant tissues, they either bacteria or fungi but they are not causing any diseases to host. The endophytic fungi are able or capable to produce what host is producing, by using these endophytes we can produce large quantity of the drug within short period by applying biotechnological aspects to meet public demand.

Calophyllum tomentosum (Calophyllacace) is endemic plant commonly known as bintangur grows in Sri Lanka and Western Ghats regions of Karnataka, India. In Ayurveda, the extracts are being practiced to treat ulcers, snake bites and eye diseases. Xnthonenes and triterpenes were identified from bark of *C. tomentosum* [1] and flavonoids, saponins and terpenoids from leaf part [2] exhibited strong α -glucosidase inhibitory activity. The *C. tomentosum* shown alkaloid, flavonoid, terpenoid, tannin, glycoside, saponin [3] are responsible for inhibition of α -glucosidase activity. The literature survey indicates that no reports on endophytic fungal species from *C. tomentosum* plant. In our lab, we have isolated three fungal endophytes from (different parts of *C. tomentosum*), analysed their phytochemicals through GC-MS and identified by molecular level using 18S rRNA (unpublished data). The present research was aimed to identify phytochemicals using qualitative, TLC, GC-MS

using methanol solvent extract of *C. uredinicola*, endophyte of *C. tomentosum*. The extracts were used to analyse antioxidant, anti-HIV, anti-diabetes and anti-Alzheimer activity *in vitro* and *in silico* experimental analysis.

Materials and Methods

Collection, identification and mass culture of endophytic fungi, *Cladosporium uredinicola* from *Calophyllum tomentosum*

The Department of Biotechnology of Shridevi Institute of Engineering & Technology, Tumakuru, Karnataka, India had provided the *C. uredinicola* culture and was inoculated in potato dextrose broth to get high mycelia mass at 26 ± 2 °C for a period of 15 days. The *C. uredinicola* was previously isolated from bark part of the *C. tomentosum*.

Extract preparation and phytochemical analysis

Methanol was used for extraction from *C. uredinicola* mycelia using microwave method two cycles of 10 min at 100 °C. The qualitative methods were employed to identify phytochemicals in *C. uredinicola* extract [4-5].

TLC

Chromatography was performed using endophytic fungal extracts [6].

Analysis of GC-MS

Separation of the phytochemicals was observed by employing GC-MS method in *C. uredinicola* extract [7] and identified each separated phytochemicals based on the parameters *viz.*, molecular weight, structures of the component, total ionic chromatograms, retention time and ionization chromatograms.

Assay of antioxidant activity in *C. uredinicola* extract

Antioxidant properties of *C. uredinicola* methanol extract was carried out using “Ferric Ion Reducing Antioxidant Power (FRAP) and 2, 2-Di Phenyl-1-Picryl Hydrazyl (DPPH)” assays.

DPPH activity

The extract of *C. uredinicola* was used for DPPH activity [8]. The freshly prepared samples were dissolved in 24 mg DPPH in 100 ml ethanol and stored at -20 °C. The sample solution of 150 μ l (10 μ l of sample and 140 μ l of distilled water) was mixed with 2850 μ l of sample containing 190 μ l of reagent and 2660 μ l of distilled water and allowed 24 h for reaction each in dark condition. The reaction was measured at 515 nm. The standard ascorbic acid curve was range of 25 to 800 μ M used to analyse test sample. To get absorbance of 1.1 ± 0.02 units at 517 nm, the 45 ml of methanol was added to stock solution (10 ml) [9]. Triplicate was maintained to all the experiments. The per cent inhibition of DPPH due to sample was measured and used the standard formula for calculation as mentioned below;

$$\text{Inhibition (\%)} = \frac{AC - AS}{AC} \times 100,$$

where AC- absorbance of DPPH activity with ethanol, AS- absorbance of DPPH activity with sample or absorbance of DPPH activity with standard.

Assay for FRAP

FRAP solution was prepared by mixing 2,4,6-tris-(2-pyridyl)-S-triazine (TPTZ) solution (2.5 ml) (10mM in 40mM HCl), acetate buffer (25ml) (300 mM, pH 3.6) and mixed the 2.5 ml FeCl_3 (20mM) water. The *C. uredinicola* (150 μ l and 0.5 mg/ml) was mixed with methanol later added 4.5 ml of FRAP, FRAP without sample was used as blank and reaction was observed at 593 nm [10-11]. The sample reaction was compared with standard ascorbic acid.

In vitro* anti-diabetic activity of *C. uredinicola* extract*Activity of inhibition of α -amylase**

The working solution contains 250 μ L of 2% (w/v) starch (250 μ L), α -amylase solution (250 μ L of 1 U/mL) and *C. uredinicola* extract (250 mL of 500 μ g/mL) was incubated for 3 min at 20 °C. To stop the reaction, dinitrosalicylic acid (500 μ L) was added to the reaction mixer, subjected to boiled water immediately added α -amylase (250 μ L) and heated the solution for 15 min. After heating, the reaction mixer was kept at 26 ± 2 °C for 3 min. To get total volume of 6000 μ l, 4500 μ L of aqua dest was mixed and homogenized the mixer in vortex. At 540 nm, the activity of α -amylase was measured in with sample or standard or without sample with the help of spectrophotometer and triplicate was maintained for each experiment. Inhibition of α -amylase activity was calculated using standard equation [11].

Inhibitory activity of α -glucosidase

The reaction mixer contains phosphate buffer solution (36 μ L), *C. uredinicola* solution (30 mL) at different concentrations of 10, 25, 50, 100 and 150 μ g/ml and 4-nitrophenyl-- α -D glycopyranoside (PNPG) substrate (17 μ l) was allowed to reaction for 5 min at 37 °C. Added the α - glucosidase solution (17 μ l of 0.15 U/mL) to each well to get 100 mL of volume after 5 min of incubation. The reaction solution allowed for 15 min and added sodium carbonate (100 μ l of 200 mM). The reaction was observed at 405 nm in micro plate reader and repeated the each experiment thrice and calculated the reaction [11].

Inhibitory activity of dipeptidyl peptidase IV

Incubated the reaction mixture of 50 μ L dipeptidyl peptidase (DPP-IV) was mixed with 25 μ L *C. uredinicola* extract for 5 min at 37 °C. Added the 100 μ L Gly-Pro-P-Nitroanilide (GPPN) (2 mM) to the reaction mixture and enzyme activity allowed for 15 min. The reaction was terminated by adding 25 μ L of acetic acid glacial (25%) and reaction activity was measured at 405 nm [11].

