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Phytochemical, *in vitro* and *in silico* screening of roots of *Jasminum auriculatum* for antioxidant activity

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Exploring the phytochemicals from traditional medicinal plants is essential for developing the novel leads for various diseases. In humans, many diseases are associated with the accumulation of free radicals. Antioxidants have the competence to scavenge free radicals and keep down their impact. Hence in the current study, we intended the identification of phytoconstituents by aiding HPLC and GC-MS methods and assessing the antioxidant ability of ethanol extract of roots of Jasminum auriculatum using *in vitro* and *in silico* approaches. Ethanol extract of roots of *Jasminum auriculatum* was prepared and inflicted to the preliminary phytochemical studies followed by HPLC and GC -MS analysis. Further *in vitro* and *in silico* studies were conducted to assess antioxidant action of ethanol extract. The results of HPLC analysis led to the presence of rutin and GC-MS analysis resulted in presence of various bioactive compounds. *In vitro* evaluation of free radical scavenging ability using DPPH, NO and ABTS techniques exhibited that the root extract possess good antioxidant activity. Further molecular docking studies performed for the identified compounds in HPLC and GC MS analysis, which substantiated the *in vitro* studies by resulting in good glide docking score with the protein targets namely NADPH oxidase and Super Oxide Dismutase. Based on these experimental findings, it can be concluded that the roots of *Jasminum auriculatum* possess good antioxidant activity.

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KEYWORDS

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Introduction

Natural products attained from various terrestrial and marine plants were being exploited as a source of classical medicines since archaic times [1,2]. Medicinal plants play an active role as one of the sources of natural products for the treatment and management of debilitating diseases [3]. The use of plant extracts and isolated pure compounds has provided the basis for the production of herbal medicines and phytopharmaceutical compounds [4,5].

Phytochemicals in plant extract encompass several bioactive compounds which exhibits propitious pharmacological activities. These phytochemicals notably cure affliction pertinent to free radicals induced stress, renal, and cardiac complaints and also, function as anti-oxidant, anti-inflammatory, anti-hyperlipidemic and hepatoprotective [6,7]. Moreover, many phytochemicals are naturally materializing antioxidants which are

promising reason behind their underlying mechanism in the therapeutic action on various diseases and disorders [8,9]. Advancement of powerful antioxidant molecule is clinching consequence in recent years as it plays the pivotal role in preventing or detaining the onslaught of certain diseased consequences such as nephrotoxicity, hepatotoxicity, and cancer.

"Medicinal plants functions as eminent antioxidant to scavenge free radicals and have immense importance as therapeutic agent in combating oxidative stress related degenerative ailments" [10,2]. *Jasminum auriculatum* (F:Oleaceae), is a medicinal plant which is valued for its benefits in the management of various ailments in traditional medicine. Roots are used as regimen in distinct ailments including skin infections especially for ringworm, eye diseases, headache, leprosy, mouth ulcers, renal calculi, burning micturition, wound healing [11]. The roots of the plant were claimed for good ethnomedicinal benefits, however till date there were no reports on phytochemical analysis and *in vitro* and *in silico* antioxidant screening roots of *Jasminum auriculatum*. Hence, the current study is focused to identify the phytoconstituents using HPLC and GC-MS analysis and evaluation of antioxidant activity of roots of *Jasminum auriculatum*.

Materials and methods

Collection & authentication of plant material

The roots of *Jasminum auriculatum* were collected from Thalkona forests of Chittoor dist. The collected plant material was authenticated by Dr. Madhavachetty (Botanist). A specimen (Voucher no. 0743) was deposited in herbarium at "Sri Venkateswara University, Tirupati, Andhra Pradesh, India". The roots were initially washed, shade dried, and grounded in Wiley mill.

Preparation of extract

After collecting of plant material, the roots are separated from it and washed under tap water for 3-4 times and shade was dried. The dried roots were powdered in Wiley mill. The extract was prepared by maceration with ethanol followed by hot extraction for 3 hours. The filtrate was separated and the same procedure was repeated twice. Three filtrates were amalgamated and distilled. The obtained extract was finally stored in desiccator.

Preliminary phytochemical studies

The prepared root extract was examined for active secondary metabolites like alkaloids, tannins, phenolic compounds, steroids, carbohydrates, flavonoids, and glycosides according to the standard methods [12].

