Research Article

Antioxidant Activity of a Popular Formulation Claimed By The Traditional Herbal Medicine Practitioners of Puducherry To Be Effective In Prophylaxis And Therapeutic Management Of Covid-19

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ABSTRACT: The aim of this research work is to evaluate the antioxidant activity of the hydroalcoholic extract of herbal formulation for COVID-19 by *in silico* and *in vitro* approach. The reported phytoconstituents of ingredients of herbal formulation were explored for molecular docking using Autodock Vina against the four targeted proteins such as lipoxygenase, NADPH oxidase, myeloperoxidase and xanthine oxidase. *In vitro* antioxidant activity was evaluated by DPPH radical scavenging and Hydroxyl Radical scavenging assay. The molecular docking study revealed that all phytoconstituents exhibited negative binding energy which indicates that they possess inhibitory activity. Among 157 phytoconstituents, Licorice-saponinA3 showed least binding energies such as-12.3Kcal/mol, -11.9Kcal/mol and -11.4Kcal/mol for the protein myeloperoxidase, NADPH oxidase, and xanthine oxidase respectively and 19-acetoxy-7, 9, 10 deacetyl-baccin VI showed least binding energy (-11.2Kcal/mol) for the protein lipoxygenase. The *in vitro* antioxidant activity revealed that the hydroalcoholic extract showed dose dependent scavenging activity and maximum activity at 1mg/ml concentration. Based on the results obtained it is concluded that herbal formulation possesses antioxidant activity.

Keywords: DPPH scavenging assay, hydroxyl scavenging assay, molecular docking and phytoconstituents.

INTRODUCTION:

COVID-19 (Corona Virus Disease -2019), a highly contagious respiratory disease which was first reported in Wuhan, China was caused by SARS CoV2 (Severe Acute Respiratory Syndrome Corona Virus-2) that lead to escalation of infection globally also death of million across the world[1,2]. SARS Cov-2 belonging to Beta coronavirus genera and subfamily ortho coronavirinae primarily causes respiratory and extra respiratory manifestation with varying severity[3].Depending upon one's age, health and comorbidities such as hypertension, diabetes etc, the severity of disease varies. Acute cardiac complication, ARDS and multiple organ dysfunction are some of the chronic condition in COVID-19 due to the response of immunological stress. This condition is due to activation of many inflammatory pathways and excess production of ROS. ACE2 receptor acts as the primary binding site for SARS CoV-2 spike protein which causes reduction of cleavage of AT II and hence its concentration increases in blood. This in turn activates MAP kinase , NF-kappa B and protein kinase C which results in NOX2 activation and upstream of cytokine and COX2. Activation of endothelial NOX2 causes excessive production of ROS resulting in oxidation of RNA, DNA and proteins which in turn signals the inflammatory response. Successively ensue in cell death and organ damage. Hence, in COVID-19 pathogenesis oxidative stress and ROS production play a major role[4]. This research work aims to explore novel herbal formulation for antioxidant activity through *insilico* and *invitro* approach.

Materials and Methods:

Insilico studies - Molecular docking:

Protein Preparation:

In the course of Arachidonic acid metabolism the enzymes such as myeloperoxidase, xanthine oxide, Lipoxygenase, NADPH oxidase and cytochrome P450 tend to rise the ROS generation which causes the oxidative stress and affects the redox homeostasis[5]. Hence these proteins serve as target for designing the drug for antioxidant property. The targeted proteins were downloaded

from protein data bank in PDB format and converted to PDBQT format using Autodock 4.2.6 software.

Ligand Preparation:

The reported 157 phytoconstituents of the formulation were downloaded from Pubchem in SDF format and converted to PDBQT format using open babel software.

Autodock Vina

Virtual screening was studied for the reorted phytoconstituents of herbal formulation using Autodock vina software where the protein ligand interaction for inhibition of protein is evaluated based on the their binding energy in comparison with standards such as zileuton, melatonin, dextromethorphan and febuxostat for the proteins lypoxygenase, myeloperoxidase, NADPH oxidase and Xanthine oxidase respectively. AutoDock vina generates 10 conformation for complex of ligand with target receptor. The active sites were predicted in protein ligand interaction profiler website.

In silico Studies

DPPH Radical Scavenging assay

The free radical scavenging potential of natural compounds is widely evaluated by DPPH radical scavenging activity. The antioxidant activity of herbal formulation was evaluated by DPPH radical scavenging assay according to Beatrice MG etal described method with slight modification[6].Briefly, 5 different concentration of hydroalcoholic extract of herbal formualtion (0.0625, 0.125, 0.25, 0.5,1 mg/ml) were prepared to 1ml with DMSO in triplicate. Same concentation of standard Ascorbic acid were prepared using DMSO. To this 3ml of 0.1mM DPPH solution was added and incubated for 20 minutes at room temperature in dark room. Blank was prepared with 1ml of DMSO and 3ml of 0.1mM DPPH solution. The absorbance of the reaction mixture is measured at 517nm using spectrophotometer. The percentage of scavenging activity was determined using the following formula:

$$\% \text{ of Radical Scavenging activity} = \frac{\text{Abs of control} - \text{Abs of Sample}}{\text{Abs of control}} \times 100$$