In vitro* HIV-1 enzymes inhibition of *C. uredinicola* methanol extract*Inhibition of activity of HIV-1 reverse transcriptase (RT)**

Using 5mM MgCl₂, 150 mM KCl, 0.05% NP-40, 5mM DTT, 0.5 mM EGTA, 0.3M Glutathione, 2.5 μ g/ml BSA, 2.5 μ g/ml Poly(rA).p(dT), 20 μ M dTTP, 0.5 μ Ci (microcurie) of [3H]TTP, 50mM Tris (pH 7.8), the 100 μ l of reaction mixture was prepared. To the reaction mixture added the 0.5 units RT enzyme and incubated for 3 h at 37 °C. By adding 0.1M EGTA (25 μ l) the enzyme activity was terminated later incubated the reaction mixer on ice for chilling. 100 μ l of *C. uredinicola* was spotted on 2.5cm Whatman filter paper (circular) and was incubated for 15 min at 26 ± 2 ° C to dry. By using 5% aqueous NaHPO₄.7H₂O, washed the filters four times later two times with double distilled water. The filters were subjected to dry and to scintillation counting. To analysis, used azidothymidine as positive control and without sample considered as negative control.

The percentage of inhibition calculated as,

$$\text{Per cent inhibition} = \frac{\text{Negative control} - \text{Test sample}}{\text{Negative control}} \times 100$$

Inhibitory activity of HIV-1 gp120 binding

ELISA kit was used to study binding of CD4 with gp120 [12]. We have studied our extract could interfere with biding of gp120 with CD4. The 5mg/ml of extract was added to gp120 (25ng) at 50 μ l of equal concentration of 50 μ l and was subjected to incubation at 26 ± 2 °C. Then transformed the reaction mixture to CD4 ligand containing microtiter plate wells and was subjected to incubation at 26 ± 2 °C incubated for 1h. By using buffer washed the reaction mixer three times. Through detector reagent, analysed the gp120 binding. For positive control, the heparin was considered as standard control and with sample used as negative control.

Calculated the percentage of inhibition as mentioned below,

$$\text{Inhibition Percentage} = [(AC-AS)/AC] \times 100,$$

A is optical density.

HIV-1 protease inhibition assay

The assay was performed based on standard procedure of Narayan and Rai [13]. Using buffer (50 mM of sodium acetate (pH 5.0), 1 mM ethylenediamine disodium (EDTA.2Na) and 2 mM 2-mercaptoethanol (2-ME), the HIV-1 PR solution was diluted and added the glycerol in the ratio 3:1. The Arg-ValNle-NH₂ (substrate peptides) was diluted with 50 mM of sodium acetate (pH 5.0). Two µl of extract, *C. uredinicola* and HIV-1 PR (4µl) was mixed with substrate solution (2µl, 2 mg/ml) and 10 µl of reaction mixture was incubated for 1h at 37°C. Without endophytic extract was used as control and terminated the reaction by keeping the reaction mixture for 1 min at 90 °C. Later, added the 20 µl of sterile water and an aliquot of 10 µl was analyzed by HPLC using RP-18 column (4.6 mm X 150 mm). The reaction mixture of 10 µl was injected to the column and eluted gradient by using 15-40 % of acetonitrile and trifluoroacetic acid (TFA) (0.2%) in water with 1.0 ml/min flow rate. Monitored the elution profile at 280 nm. The HIV-1 PR inhibitory assay was analysed using following formula:

$$\% \text{ inhibition} = (AC-AS)/AC \times 100,$$

for positive control the acetyl pepstatin was used.

HIV-1 integrase inhibition assay

The biotinylated long term repeat donor DNA (LTR-D) sequence of 3'-GAAAATCAGTCACACCTTTTAGAGATCGTCA-5' (LTR-D2) and 5'-biotin-ACCCTTTTAGTAGTGTGGAAATCTCTAGCAGT-3' (LTR-D1) and were unlabelled complements. These targets the digoxigenin-labelled DNA (TS1) and its 3'-labelled component were 5'-TGACCAAGGGCTAATTCACCT-digoxigenin-ACTGGTTCCTATTAAGTGA-5' (TS2). 12 µl of integrase buffer containing 25% of glycerol, 500 µg/ml of bovine serum albumin, 5mM of dithiothritol (DTT), 75mM of MnCl₂, 150 mM of 3-(N-morpholine) propane sulfonic acid, pH 7.2 (MOPS) and added 1µl of digoxigenin labelled target DNA (5pmol/mol) and sterilized water (32 µl) and these mixture were transferred to each well of a 96 well plate. Added the 1/5 dilution of integrase enzyme (9 µl) and sample solution (6 µl) to the reaction mixture and the reaction plates were subjected to incubation at 37° C for 80 min. Using PBS, washed the wells four times, incubated the reaction mixture at 37° C for 1 h after adding 100 µl of alkaline phosphatase (AP) labelled anti-digoxigenin antibody (500 mU/ml). Using washing buffer, the plates were washed four times. 150 µl of AP buffer [5mM MgCl₂, 10mM of p-nitrophenyl phosphate, 100mM of NaCl, 100 mM of Tris-HCl (pH 9.5)] was added to each well containing reaction solution and subjected to incubation at 37°C for 1h. Using microplate reader, the reaction was measured at 405 nm. 50% of DMSO and an integrase containing negative solution was referred as negative, buffer-E containing 20 mM MOPS (7.2), 20% of glycerol, 0.1% of nonidet-P40, 4M of urea without the integrase enzyme, 400 mM of potassium glutamate and 1mM of ethylenediamine tetra acetate disodium salt (EDTA-2Na) was referred as blank. For positive control, the suramin (polyanionic HIV-1 integrase inhibitor) was used.

$$\text{Inhibition percentage of integrase} = OD_{\text{control}} - OD_{\text{sample}} / OD_{\text{control}} \times 100$$

Inhibition of protease enzyme

In a 500µl of reaction mixture, 800µg haemoglobin, *C. redinicola* extract and 50µg pepsin was incubated for 20 min at 37° C to allow proper mixing and to stop the reaction added the 5% of TCA. Centrifuged the reaction for 5 min at 14000 g and the supernatant OD was recorded at 280 nm. To compare the reaction effect of our sample, both negative (pepstatin A, a protease inhibitor) and positive controls (enzyme and standard substrate) were used. Triplicate was maintained for each sample.

***In vitro* anti-Alzheimer activity**

Acetyl and butyryl cholinesterase inhibition assay

96-well microplate reader was used to carry out acetylcholinesterase assay. The ChE enzyme (10 mL volume, diluted 100 times in phosphate buffer, pH 7.4) was mixed with DTNB (5,50-dithiobis-(2-nitro-benzoic acid)) (104 M concentration), 70 mM of phosphate buffer (Na₂HPO₄/NaH₂PO₄, pH 7.4) and ATCh (1.35 X 10⁻⁴ M concentration) in plate wells and allowed for reaction at 37° C and reaction was measured for 5 min at 412 nm. For each experiment, three replicates were maintained. The enzyme inhibition percentage was calculated by comparing with negative and positive control [14].