HPLC analysis

HPLC qualitative analysis was performed for Ethanol extract of roots of *Jasminum auriculatum* (EEJA) using C_{18} Phenomenox 5u, 4.6×250 mm column. Methanol: Water is used as mobile phase. The sample was dissolved in ethanol and injected at volume of 20 µl. Flow rate at 1 mL/min is maintained and detection is carried at 254 nm.

GC-MS analysis

GC-MS analysis of EEJA was performed using Clarus 680 GC. "Gas chromatograph was equipped and coupled to a mass detector Turbo mass gold-Perkin Elmer with turbomass version 5.2.0 spectrometer with an Elite-5MS (5% Phenyl 95% dimethyl Polysilioxane), 30 m x 500 µm id capillary column". The instrument adjusted to the initial temperature 60 °C. Then, the oven's temperature was increased to 300 °C at the inclination rate is 10 °C/min, and sustained for 6 min. Helium flow rate was -1.0 mL/min and ionization voltage was –70 eV. The samples at 1:10 were injected at split mode. The

compounds' spectrums were compared with GC-MS NIST (2008) library. Further bioactivity of the compounds was predicted based on Dr.Dukes ethnomedicinal database.

In vitro antioxidant activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay

EEJA in concentrations of 1 to 5 mg/mL was taken and 195 µL of 0.004% (w/v) DPPH was affixed. The mixture then was incubated for 30 min at room temperature in a relatively dark place and absorbance was measured at 517 nm. Ascorbic acid (Vitamin C) was used as the standard. The percentage inhibition is calculated as per the standard methods [13].

ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity:

The working solution was processed by blending 25 mL of ABTS with 0.1 mL of potassium persulfate solution. The mixture was confirmed to revert for 12 h in dark at room temperature. The solution was diluted by combining it with ethanol to attain absorbance of 0.706±0.001 at 734 nm employing spectrophotometer. To 1 mL of the plant extract, 3 mL of ABTS solution was put inside and permitted to react for 6 min. Absorbance was scaled at 734 nm. The ABTS scavenging ability of EEJA was correlated accordingly with ascorbic acid's and percentage inhibition assessed [14].

Nitric oxide scavenging assay

EEJA at a concentration of 1 to 5 mg/mL was taken and 1 mL of sodium nitroprusside solution and phosphate buffer was added. The mixture was then incubated for about 2 hrs at 27 °C. This reaction mixture was treated with 1.2 mL Griess reagent. Absorbance was measured at 550 nm and compared with the standard (Ascorbic acid). Using standard formula percentage inhibition was calculated [15].

In silico molecular docking studies

Ligand preparation

The 2D structures of the draw up ligand were computerized in the SDF set out from online data base (Pubchem). These molecules were then processed in Schrodinger Ligprep wizard as per the standard methods and ultimately, ligand directory files were constructed.

Protein preparation

The protein structural codes for the NADH oxidase (NOX 4) (3A1F) and superoxide dismutase (SOD1) (5YTO) were attained out of the protein data bank. The construction of proteins was accomplished by adopting the standard methods [16].

Grid preparation & molecular docking

Using Maestro search grid was prepared for each protein and ligands were docked and the docked conformers were evaluated by availing Glide Score (G).

Results and discussion

Preliminary phytochemical studies

The preliminary phytochemical assessment divulged the presence of varied bioactive phytoconstituents namely flavonoids, tannins, glycosides, steroids, and terpenoids which were immensely beneficial for assessing the pharmacological activity of these roots.

HPLC analysis

HPLC was used for characterization of secondary metabolites in plant extracts [17]. EEJA was subjected to HPLC analysis in solvent system Chloroform: Methanol (7:3). A peak at R_t of 5.189 was identified and it found to be matched with retention time of rutin (Table 1 and Figure 1).

