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'BhAVI-23'-A spice-herb based dietary infusion possessing *in-vitro* anti-viral potential



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ABSTRACT

Background: Viruses cause many life threatening human diseases. Recently, COVID-19 pandemic has challenged the health care systems worldwide. As a disease preventive approach and to bring relief to the severity of the symptoms, a infusion termed as Bhabha Anti-Viral Infusion-23 ('BhAVI-23') was conceptualized and formulated which comprised of 23 selected spices and herbals.

Objective: The present study was conducted to assess the *in vitro* antiviral potential of the formulation, BhAVI-23.

Material and methods: The *in-vitro* anti-viral potential of BhAVI-23 was assessed through inhibition of HIV1 reverse transcriptase (RT) as well as through a novel P1 (virulent) bacteriophage based screening assay system. Anti-diabetic potential was assessed by non-enzymatic glycosylation of haemoglobin and the bioactive volatile components were detected through headspace gas chromatography followed by molecular docking analysis.

Results: The infusion displayed prominent anti-viral activity as evident from significant (57%) inhibition of the HIV1-RT as well as through reduction in the infectivity of P1 (virulent) bacteriophage. The infusion also exerted profound protection (~64%) to non-enzymatic glycosylation of haemoglobin. Headspace gas chromatography and mass spectrometric analysis confirmed the presence of at least 47 major compounds. Docking analysis indicated possible interaction of α -pinene and eugenol with SARS-CoV spike protein.

Conclusion: This 'BhAVI-23' infusion displayed prominent *in-vitro* anti-viral and anti-diabetic potential in different model systems. These attributes have relevance as diabetic patients are more prone to COVID-19 morbidity. 'BhAVI-23' opens the avenue for its potential inclusion as a supportive health care system upon due regulatory approval during the current pandemic.

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1. Introduction

Viruses are known to be responsible for various human pathogenesis and recently, the coronavirus breakout was notified as a Public Health Emergency of International Concern and subsequently a pandemic by the WHO. COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has challenged health care systems worldwide [1,2]. SARS-CoV-2 is an enveloped coronavirus that possess single-stranded plus sense RNA

genome and its cellular attachment is mediated through interaction of specific viral surface protein with cell surface ACE2 receptor [3,4]. Typical symptoms shown by the COVID-19 patients include coughing, chest congestion, fever, lung damage as well as some other associated systemic disorders [5].

Therefore a wide range of health preventive strategies are being deployed and one of the main treatment modalities in the absence of specific anti-COVID therapeutics is supportive care only. Under such prevailing circumstances, ancient system of Indian complementary herbal medicine 'Ayurveda' has the potential to provide relief to the patients through various mechanisms as well as boosting the immune system [6,7]. Based upon the scientific & medicinal information documented in 'Charak Samhita' and

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'Sushruta Samhita' many herbals could exert health benefiting effects including anti-viral, anti-pyretic, anti-inflammatory, anti-haemorrhoid and anti-emetic properties [8]. Realizing the health benefiting properties associated with herbal medicine, WHO has indicated that traditional medicine could have important contributions towards achieving health security to the global population [9]. The Ministry of AYUSH, Government of India, has also released an advisory to deploy the Ayurvedic, Homeopathic and Unani ways of managing the COVID-19 pandemic and listed out a number of herbs and other combinations that could be adopted for countering the COVID-19 infection and subsequently suppression of associated life threatening symptoms [10]. Many individuals are increasingly turning to ayurvedic medicines and herbal products to augment their immunity [11]. Also, as the COVID-19 severity has been reported to be higher in people with diabetes, therefore inclusion of anti-diabetic-herbals in the treatment regime has been duly considered.

In these contexts, as an add-on supportive system and from disease preventive and health benefiting point of view, a spice-herb infusion named as 'BhAVI-23' (Bhabha Anti-Viral Infusion-23) comprising of 23 ingredients has been conceptualized & formulated. The basis of inclusion of these selected herbals & spices is reported scientific evidences [12–36]. This formulation is proposed to have broad-spectrum *in-vitro* antiviral attributes and anti-diabetic activity, therefore a prospective counter-measure for the betterment and symptomatic relief of patients suffering from COVID-19.

2. Materials and methods

2.1. Preparation of 'BhAVI-23' infusion

Different spices and herbals (23 in nos.) were used to prepare 'BhAVI-23' mix. Many of these were picked from the centre's botanical garden and whole spices were procured from authentic spice whole-seller in Mumbai, Maharashtra, India registered with Food Safety & Standards Authority of India (FSSAI). Thoroughly washed and solar dried ingredients were powdered and strained. All the 23 ingredients were added in a proportion (total wt. 57.85 g) as detailed in Table S1. For preparation of infusion, approximately 1 small tablespoon (~1 g) of the powder was suspended in ~200 ml of water. This suspension was further boiled for 5 min and strained to obtain ready-to-be consumed hot beverage.