***In silico* antioxidant, anti-HIV, anti-diabetic and anti-cholinesterase activity**

Selection of proteins/ receptors/ enzymes

The oxidant proteins, 1cb4 (SOD), 1qqw (Catalase), 1spd (SOD), 2cag (Catalase), 2he3 (gpx), 2p31 (Glutathione peroxidase 7), 3mng (human oxidant enzyme), the HIV-1 proteins 1bi4 (integrase), 1c0u (RT), 1dmp (protease), 1eby (protease), 1ex4 (integrase), 1exq (integrase), 1gc1 (gp120), 1rev (RT), 1w5x (protease), 2buo (capsid), 2m8n (capsid), 2nxy (gp120), 2ny7 (gp120), 3h47 (capsid), 3kk2 (RT), 3ndw (protease), 3p05 (capsid), the diabetic proteins 1ppi (amylase), 1uok (glucosidase), 2g5p (pppiv), 3q6e (human insulin), 3w37 (glucosodase), 4gqr (human pancreatic amylase), 4x9y (amylase) and acetyl and butyryl cholinesterase proteins, 1b41 (AChE), 1gqr (AChE), 1xlw (BChE), 2ace (AChE), 2j4c (BChE), 2x8b (AChE), 4aqd (BChE), 4bds (BChE), 4pqe (AChE) were used for the molecular interaction studies.

Preparation of ligands

All the seven phytochemicals of *C. uredinicola* structures were obtained from NCBI PubChem database and their canonical smiles were used to generate 3D structure from www.mn-am.com/online_demos/corina_demo. The pharmacokinetic properties such as carcinogenicity, toxicity, inhibitory properties and various other properties were screened using admet-SAR device. Sedate likeliness, Adsorption, Dissolution, Metabolism, Excretion profile, toxicity and adverse factors of the ligand was anticipated. The ADME incorporates rate of retention, metabolism, digestion system and excretion. The admet-SAR employs Caco-2-cell (human epithelial colorectal adenocarcinoma cell lines) and MDCK (Madin-Darby Canine Kidney) cell models for oral medication, retention, skin porousness and human intestinal absorption to demonstrate oral and transdermal medical assimilation. Pre-ADMET predicts poisonous quality in view of the ADMET parameters and Rat acute toxicity [15-16].

Docking studies/Virtual screening

Molecular docking is the study utilized to predict the binding interaction of a molecule (ligands/drug candidates) to target proteins/receptors to discern the fitness of the ligand in the active site of the receptor. Consequently, the knowledge about the affinity and activity of the ligand can be determined. The 3D structure of all the seven ligands obtained from GC-MS study were developed using the CORINA tool (<http://mn-am.com/online-demos/corina-demo>), by entering the chemical structures as SMILES strings. CORINA is a fast and powerful tool which generates single, high-quality and low energy 3D structures of drug like molecules used for *in silico* profiling. The receptors were prepared by eliminating the water molecules from the PDB structures. Docking was carried out using iGEMDOCKv2.1. It is a tool used to study the interactions of pharmacologically important drugs. It provides basic idea on interactive or binding sites of receptor or proteins. After docking is completed a protein-ligand complex is generated along with the interaction profile which is used to rank the ligands based on the pharmacological energy. We have used two different methods to evaluate the docking scores, which accounts for biasing by dividing by molecular weight and non-hydrogen atoms [17].

AdmetSAR test

The ADMET (Absorption Distribution Metabolism Excretion Toxicity) profiling describes the disposition of pharmaceutical compound within an organism. The inhibitory properties of phytochemicals were studied through ADMET profile by submitting the canonical smiles downloaded from PubChem in admetSAR (admetSAR@LMMD). Human intestinal absorption, human oral bioavailability, penetration of blood–brain barrier, binding of plasma protein, volume of distribution, cytochrome P450 substrate, inhibitor, inducer, activator, half time (t_{1/2}), renal clearance, drug induced toxicity, genomic toxicity, aquatic and terrestrial toxicity, reproductive toxicity, environmental factor – biodegradability.

Drug-likeness studies

Drug likeliness properties of each ligand were studied using Molsoft L.L.C. online portal (www.molsoft.com) [18].

Results and Discussion

The methanol extract of *C. uredinicola* shows important phytochemicals viz., flavonoids, tannins, alkaloids, glycosides, phenols and coumarins but it not shown the saponin, anthraquinones (Table 1). The *C. uredinicola* reported as endophytic fungi from *Tinospora cordifolia* [19], *Psidium guajava* [20], Guava fruit [21]. Similar kinds of phytochemicals were noticed from endophytic fungi, *Neurospora crassa* [22] and *Penicillium* species [23].

Table 1. Phytoconstituents present in methanol extract of *Cladosporium uridinicola*

Phytochemicals	<i>Cladosporium uridinicola</i> extract
Anthraquinones	-
Flavonoids	+
Saponins	-
Tannins	+
Alkaloids	+
Glycosides	+
Phenols	+
Terpenoids	+
Coumarins	+

*Repeated the each experiment thrice, + =Presence and - = Absence

The obtained pure culture (Fig. 1A) was mass cultured in PDB (Fig. 1B) and the TLC have shown well separated many compounds in the extract (Fig. 1C). The GC-MS results have exhibited seven different phytochemicals they are 2H-1-Benzopyran-2-one (1), 3-Benzofurancarboxylic acid (2), Hymecromone (3), 16-Octadecanoic acid, methyl ester (4), 4-Hydroxy-9-(3-methyl-2-butenyl)furo(3,2-g)chromen-7-one (5), Z,E-2-Methyl-3,13-octadecadien-1-ol (6), [Z]-Cinnamic acid, 2-diphenylmethyleneamino-, methyl ester (7) and identified based on retention time using phytochemical library (Fig. 1D and Table 2).