TABLE 1 HPLC studies of EEJA

Peak	Retention time	Area	Height	Area %
1	2.960	10175665	960493	4.019
2	3.403	19562852	3029029	7.727
3	3.537	52682513	3504430	20.810
4	4.005	26291718	1558323	10.385
5	4.299	39099346	1764693	15.444
6	4.805	30254858	1623437	11.951
7	4.935	20220171	1559229	7.987
8	5.189	8797070	497378	3.475
9	6.207	1188046	57501	0.469
10	7.432	705697	41405	0.279
11	8.312	33217082	1347198	13.121
12	9.740	1408727	40214	0.556
13	10.462	9044106	6973	3.572
14	13.457	164392	14463	0.065
15	16.240	349901	16422427	0.138
Total		253162145	16422427	100.000

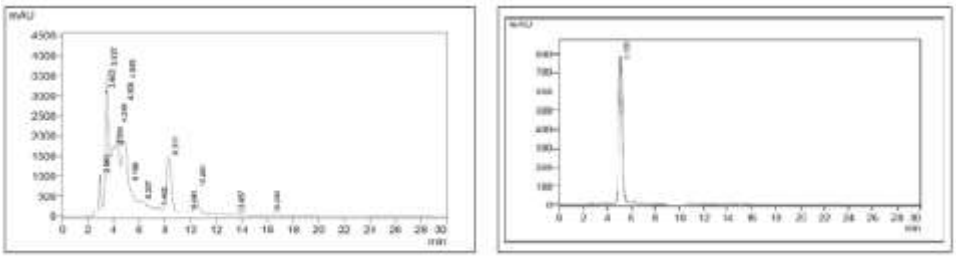

FIGURE 1 HPLC analysis of roots of *Jasminum auriculatum*

GC-MS analysis of ethanol root extract of Jasminum auriculatum

The identification of 15 phytoconstituents in EEJA by GC-MS analysis confirmed the presence of notable amounts of phytoconstituents which may contribute to various pharmacological activity of EEJA extract (Figure 2 and Table 2).