Hydroxyl Radical Scavenging activity

Hydroxyl radical scavenging activity of hydroalcoholic extract was evaluated according to Kalaisezhiyen et al with slight modification[7]. 5 different concentration of sample and standard (0.0625, 0.125, 0.25, 0.5,1 mg/ml) were prepared using DMSO. To this 1ml of 0.13% of ferrous ammonium sulphate (Iron EDTA solution), 0.5ml of 0.018% of EDTA and 0.5ml of 0.018% of EDTA and 0.5ml of 0.22% ascorbic acid were added. The test tubes were carefully heated in water bath at 80 to 90ºC for 15 minutes. To the above mixture ice cold 17.5% of TCA(1ml) was added to terminate the reaction and 3ml of nash reagent was added and incubated for 15 minutes at room temperature. The absorbance was measured at 412nm against the blank. triplicate of standard and test sample were prepared. The percentage of inhibition is determined by following formula:

$$\% \text{ of Radical Scavenging activity} = \frac{\text{Abs of control} - \text{Abs of Sample}}{\text{Abs of control}} \times 100$$

Statistical Method :

Mean±SEM was calculated followed by Student t test with two tailed distribution with unequal variance with p value less than 0.05

Results and Discussion

Molecular docking:

Molecular docking reveals that all phytoconstituents showed negative binding energy. The standand zileuton showed -7.5Kcal/mol against the targeted protein lypoxygenase and the binding energies of phytoconstituents were represented in figure1. The standard Melatonin showed binding energy -5.8Kcal/mol for the protein myeloperoxidae and the binding enegies for the phytoconstituents were represented in figure2. The standard dextromethorphan exhibited -7.4Kcal/mol for the protein NADPH oxidae and figure3 represent the binding energies of phytoconstitutents. The standard febuxostat exhibited -8.2Kcal/mol for the protein xanthine oxidase and the figure4 represent the binding enegies for the reported phytoconstituents. Among 157 phytoconstituents , the constituent 135 showed least binding energies for the protein targets myeloperoxidase(-12.3Kcal/mol), NADPH oxidase (-11.9Kcal/mol) and Xanthine oxidase (-11.4Kcal/mol) and the constituent 27 exhibited least binding energy for the protein lypoxygenase(-11.2Kcal/mol).

DPPH Radical Scavenging assay:

The principle behind the DPPH radical Scavenging assay is the acceptance of electron from antioxidant compound by DPPH radical which is indicated by colour change from violet to yellow followed by measurement of absorbance. The comounds which have the ability to carryout this reaction are considered as radical scavengers or antioxidants.

In vitro DPPH radical scavenging assay revealed the increased dose dependent scavenging activity for hydroalcoholic extract and maximum scavenging activity was observed for 1mg/ml. L-ascorbic acid (standard) exhibited higher scavenging activity than the extract. The results were represented in table 1 and figure 5.

Hydroxyl Radical Scavenging activity

Hydroxyl radical acts as potent oxygen reactive species in biological system. In hydroxyl radical scavenging assay ascorbic acid and iron EDTA generates hydroxyl radical by reacting with DMSO (oxidation reaction) which result in formation of formaldehyde, that helps in detection of hydroxyl radical when treated with nash reagent.

In vitro Hydroxyl radical scavenging assay revealed the increased dose dependent scavenging activity for hydroalcoholic extract and maximum scavenging activity was observed for 1mg/ml. Gallic acid (standard) exhibited higher scavenging activity than the extract. The results were represented in table 2 and figure 6.

Acknowledgement

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Tables

Table 1 represent the scavenging activity of standard ascorbic acid and hydroalcoholic extract of herbal formulation by DPPH scavenging activity assay.

Table 1: Invitro DPPH scavenging activity of hydrochloric extract of herbal formulation.

Table1: Invitro DPPH scavenging activity of Hydroalcoholic extract of herbal formulation

Concentration in mg/ml	Percentage of Inhibition (in %)	
	L-ascorbic acid	Hydroalcoholic extract
0.0625	28.50±0.17	10.90±0.30
0.125	36.29±0.36	17.20±0.13
0.25	50.47±0.33	31.51±0.18
0.5	70.78±0.18	56.09±0.18
1	84.84±0.15	77.23±0.16

The values are expressed as mean±SEM

Table 2 represent the hydroxyl scavenging activity of gallic acid hydroalcoholic extract of herbal formulation

Table 2: Invitro hydroxyl scavenging activity of Hydroalcoholic extract of herbal formulation

Concentration in mg/ml	Percentage of Inhibition (in %)	
	Gallic acid	Hydroalcoholic extract
0.0625	24.36±0.13	18.99±0.20
0.125	39.62±0.29	33.73±0.10
0.25	57.63±0.13	47.38±0.17
0.5	68.98±0.12	59.56±0.13
1	82.93±0.07	73.09±0.20