Figure 1. A. Pure culture of *Cladosporium uredinicola* on PDA media, B. *C. uredinicola* grown in liquid media, C. TLC shows separated phytochemicals, and D. methanol extract of *C. uredinicola* showing 7 biologically important phytochemicals in GC-MS. 1-2H-1-Benzopyran-2-one, 2-3-Benzofurancarboxylic acid, 3- Hymecromone, 4-16-Octadecanoic acid, methyl ester, 4-4-Hydroxy-9-(3-methyl-2-butenyl)furo(3,2-g)chromen-7-one, 5-4-Hydroxy-9-(3-methyl-2-butenyl)furo(3,2-g)chromen-7-one, 6- Z,E-2-Methyl-3,13-octadecadien-1-ol, 7-[Z]-Cinnamic acid, 2-diphenylmethylethylamine-, methyl ester

Table 2. Bioactive compounds of methanol fraction of *Cladosporium* species BCt of *Calophyllum tomentosum*

Peak No	RT (min)	Identified compound Name	Structure	Synonyms
1	16.05	2H-1-Benzopyran-2-one		1) Coumarin 2) 2H-1-Benzopyran-2-one 3) 2H-Chromen-2-on 4) 2H-Chromen-2-one 5) 2H-Chromén-2-one 6) Chromen-2-one
2	17.22	3-Benzofurancarboxylic acid		1) Coumarilic acid, 2) Coumarone-2-carboxylic acid, 3) 2-Benzofurancarboxylic acid 4) 1-benzofuran-3-carboxylic acid
3	17.75	Hymecromone		1) 2H-1-Benzopyran-2-one, 7-hydroxy-4-methyl- 2) Coumarin, 7-hydroxy-4-methyl- 3) β -Methylumbelliferone 4) Bilcolic

				5) Bilicante 6) Cantabilin 7) Coumarin 4 8) Eurogale 9) 4-Methyl-7-Hydroxycoumarin 10) 4-Methylumbelliferone 11) 7-Hydroxy-4-methylcoumarin 12) 7-Hydroxy-4-methyl-2-oxo-3-chromene 13) Cholestil 14) Cholonerton 15) Crodimon 16) Cumarote-C 17) 2H-1-Benzopyran, 7-hydroxy-4-methyl-2-oxo- 18) 2H-1-Benzopyren-2-one, 7-hydroxy-4-methyl- 19) 7-Hydroxy-4-methyl-2-oxo-2H-1-benzopyran 20) 7-Hydroxy-4-methyl-2H-1-benzopyran-2-one 21) Cholspasmin 22) Himecol
4	18.93	16-Octadecanoic acid, methyl ester		1) 16-Octadecenoic acid methyl ester 2) Methyl (E)-octadec-16-enoate
5	21.12	4-Hydroxy-9-(3-methyl-2-butenyl)furo(3,2-g)chromen-7-one		1) 4-Hydroxy-9-(3-methyl-2-butenyl)-7H-furo[3,2-g]chromen-7-one, 2) 4-Hydroxy-9-(3-methyl-2-butenyl)furo(3,2-g)chromen-7-one, 3) 4-hydroxy-9-(3-methylbut-2-enyl)furo[3,2-g]chromen-7-one, 4) <u>Alloisioimperatorin</u>
6	25.47	Z,E-2-Methyl-3,13-octadecadien-1-ol		1) 3E,13E)-2-Méthyl-3,13-octa déca dién-1-ol 2) 3,13-Octadecadien-1-ol, 2-methyl-, (3E,13E)
7	27.95	[Z]-Cinnamic acid, 2-diphenylmethylen amino-, methyl ester		1) (E)-Cinnamic acid- α -diphenylmethylen amino-, methyl ester 2) Methyl (2Z) -2- [(diphenyl methylene) amino]-3-phenyl-2-propenoate, 3) methyl (Z)-2- di (phenyl) methylidene amino] -3-phenylprop-2-enoate, 4) (Z)-2- di (phenyl) methylene amino] -3-phenyl- acrylic acid methyl ester,

The similar types of phytochemicals were also observed from different endophytes 2H-1-Benzopyran-2-one [24], 3-Benzofurancarboxylic acid [25], Hymecromone [26], 16-Octadecanoic acid, methyl ester [27], alloisioimperatorin [28], Z,E-2-Methyl-3,13-octadecadien-1-ol [29], [Z]-Cinnamic acid, 2-diphenylmethylen amino-, methyl ester [30].

These endophytic fungal phytochemicals are responsible for *in vitro* antioxidant activity [31-32]. The *in vitro* antioxidant method, DPPH is universally considered to screen the antioxidant compounds and it is causing any effect on enzyme inhibition and metal [33]. The methanol extract of *C. uredinicola* was studied free radical scavenging activity using DPPH method and standard ascorbic acid was used. The *C. uredinicola* extract had shown significant antioxidant activity and it was concentration dependent. Fig. 2 represents the antioxidant activity of endophytic activity was compared with ascorbic acid. The phytochemicals present in extract of *C. uredinicola* are responsible for antioxidant properties. The endophytic fungal phytochemicals donate electrons to DPPH and they are responsible for reduction of purple coloured DPPH to colourless solution [34]. Our findings are agreement with results of Manjunath et al. [35], Hulikere et al. [31]. The methanol extract of *C. uredinicola* phytochemicals reduced the Fe^{3+} TPTZ complex to Fe^{2+} -tripyridyltriazine (blue coloured complex) by donating electron at low pH and the reaction was observed and measured in absorbance at 593 nm. The *C. uredinicola* phytochemicals are acts strong as antioxidants agents in reducing power potential (Fig. 3) [36-38].

Figure 2. DPPH scavenging activities of *Cladosporium uredinicola* methanol extract compared the values with standard ascorbic acid

Figure 3. Total antioxidant activity of *Cladosporium uredinicola* methanol extract compared with standard ascorbic acid

The *C. uredinicola* methanol extract significantly reduced the activity of α -amylase, α -glucosidase and dipeptidyl peptidase IV activity *in vitro* condition (Fig. 4). The result confirms that inhibitory activities of diabetic enzymes are dependent on concentration of the sample. The *C. uredinicola* phytochemicals strongly inhibited the activity of α -amylase and it was significant when compared to positive control standard drug acarbose. Our results are confirmation with the findings of endophytic fungal α -glucosidase inhibitory activity [39-40]. The *C. uredinicola* extract inhibited the α -glucosidase activity at maximum level. The activity of DPP-IV significantly inhibited by *C. uredinicola* extract and it was compared with standard drug diprotin. The literature survey indicates that no results were found in dipeptidyl peptidase IV inhibitory activity using fungal extracts. The obtained results proving significant inhibition of dipeptidyl peptidase IV by *C. uredinicola* extract and are confirmation with the results of Kumar et al. [41]. The plant extracts also exhibited the dipeptidyl peptidase IV inhibitory activity [41-42].