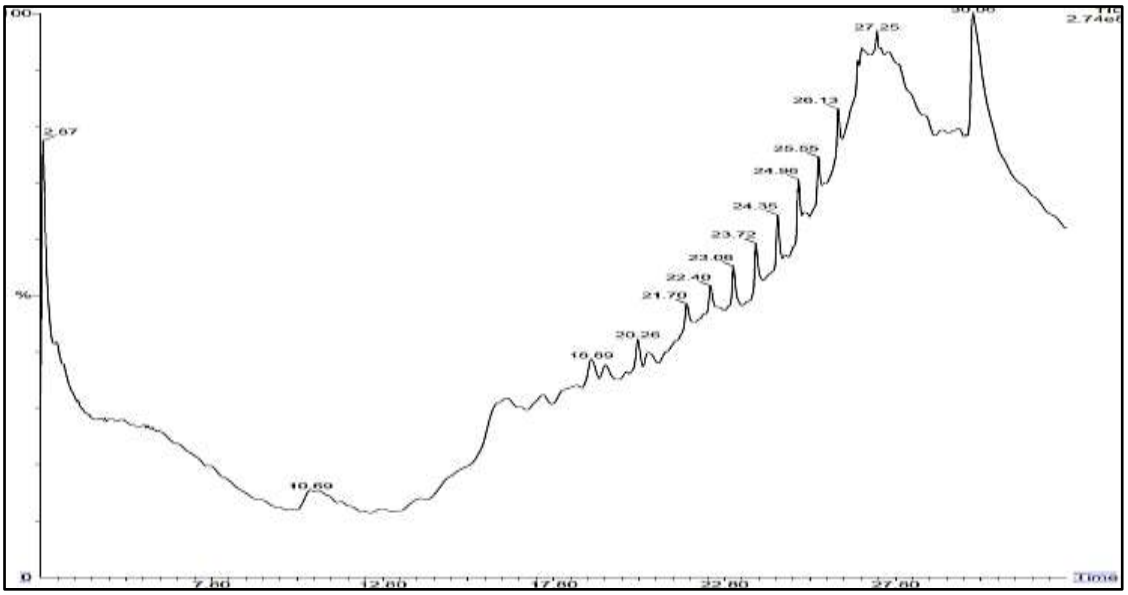

FIGURE 2 GC-MS chromatogram of EEJA

TABLE 2 GC-MS analysis of EEJA

S.NO	Retention time	Name of the compound	MF	MW.	%Peak area	*Biological activity
1.	20.245	Ether hexyl isopropyl	$C_9H_{20}O$	144	5.73	Nf
2.	23.031	Hexadecane 1-bromo	$C_{16}H_{33}Br$	304	7.28	Nf
3.	23.702	Endo-2,3-o-ethylidene-beta.-d-erythrofuranose.	$C_6H_{10}O_4$	146	7.37	Platelet adhesion, endo anaesthetic, beta blocker, anti-cancer, Nitric oxide inhibitor, odontolytic, CNS depressant diaphoretic, dopaminergic.
4.	24.332	n-tetradecyl trichlorosilane	$C_{14}H_{29}CI_3Si$	330	6.03	Anti-tumor, increase NK Cell Activity, nephroprotective, NO scavenger.
5.	24.942	2-[1,2-dihydroxyethyl]-9-[.beta.-d-ribofuranosyl]hypoxanthine	$C_{12}H_{16}N_4O_6$	212	7.73	Nf
6.	25.542	1,3-bis-t-butylperoxy-Phthalan	$C_{16}H_{24}O_5$	296	6.09	Anti-tumor, catechol -o-methyl transferase, Glutathione transferase inhibitor, Tachycardiac.
7.	26.113	2-[1,2-dihydroxyethyl]-9-[.beta.-d-ribofuraosyl]hypoxanthine	$C_{12}H_{16}N_4O_6$	212	15.10	Nf
8.	26.628	Heptasiloxane 1,1,3,3,5,5,7,7,9,9,11,11 13,13- tetra decamethyl	$C_{14}H_{44}O_6Si_7$	504	29.77	Nf
9.	26.803	Octa,15-Siloxane, 1,1,3,3,5,5,7,7,9,9,11,11 13,13,15,15-Hexadecamethyl	$C_{16}H_{50}O_7Si_8$	578	56.02	Abortifacient, lipoxygenase inhibitor, Alpha reductase inhibitor, 5-HT inhibitor.
10	27.258	Silicic acid diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296	100.02	Antitumor, nephroprotective, Diuretic, increase superoxide dismutase activity.
11.	27.913	Benzene,2-[(tertbutyldimethylsilyl)oxy]-1- isopropyl-4-methyl	$C_{24}H_{38}O_2Si_2$	414	28.50	Nf
12.	28.153	Hexestrol di-tms	$C_{24}H_{38}O_2Si_2$	414	16.98	Diuretic, antidote Antidote, Antitumor, Glucosyl and glutathione transferase inhibitor TNF alpha inhibitor, tranquilizer, topoisomerase -II inhibitor.
13.	28.359	1,1,1,3,5,5,5 heptamethyltrisiloxane	$C_7H_{22}O_2Si_3$	222	14.04	
14.	28.679	Trimethyl [4-(2-oxo pentyl) Phenoxy)silane	$C_{15}H_{24}O_2Si$	264	8.77	Nf
15.	29.159	Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si$	222	9.36	Nf

16.	30.054	cyclotrisiloxane, hexamethyl	$C_6H_{18}OSi_3$	222	52.57	Lipoxygenase inhibitor, alpha reductase inhibitor, analeptic, ngiotensin receptor blocker, ANS stimulant, anthiarythmic, anticancer, antiasthmatic, protease inhibitor, reverse transcriptor inhibitor.

Nf -Not found

***Biological activity Source:** Dr. Duke's phytochemical and ethnomedicinal database

In vitro antioxidant studies

In pathological states like hypoglycemia, cancer, autoimmune and cardiovascular maladies the fructification of free radicals is overwhelmed [18,19]. These awfully instinctual radicals can oxidatively amend an array of biomolecules, induce to cellular oxidative strain and demise. At these pathological conditions, the endogenous antioxidants will not be sufficient to scavenge the produced free radicals and thus the exogenous antioxidants are required [20,21]. Phytochemicals are naturally befalling antioxidants which could be contemplated as one of the optimistic materials used in indiscriminate pathological conditions with underlying free radicals production [22]. The antioxidant activity of root extract of *Jasminum auriculatum* was assessed by three different assays and the results are given in Figure 4 and Table 3. The IC_{50} of EEJA in DPPH was found to be 4.26 mg/mL, in NO scavenging activity 1.35 mg/mL and in ABTS 1.009 mg/mL.