The values are expressed as mean±SEM

Figures

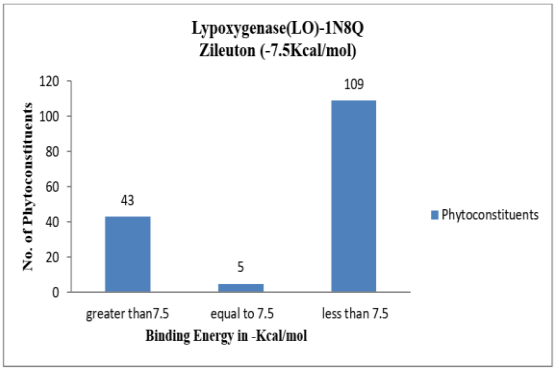

Figure1: Binding energies of phytoconstitutents against the protein lypoxygenase. The standard Zileuton showed binding energy – 7.5Kcal/mol. 109 phytoconstituents showed less binding energy than the standard which indicates that the possess better inhibitory activity than the standard.

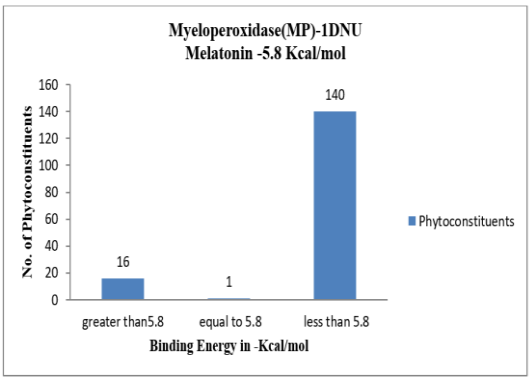

Figure2: Binding energies of phytoconstitutents against the protein myeleperoxidase. The standard Melatonin showed binding energy – 5.8 Kcal/mol. 140 phytoconstituents showed less binding energy than the standard which indicates that the possess better inhibitory activity than the standard.

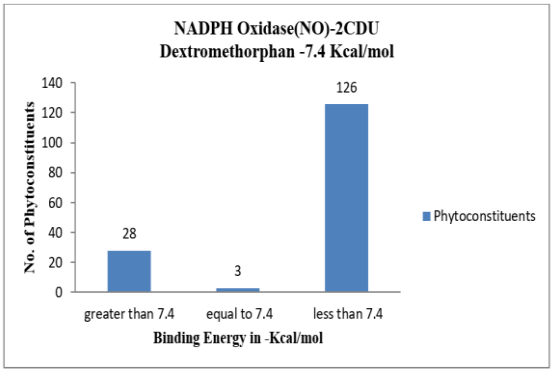

Figure3: Binding energies of phytoconstitutents against the protein NADPH oxidase. The standard dextromethorphan showed binding energy – 7.4 Kcal/mol. 126 phytoconstituents showed less binding energy than the standard which indicates that the possess better inhibitory activity than the standard.

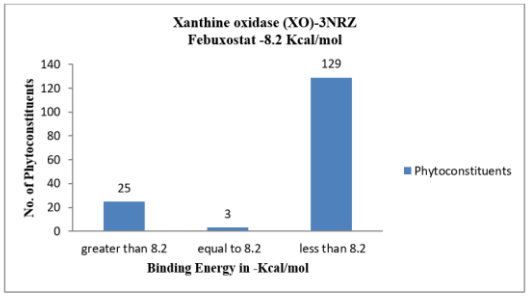

Figure4: Binding energies of phytoconstitutents against the protein Xanthine oxidase. The standard febuxostat showed binding energy -8.2 Kcal/mol. 129 phytoconstituents showed less binding energy than the standard which indicates that the possess better inhibitory activity than the standard.

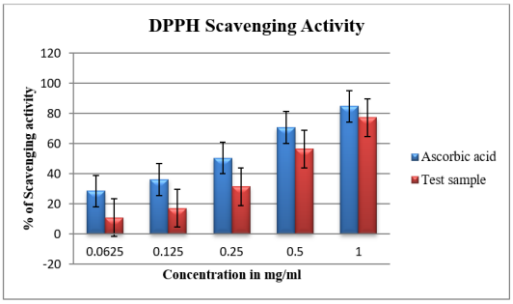

Figure5: In vitro DPPH scavenging activity of hydroalcoholic extract which is represented in mean±SEM and the values are not significantly different (p>0.05)

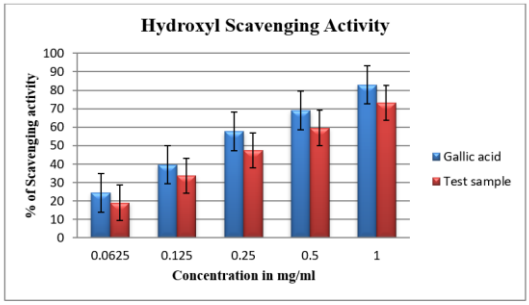

Figure6: In vitro Hydroxyl scavenging activity of hydroalcoholic extract which is represented in mean±SEM and the values are not significantly different (p>0.05)

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