Figure 4. Per cent inhibition of diabetic enzymes by *C. uredinicola* phytochemicals

The *C. uredinicola* extract had shown inhibition of HIV-1 proteins viz., protease, RT, protease and gp120. The *C. uredinicola* was inhibited RT activity strongly (83.81 ± 2.14) and it was high compared to standard AZT (74.36 ± 1.89) (Table 3) [27, 43]. Similarly, the gp120 (80.24 ± 2.4) (Table 4), protease (77.63 ± 2.14) (Table 5), integrase (79.43 ± 2.14) (Table 6) proteins activity was decreased due to *C. uredinicola* extract. The HIV-1 proteins activity was inhibited due to potent phytochemicals of *C. uredinicola* and the activity may in combination of all the phytochemicals or any single potent phytochemical [41-42].

Table 3. Per cent inhibition of RT by *Cladosporium uredinicola* methanol extract compared with respective standard drugs

Treated agents	Per cent inhibition of RT (mean $\pm$ SD)
<i>Cladosporium uredinicola</i> extract	83.81 ± 2.14
AZT (0.0016 mg/ml)	74.36 ± 1.89

Note: inhibition $\geq 50\%$ is considered as significant

Table 4. Inhibition percentage of gp120 by *Cladosporium uredinicola* methanol extract compared with respective standard drugs

Treated agents	Per cent inhibition of gp120 (mean $\pm$ SD)
<i>Cladosporium uredinicola</i> extract	80.24 ± 2.31
Heparin (12.5 units)	76.48 ± 1.69

Note: inhibition $\geq 50\%$ is considered as significant

Table 5. Inhibition percentage of protease by *Cladosporium uredinicola* methanol extract compared with respective standard drugs

Treating agents	IC ₅₀ (μg/ml)±SD
<i>Cladosporium uredinicola</i> extract	77.63±2.14
Acetyl pepstatin (positive control for protease)	82.91±2.14

Table 6. Inhibition percentage of integrase by *Cladosporium uredinicola* methanol extract compared with respective standard drugs

Treating agents	IC ₅₀ (μg/ml)±SD
<i>Cladosporium uredinicola</i> extract	79.43 ±2.14
Suramin (positive control for integrase)	81.97±2.14

The *C. uredinicola* methanol extract significantly inhibited the AChE and BChE activity *in vitro*. The study explains that AChE (33.47±2.8) was strongly inhibited due to the endophytic fungi extract compared with BChE (56.52±2.8) and these results were compared with standard drug Galantamine (Table 7). The inhibition of these enzymes may be due to the *C. uredinicola* phytochemicals may adsorbed or interact with AChE or BChE protein [44-45].

Table 7. Anticholinesterase activity of *Cladosporium uredinicola* methanol extract compared with respective standard drugs

Samples	AChE assay IC ₅₀ (mg/ml)	BChE assay IC ₅₀ (mg/ml)
<i>Cladosporium uredinicola</i> extract	33.47±2.8	56.52±1.08
Galantamine	39.81±2.8	2.6±1.1

IC₅₀ values represent the means ± standard deviation of three parallel measurements

The molecular interaction studies were carried out between *C. uredinicola* phytochemicals with oxidant, HIV-1, diabetes and acetyl-cholinesterase proteins. The seven different phytochemicals have shown different binding energy with different oxidant proteins. The [Z]-Cinnamic acid, 2-diphenylmethyleamino methyl ester had shown highest interaction with all the oxidant proteins followed by alloisioimperatorin and hymecromone. The 2-diphenylmethyleamino methyl ester have interacted with val165 of 2p31 of 2p31 (-97.3) followed by 2cag (-96.14), 2he2 (-90.02), 1cb4 (-88.38), 1qqw (-88.25). The alloisioimperatorin ester had shown highest interaction with 2cag proteins (-97.56) at ser336, tyr337 followed by 1spd (-86.41), 3mng (-84.75), 1qqw (-83.18) (Fig. 5) (Table 8). No reports on 2-diphenylmethyleamino methyl ester antioxidant activity but alloisioimperatorin [46] and hymecromone [47] had shown strong antioxidant activity *in vitro* condition but no reports on *in silico* antioxidant activity.

Figure 5. *In silico* molecular docking diphenylmethyleamino methyl ester with oxidant, diabetic, HIV-1 and cholinesterase proteins

Table 8. Molecular interaction study between *C. uredinicola* phytochemicals with oxidant proteins

PDB number	<u>Alloisioimperatorin</u>			<u>[Z]-Cinnamic acid, 2-diphenylmethyleamino methyl ester</u>			<u>Hymecromone</u>		
	1	2	3	1	2	3	1	2	3
1cb4	-77.89	-69.08	-8.81	-88.38	-88.38	0	-72.97	-58.47	-14.5
1qqw	-83.18	-63.74	-19.44	-88.25	-82.79	-5.47	-85.76	-64.84	-20.92
1spd	-86.41	-67.24	-19.17	-84.81	-75.18	-9.63	-79.29	-56.67	-22.63
2cag	-97.56	-81.57	-15.99	-96.14	-90.33	-5.81	-85.69	-63.15	-22.54
2he3	-76.44	-63.73	-12.7	-90.02	-84.12	-5.9	-68.6	-51.65	-16.95
2p31	-75.32	-54.71	-20.6	-97.3	-90.76	-6.54	-93.44	-78.1	-15.34
3mng	-84.75	-73.83	-10.92	-86.48	72.21	14.27	-74.94	-56.95	-17.99

1- Binding energy (kJ/mol), 2-VDW, 3-H-bond

The diphenylmethyleamino methyl ester had shown strong interaction with HIV-1 protease (1w5x:-103.41; 1eby:-102.58; 3ndw:-100), HIV-1 gp120 (2ny7:-108.47; 2nxy: -92.57; 1gc1:-84.24), HIV-1 RT (3kk2: -98.69; 1rev:-92.88; 1c0u:-88.41) and HIV-1 integrase (1ex4:-80.66; 1exq:-80.45; 1bi4:-79.23) followed by hymecromone and alloisioimperatorin (Table 9) (Fig. 5) with high binding energy. The diphenylmethyleamino methyl has exhibited strong *in vitro* anti-HIV activity [48]. There is no report on *in vitro* and *in silico* anti-HIV activity of alloisioimperatorin and hymecromone. The diphenylmethyleamino methyl ester had ability to interact with HIV-1 integrase, protease, RT, gp120 proteins with high binding energy leads to confirmation changes to inhibit their functions.