TABLE 3 The effect of EEJA on *In vitro* antioxidants

Concentration (mg/mL)	% inhibition of DPPH (Mean±SEM)	% inhibition NO (Mean±SEM)	% inhibition ABTS (Mean±SEM)
1	39.2±0.10	50.26±0.09	48.93±0.31
2	41.05±0.50	50.53±0.18	55.28±0.10
3	46.16±0.08	51.36±0.15	61.36±0.12
4	50.63±0.21	52.13±0.46	67.91±0.30
5	51.4±0.16	52.43±0.15	68.95±0.32
Standard (Vitamin C)	43.9±0.14	55.2±0.10	55.96±0.15
IC_{50}	4.26	1.35	1.009

In silico screening

"In silico prediction is a valid alternate to the experimental studies and plays a vital role in selecting hit molecules from huge library in drug discovery process" [23,24]. Molecular docking was carried out to predict the preferred orientation and binding affinity of molecules to a receptor/a binding site/an enzyme. The compounds identified in GC MS and HPLC analysis were docked against two targets (proteins) namely; NADPH oxidase and Super oxide dismutase (SOD). These two selected proteins i.e. NADPH oxidase and SOD play a crucial role in cellular antioxidant complex by aiding in further reducing the oxidized antioxidant molecules and scavenging the free radicals (super oxide anion), respectively. The binding affinity of ligand with proteins was represented in the terms of Glide docking score and mentioned in Tables 4 and 5. The ligand interactions are illustrated in Ligand interaction tool of maestro and it was noticed that with both

targeted some compounds exhibiting potent score which were contrasted with the standard. Many compounds demonstrated both hydrogen bond and strong hydrophobic interactions at the active site of proteins. The more negative values of the glide docking score represented tighter binding to the targets. Among all the compounds "Rutin and Trimethyl [4-(2-methyl-4-oxo-2pentyl) phenoxy] silane" displayed the admirable G score with both the proteins (Figure 3).

TABLE 4 Molecular docking studies of identified compounds with NADPH OXIDASE

Molecules	Residues involved in polar and non-polar interactions	G Score	Glide emodel score
Rutin 5280805	Ser101, Leu80, Glu575, Arg77, Phe577, Arg102, Ser576, Lys574, Met75, Phe190, Tyr338, Asp99	-11.559	-75.543
n-tetradecyl tri chloro silane; 87626	Ile175, Met174, Cys113, Ser178, Leu171, Ile112, Phe200, Ile71, Tyr201, Phe197, His194, Leu72, His105, Gly109	-7.774	-42.922
Hexe sterol Di- TMS; 610041	Ser178, Leu171, Cys113, Ile112, Gly109, Met174, Tyr201, Phe200, Phe197, Tyr294, Met75, Leu72, Ile71, His105, Phe190, Ile106, Arg183	-7.205	-16.439
Hexadecane-1 Bromo; 8213	His194, Ser178, Ile175, Gly109, Leu72, Phe197, Tyr201, Ile71, Phe200, Met174, Leu171, Ile112, His105	-7.098	-36.024
Ascorbic acid; 54670067	Ser101, Tyr338, Gly414, Arg77, Arg102, Leu80, Asp99, Ser415	-6.564	-25.043
Trimethyl- [4-(2-methyl-4-oxo-2-pentyl) Phenoxy] silane; 610039	Cys68, Leu171, Met174, Ser178, Ile175, Val110, Cys113, Gly109, Ile71, Ile112, Tyr201	-5.640	-33.982
1,2-bis(trimethylsilyl)benzene; 519794	Cys18, Phe104, Leu97, Leu98, Ser101, Leu 80	-5.099	-29.868
1,3-bis-t-butylperoxy-phthalan; 552032	Ser575, Phe577, Arg102, Glu575, Arg77, Gly414, Leu80, Leu98, Ser101, Tyr338, Asp99, Lys574	-3.319	-47.209
Benzene,2- [(tert-butyldimethyl silyl) oxy]-1-isopropyl-4-methyl; 610042	Arg102, Glu575, Phe577, Tyr338, Arg77, Ser101, Leu80, Leu98, Asp99	-3.028	-34.115
Silicic acid, dimethyl bis(trimethyl Sillyl) ester; 77092	Tyr388, Ser101, Arg77, Met 75, Pro 74, Arg 102, Leu 98, Gly414	-2.769	-36.172
Cyclo trisiloxe, hexamethyl-10914	Asp99, Arg102, Arg77, Tyr 338, Ser 101, Glu 575	-1.402	-20.231
Octa siloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadeca methyl; 6329087	Tyr338, Lys 574, Ser101, Pro74, Ser 576, Gly 414	-0.892	-82.082
Heptasiloxane 1,1,3,3,5,5,7,7,9,9,11,1	Arg102, Phe 577, Ser576, Arg 183, Gly414, Leu80	-0.445	-68.555