Table 9. Molecular interaction study between *C. uredinicola* phytochemicals with HIV-1 proteins

PDB number	<u>Alloisioimperatorin</u>			[Z]-Cinnamic acid, 2-diphenylmethyleamino methyl ester			Hymecromone		
	1	2	3	1	2	3	1	2	3
HIV-1 integrase									
1bi4	-77.59	-61.05	-16.54	-79.23	-74.71	-4.53	-85.72	-79.35	-6.37
1ex4	-79.08	-69.7	-9.38	-80.66	-71.05	-9.62	-65.5	-63	-2.5
1exq	-84.39	-78.92	-5.47	-80.45	-70.88	-9.56	-64.09	-60.59	-3.5
HIV-1 protease									
1dmp	-86.81	-75.21	-11.6	-88.92	-85.42	-3.5	-65.71	-55.03	-10.68
1eby	-85.19	-72.1	-13.09	-102.58	-105.28	0	-80.74	-73.28	-7.46
1w5x	-85.38	-83.61	-1.77	-103.41	-103.41	0	-81.1	-71.74	-9.36
3ndw	-80.74	-62.48	-18.26	-100	-93	-7	-70.78	-70.78	0
HIV-1 gp120									
1gc1	-86.57	-60.57	-26.08	-84.24	-80.74	-3.5	-83.66	-83.66	0
2nxy	-91.24	-75.79	-15.46	-92.57	-82.07	-10.5	-71.57	-81.57	0
2ny7	-105.91	-97.41	-8.49	-108.47	-105.97	-2.5	-95.23	-95.23	0
HIV-1 RT									
1c0u	-77.3	-73.8	-3.5	-88.41	-83.23	-5.17	-76.02	-72.52	-3.5
1rev	-89.9	-86.79	-3.11	-92.88	-92.88	0	-83.35	-77.19	-6.16
3kk2	-94.51	-88.64	-5.87	-98.69	-92.41	-6.28	-87.06	-87.06	0

1- Binding energy (kJ/mol), 2-VDW, 3-H-bond

The alloisioimperatorin was shows highest interaction with all the three diabetic enzymes (α -amylase, β -glucosidase and DPPIV) by showing highest binding energy compared to diphenylmethyleamino methyl ester and hymecromone (Table 10) (Fig. 6). The alloisioimperatorin have firmly interacted with α -amylase and DPPIV followed by β -glucosidase. The alloisioimperatorin had interacted with val234, glu233 of 4x9y and showed highest binding energy. No reports are available on these compounds as antidiabetic activity from *in vitro* and *in silico* assays.

Table 10. Molecular interaction study between *C. uredinicola* phytochemicals with diabetic proteins

PDB number	<u>Alloisioimperatorin</u>			[Z]-Cinnamic acid, 2-diphenylmethyleamino methyl ester			Hymecromone		
	1	2	3	1	2	3	1	2	3
α-amylase									
1ppi	-83.35	-65.71	-17.64	-86.84	-86.84	0	-71.5	-57.62	-13.87
4x9y	-98.81	-82.91	-15.89	-87.14	-87.14	0	-85.97	-70.51	-15.46
4gqr	-92.71	-76.41	-16.3	-85.66	-82.36	-3.26	-71.24	-61.75	-9.49
β-glucosidase									
1uok	-80.96	-70.93	-10.04	-83.43	-77.05	-6.38	-70.71	-56.6	-14.1
3w37	-80.73	-74.73	-6	-90.63	-83.22	-7.41	-79.01	-55.12	-23.89
DPPIV									
2g5p	-83.58	-68.64	-14.94	-86.83	-70.9	-15.93	-82.15	-59.58	-22.58
3c45	-92.44	-73.55	-18.89	-91.47	-84.11	-7.36	-80.46	-59.54	-20.92

1- Binding energy (kJ/mol), 2-VDW, 3-H-bond

Figure 6. *In silico* molecular docking of alloisomperatorin with oxidant, diabetic, HIV-1 and cholinesterase proteins

The alloisomperatorin had shown strong interaction with AChE and BChE proteins with binding energy and results shows that the compounds have firm interaction with AChE. The alloisomperatorin have ability to bind with tyr124, ser125, thr83, gly82, tyr337 of 4pqr. No reports on *in vitro* and *in silico* acetylcholinesterase activity of alloisomperatorin (Fig. 6) (Table 11). From *in silico* results, we have discussed only three best phytochemicals possessing antioxidant, anti-diabetic, anti-HIV and anti-acetylcholinesterase activity. Out of seven phytochemicals of *C. uredinicola*, the diphenylmethylethylamine methyl ester showed strong anti-oxidant and anti-HIV

activity and alloisoimperatorin shows potent anti-diabetic and anti-cholinesterase activity in both *in vitro* and *in silico* experimental analysis.

Table 11. Molecular interaction study between *C. uredinicola* phytochemicals with cholinesterase proteins

PDB number	Alloisoimperatorin			[Z]-Cinnamic acid, 2-diphenylmethyleamino methyl ester			Hymecromone		
	1	2	3	1	2	3	1	2	3
AChE									
1b4l	-90.52	-75.54	-14.98	-84.25	-83.06	-1.18	-81.33	-68.44	-12.88
1gqr	-101.5	-92.53	-8.98	-94.87	-92.37	2.5	-84.18	-70.34	-13.83
2ace	-103.69	-97.77	-5.92	-84.73	-84.73	0	-71.08	-52.14	-18.94
2x8b	-99.13	-88.17	-10.97	-94.46	-80.51	-13.94	-82.46	-66.1	-16.36
4pge	-103.95	-93.87	-10.08	-90.89	-84.77	-6.12	-81.68	-69.69	-11.99
BChE									
1xlw	-92.42	-79.56	-12.86	-87.03	-87.03	0	-72.73	-60.13	-12.6
2j4c	-85.41	-72.21	-13.2	-83.64	-83.64	0	-78.19	-64.88	-13.31
4aqd	-93.6	-75.1	-18.5	-84.69	-82.19	-2.5	-76.46	-72.96	-3.5
4bds	-91.51	-78.23	-13.27	-87.16	-87.16	0	-75.9	-65.42	-10.48

1- Binding energy (kj/mol), 2-VDW, 3-H-bond

The admet-SAR results confirm that these three promising compounds are non-AMES toxic, non-carcinogens, readily biodegradables (Table 12A-B). They also showed drug likeliness property in Molsoft (Table 13 and Fig. 7). Further, *in vivo* work will be carried out in future.

Table 12A. ADMET Predicted profile of the potent phytochemicals of *Cladosporium uredinicola* species

Property	Cinnamic acid, 2-diphenylmethylene		2H-1 Benzopyran-2-one ND		3- Benzofurancarboxylic acid ND		4 Hydroxy-9 (3-methyl-2-butenyl)	
	Value	Probability	Value	Probability	Value	Probability	Value	Probability
Blood Brain Barrier	BBB+	0.9526	BBB+	0.9607	BBB+	0.9607	BBB+	0.7646
Human Intestinal absorption	HIA+	0.9945	HIA+	0.9967	HIA+	0.9967	HIA+	0.9910
Caco-2-permeable	CaCo2-	0.9097	CaCo2+	0.5651	CaCo2+	0.5651	CaCo2+	0.5382
P-glycoprotein-substrate	Substrate	0.8286	Non-substrate	0.7543	Non-substrate	0.7543	Substrate	0.6445
P-glycoprotein-inhibitor I	Non-inhibitor	0.9861	Non-inhibitor	0.9394	Non-inhibitor	0.9394	Non-inhibitor	0.6210
	Inhibitor	0.9906	Non-inhibitor	0.8348	Non-inhibitor	0.8348	Non-inhibitor	0.7706
Renal organic cation transporter	Non-inhibitor	0.9145	Non-inhibitor	0.9014	Non-inhibitor	0.9014	Non-inhibitor	0.8232
Distribution								
Subcellular localization	Plasma membrane	0.5803	Plasma membrane	0.4393	Plasma membrane	0.4393	Mitochondria	0.7391
Metabolism								
CYP450 2C9 Substrate	Non-substrate	0.7768	Non-substrate	0.8051	Non-substrate	0.8051	Non-substrate	0.7767
CYP450 2D6 Substrate	Non-substrate	0.9644	Non-substrate	0.9225	Non-substrate	0.9225	Non-substrate	0.8912
CYP450 3A4 Substrate	Substrate	0.8188	Non-substrate	0.7867	Non-substrate	0.7867	Non-substrate	0.5203
CYP450 1A2 Substrate	Non-inhibitor	0.8383	Non-inhibitor	0.6553	Non-inhibitor	0.6553	Non-inhibitor	0.5799
CYP450 2C9 Inhibitor	Non-inhibitor	0.9763	Non-inhibitor	0.9574	Non-inhibitor	0.9574	Non-inhibitor	0.7026
CYP450 2D6 Inhibitor	Non-inhibitor	0.9546	Non-inhibitor	0.9641	Non-inhibitor	0.9641	Non-inhibitor	0.6105
CYP450 2C19 Inhibitor	Non-inhibitor	0.9724	Non-inhibitor	0.9395	Non-inhibitor	0.9395	Non-inhibitor	0.6196
CYP450 3A4 Inhibitor	Non-inhibitor	0.9702	Non-inhibitor	0.9727	Non-inhibitor	0.9727	Non-inhibitor	0.6850
CYP Inhibitory Promiscuity	Low CYP inhibitory	0.9687	Low CYP inhibitory	0.9416	Low CYP inhibitory	0.9416	Low CYP Inhibitory	0.7251
Excretion-Toxicity								
Human Ether-a-go-Related Gene Inhibition	Weak inhibitor	0.9620	Weak inhibitory	0.9822	Weak inhibitor	0.9822	Weak inhibitory	0.8989
	Non-Inhibitor	0.9899	Non-inhibitor	0.9694	Non-inhibitor	0.9694	Non-inhibitor	0.9525
AMES Test	Non- AMES toxic	0.9722	Non AMES toxic	0.8589	Non AMES toxic	0.8589	Non AMES toxic	0.8380
Carcinogens	Non-carcinogens	0.5927	Non-carcinogens	0.8669	Non-carcinogens	0.8669	Non-carcinogens	0.9384
Fish Toxicity	High FHMT	0.8969	High FHMT	0.6986	High FHMT	0.6986	High FHMT	0.9962

Tetrahumena Pyriformis Toxicity	High TPT	0.9585	High TPT	0.8298	High TPT	0.8298	High TPT	0.9793
Honey Bee Toxicity	High HBT	0.7685	High HBT	0.7662	High HBT	0.7662	High HBT	0.8708
Biodegradation	Not ready biodegrad able	0.7942	Ready biodegrad able	0.7250	Ready biodegradab le	0.7250	Ready biodegradabl e	0.8852
Acute Oral Toxicity	III	0.8487	IV	0.7714	III	0.7714	III	0.5070
Carcinogenicity (Three class)	Non- required	0.7458	Non- required	0.4655	Non required	0.4655	Danger	0.4513
Absorption								
Aqueous solubility	-2.4174	LogS	-1.8449	LogS	-1.8449	LogS	-4.1694	LogS
CaCo2 Permability	1.8973	LogPap p, cm/s	-1.1038	LogPap p, cm/s	1.1038	LogPa pp, cm/s	0.8455	LogPap p, cm/s
Distribution, Metabolism, Excretion, Toxicity								
Rat acute toxicity	1.7416	LD50, mol/kg	2.0566	LD50, mol/kg	2.0566	LD50, mol/kg	3.0836	LD50, mol/kg
Fish Toxicity	1.6417	pLC50, mg/L	1.6850	pLC50, mg/L	1.6850	pLC50 , mg/L	-1.1036	pLC50, mg/L
Tetrahymena pyriformis Toxicity	0.1061	pIGC5 0, ug/L	-0.3931	pIGC50 , ug/L	-0.3931	pIGC5 0, ug/L	0.8258	pIGC50 , ug/L

Table 12B. ADMET Predicted profile of the potent phytochemicals of *Cladosporium uredinicola* species

Property	16-octadecanoic acid, methyl ester ND		Hymercromone ND		Z, E-2-Methyl-3,13-octadecadine-1-01 ND	
	Value	Probability	Value	Probability	Value	Probability
Blood Brain Barrier	BBB+	0.9848	BBB+	0.9488	BBB+	0.9488
Human Intestinal absorption	HIA+	0.9881	HIA+	0.9888	HIA+	0.9888
Caco-2-permeable	CaCo2+	0.8141	CaCo2+	0.8326	CaCo2+	0.8326
P-glycoprotein-substrate	Non-Substrate	0.7061	Non-substrate	0.6321	Non-substrate	0.6321
P-glycoprotein-inhibitor I	Non-inhibitor	0.8951	Non-inhibitor	0.9598	Non-inhibitor	0.9598
	Non-Inhibitor	0.7988	Non-inhibitor	0.9277	Non-inhibitor	0.9277
Renal organic cation transporter	Non-inhibitor	0.8908	Non-inhibitor	0.9266	Non-inhibitor	0.9266
Subcellular localization	Mitochondria	0.4276	Mitochondria	0.5152	Mitochondria	0.5152
CYP450 2C9 Substrate	Non-substrate	0.8648	Non-substrate		Non-substrate	0.7886
CYP450 2D6 Substrate	Non-substrate	0.8885	Non-substrate	0.7886	Non-substrate	0.8956
CYP450 3A4 Substrate	Substrate	0.6454	Non-substrate	0.8956	Non-substrate	0.6982
CYP450 1A2 Substrate	Non-inhibitor	0.5548	Non-inhibitor	0.6982	Non-inhibitor	0.8326
CYP450 2C9 Inhibitor	Non-inhibitor	0.9329	Non-inhibitor	0.8326	Non-inhibitor	0.8808
CYP450 2D6 Inhibitor	Non-inhibitor	0.9502	Non-inhibitor	0.8808	Non-inhibitor	0.9554
CYP450 2C19 Inhibitor	Non-inhibitor	0.9524	Non-inhibitor	0.9554	Non-inhibitor	0.9578
CYP450 3A4 Inhibitor	Non-inhibitor	0.9773	Non-inhibitor	0.9484	Non-inhibitor	0.9484