1,13,13-Tetradecamethyl; 6329088			
2-[1,2-dihydroxyethyl-9-[beta.-d-ribofuranosyl] hypoxanthine; 135599152	Tyr294, His 194, Met174, Phe197, Met75, His105	-8.223	-62.980
Endo-2,3-ethyldiene beta-D-erythro Furanose; 22213396	His194, Ser178, Ile175, Met174, Leu171	-4.129	-26.243
Ether,hexyl Isopropyl; 538307	Tyr338, Phe413, Gly414, Arg77, Glu575, P	-1.769	-23.435

TABLE 5 Molecular docking studies of identified compounds with Super oxide dismutase

Molecules	Residues involved in polar and non-polar interactions	G Score	Glide emodel score
Trimethyl[4-(2-methyl-4-oxo-2 pentyl) phenoxy]silane; 610039	Val87, Leu84, Glu100, Ser102, Asp101, Ile99, Asn86	-3.794	-25.579
Heptasiloxane 1,1,3,3,5,5,7,7,9,9,11,11,13,13-Tetradecamethyl; 6329088	Arg79, Val103, Ser102, Asp101, Ile99, Leu84, Asp83, Pro74, Lys75, Asp76, Glu77	-3.020	-41.981
Rutin; 5280805	Arg79, Val87, Asp101, Phe45, Asn86, Val197, Ile99, Glu100, Pro74, Arg79	-4.829	-47.751
Benzene, 2-[(tert-butyldimethyls ilyl)oxy]-1-isopropyl-4-methyl; 610042	Leu84, Pro74, Asp101, Vall103, Arg79, Glu100, Asn86, Ile 99	-1.350	-0.986
2-[1,2-dihydroxyethyl-9-[.beta.-d-ribofuranosyl] hypoxanthine; 135599152	Glu100, Arg79, Asp101, Asn86, Gly85, Pro74, Leu84, Ile99	-4.872	-39.929

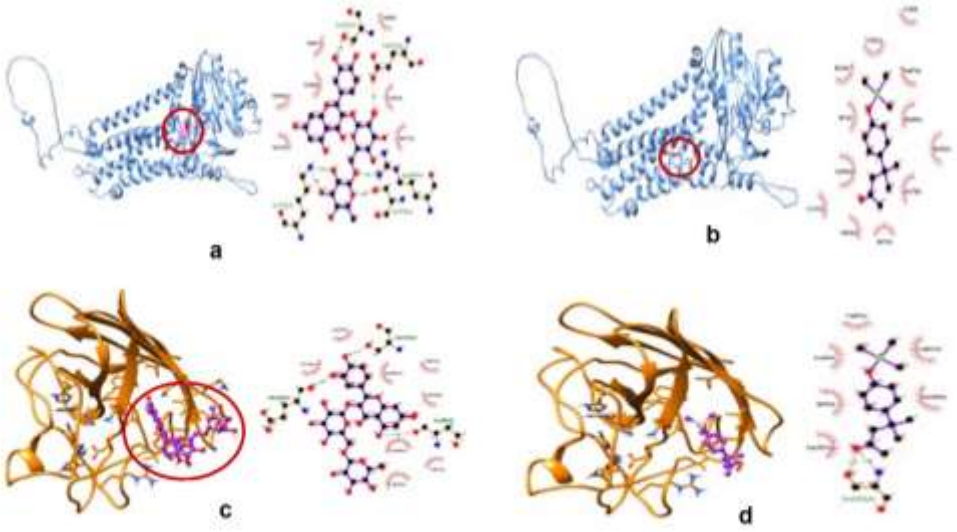

FIGURE 3 Molecular docking studies- a and b-Binding interactions of Rutin and Trimethyl[4-(2-methyl-4-oxo-2pentyl) phenoxy] silane with NADPH Oxidase; c and d- Binding interactions of Rutin and Trimethyl[4-(2-methyl-4-oxo-2pentyl) phenoxy] silane with Super oxide dismutase

Conclusion

The findings of the study clearly evidenced potent antioxidant activity in both *in vitro* and *in silico* screening which may be due to the presence of bioactive phytoconstituents and the desired antioxidant properties in roots of *Jasminum auriculatum.* Furthermore, it supports the ethnomedicinal usage of these roots in various diseases associated with the oxidative stress.

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