CYP Inhibitory Promiscuity	Low CYP inhibitory	0.9176	Low CYP inhibitory	0.9647	Low CYP inhibitory	0.9647
Human Ether-a-go-Related Gene Inhibition	Weak inhibitor	0.9104	Weak inhibitor	0.9322	Weak inhibitor	0.9322
	Inhibitor	0.8787	Non-inhibitor	0.8868	Non-inhibitor	0.8868
AMES Test	Non- AMES toxic	0.9765	Non AMES toxic	0.9865	Non AMES toxic	0.9865
Carcinogens	Non-carcinogens	0.5347	Non-carcinogens	0.6452	Non-carcinogens	0.6452
Fish Toxicity	High FHMT	0.8790	High FHMT	0.9144	High FHMT	0.9144
Tetrahymena Pyriformis Toxicity	High TPT	0.8990	High TPT	0.9990	High TPT	0.9990
Honey Bee Toxicity	High HBT	0.7623	High HBT	0.6691	High HBT	0.6691
Biodegradation	Not ready biodegradable	0.8747	Ready biodegradable	0.8795	Ready biodegradable	0.8795
Acute Oral Toxicity	III	0.8589	IV	0.6378	IV	0.6378
Carcinogenicity (Three class)	Non-required	0.7269	Non-required	0.7057	Non required	0.7057
Aqueous solubility	-3.3987	LogS	-3.5022	LogS	-3.5022	LogS
CaCo2 Permability	1.2386	LogPapp, cm/s	1.3950	LogPapp, cm/s	-1.3950	LogPapp,cm/s
Rat acute toxicity	1.4915	LD50, mol/Kg	1.3275	LD50, mol/Kg	1.3275	LD50, mol/kg
Fish Toxicity	0.8236	pLC50, mg/L	1.8920	pLC50, mg/L	1.8920	pLC50,mg/L
Tetrahymena pyriformis Toxicity	0.6648	pIGC50, ug/L	0.3852	pIGC50, ug/L	0.3852	Pigc50, ug/L

Table 13. Physiochemical properties of *Cladosporium uridinicola* phytochemicals

Phytochemicals	MF	MW (<500)	HBA (>10)	HBD (<5)	LogP	LogS (Log (moles/L))	PSA (Å ²)	MV (Å ³)	NSC
2H-1-Benzopyran-2-one	C ₉ H ₆ O ₃	162.03	3	1	2.27	0.320	36.94	141.66	0
3-Benzofurancarboxylic acid	C ₁₅ H ₁₅ NO ₃	257.11	3	1	1.93	-3.09	43.86	262.08	0
Hymecromone	C ₁₆ H ₃₂ O ₂	256.24	2	1	6.65	-5.66	28.89	305.93	0
16-Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	298.29	2	0	7.97	-6.78	21.09	364.74	0
4-Hydroxy-9-(3-methyl-2-butenyl)furo(3,2-g)chromen-7-one	C ₁₆ H ₁₄ O ₄	270.09	4	1	3.55	-4.93	44.24	296.33	0
Z,E-2-Methyl-3,13-octadecadien-1-ol	C ₁₉ H ₃₈ O ₂	298.29	2	1	7.93	-6.64	28.92	356.87	1
[Z]-Cinnamic acid, 2-diphenylmethyleneamino-, methyl ester	C ₉ H ₈ O ₂	148.05	2	1	2.33	-2.61	28.62	151.60	0

MF: Molecular Formula, MW-Molecular Weight, HBA-Hydrogen Bond Acceptor, HBD-Hydrogen Bond Donor,

MolLogP-MolLogS-, MolPSA-, MolVol-, NSC-Number of Stereo Centers

Figure 7. MolSoft drug-likeness properties of the *Cladosporium uridinicola* phytochemicals used

The *C. uridinicola* extract biologically phytochemicals are responsible for strong antioxidant, anti-HIV, anti-diabetes and anti-cholinesterase activity. The *in silico* experiment clearly understands that the three phytochemicals (diphenylmethylenamino methyl ester, alloisioimperatorin, hymecromone) have the ability to interact with oxidant, HIV-1, diabetic and cholinesterase proteins with highest binding energy. The admetSAR and Molsoft proven that these three compounds are non-toxic, non-carcinogens, easily biodegradable and having drug likeliness properties.

The oxidant proteins SOD, catalase, gpx, glutathione peroxidase 7, human oxidant enzyme are inhibited by endophytic fungal extract. All these enzymes break down potentially harmful molecules in cells and these oxygen molecules play a role in disease or cell damage.

The diabetic proteins, α -amylase, β -glucosidase and DPP-IV are strongly inhibited by the *C. uridinicola* extract. The α -amylase and β -glucosidase are involved in digestion of carbohydrates lead to increase of blood glucose level in diabetes -2. The DPP-IV increases glucagon and blood glucose level. These enzymes play vital role progression of diabetes-2. HIV-1 RT (essential step in retroviral replication), protease (play crucial role HIV life cycle and it cleaves the newly synthesized polypeptides to obtain mature components of protein of an infective HIV), integrase (requires for multidomain enzyme essential for the viral DNA in the host genome), gp120 (essential for virus entry into cells and helps in attachment to specific cell surface receptors) are greatly inhibited by the endophytic fungal extract. The AChE and BChE are pathogenesis of Alzheimer disease and progression.

Conclusion

Based on outcomes of our *in vitro* and *in silico* research clearly indicates that the endophytic fungi *C. uredinicola* have shown biologically important phytochemicals in methanol extract and these are responsible for antioxidant, anti-HIV, anti-diabetes and anticholinesterase activity and suggested their possible role. Further *in vivo* work is needed to justify our research.

Acknowledgements

Author Dr Govindappa M greatly acknowledges SERB-DST, New Delhi for financial support under Empowerment and Equity (SB/MEO-355/2013 dated 29-10-2013). We thank Dr K. G. Bhat, Taxonomist, Udupi, helping in plant identification and GC-MS unit, SAIF of Indian Institute of Technology, Chennai, Tamil Nadu, India.

Conflicts of Interest

The authors declare no conflict of interest.

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