2.2. Assessment of the anti-viral potential

2.2.1. Anti-retroviral assay

2.2.1.1. HIV reverse transcriptase inhibition assay.

The anti-retroviral potential of 'BhAVI-23' extract was assessed through the colorimetric reverse transcriptase (RT) inhibition assay kit (Roche, Switzerland). This colorimetric assay utilizes the property of reverse transcriptase enzyme to synthesize fresh DNA from the template/primer hybrid incorporating digoxigenin and biotin labeled nucleotides. As a measure of the RT activity, the detection as well as quantification of the newly synthesized labeled DNA is achieved through enzyme-linked immunosorbent assay (ELISA). The surface of the microplate is precoated with streptavidin which serves as a high affinity binding platform for the biotin-labeled DNA. Subsequently, the bound DNA is subjected to antibody treatment in which the specific antibody to digoxigenin conjugated with peroxidase (anti-DIG-POD) gets bound to the digoxigenin labeled DNA that is already affixed to streptavidin. For the detection, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) is added that serves as a chromogenic substrate for the POD which oxidizes it leading to the formation of a colored reaction product

(stable green radical) whose absorbance (at 405 nm) is quantified in an ELISA reader.

Reaction mix consisted of 20 µL diluted HIV-1-RT (6 ng) to which 20 µL of 'BhAVI-23' extract was added with varying concentrations. The mixture was incubated for 5 min followed by addition of 20 µL of reaction mixture containing dNTPs, template and primers in a reaction buffer. The samples were incubated (1 h at 37 °C) at room temperature. HIV-1-RT (6 ng, 20 µL) without any extract acted as a positive control. Post incubation, whole reaction mix (60 µL) was transferred into streptavidin coated well of micro-plate, and mixture was further incubated (1 h, 37 °C) in dark. After the removal of solution, wells were gently washed using washing buffer. 200 µL working solution of anti-DIG-POD (digoxigenin-peroxidase) was added per well followed by plate incubation (37 °C, 1 h). After the incubation, the wells were washed with washing buffer. Finally, to each well 200 µL of ABTS substrate solution was added and the plate was incubated at room temperature for 45 min. Quantification of the sample absorbance was done employing a microplate reader at 405 nm.

2.2.2. P1 bacteriophage based qualitative screening assay

Anti-viral potential of the infusion was also evaluated using a novel P1 (virulent) bacteriophage system. The P1 (virulent) bacteriophage is known to form plaques in host bacteria through host cell lysis. This property was employed to assess infectivity lowering potential of 'BhAVI-23' infusion against P1 virulent phage. Four different experimental conditions were set: A) *Escherichia coli*; B) *E. coli* + P1 (vir) lysate; C) *E. coli* + P1 (vir) lysate + 'BhAVI-23' extract (1.75%); D) *E. coli* + 'BhAVI-23' extract. These cultures were incubated (1 h, 37 °C) followed by pour plating using soft agar on LB agar plates. These plates were then incubated at 37 °C for 24 h for qualitative evaluation of the antiviral property of the 'BhAVI-23' extract.

2.3. Assay for anti-glycation property of infusion

Haemoglobin (Hb) undergoes non-enzymatic chemical linkage with sugars including glucose leading to the formation of glyated Hb and under hyperglycemic conditions the level of glyated Hb significantly rises. Therefore, the anti-diabetic potential of the 'BhAVI-23' infusion was assessed through non-enzymatic glycosylation assay of haemoglobin (Hb) [37]. The solutions of ampicillin (0.02%), glucose (2%), and haemoglobin (0.06%) were prepared in phosphate buffer (0.01 M, pH 7.4). Later, 1 ml each of these solutions were mixed followed by addition of 1 mL of 'BhAVI-23' extract at varying concentrations. The reaction mixture was incubated (72 h in dark) at room temperature followed by spectrophotometric measurement of haemoglobin glycosylation at 520 nm. Percentage inhibition of glycosylation was calculated based upon the absorbance of the samples along with control (without any extract).

2.4. Headspace gas chromatography-mass spectrometric analysis (HS-GCMS)

Headspace gas analysis was performed as per the method reported earlier [38]. A 15 ml of herbal extract (0.55%) was prepared (section 2.1) and taken in 40 ml SPME vial. In this extract 4.5 g of NaCl was added. 2-octanol (82 µg/L) was added as an internal standard. Samples were kept on a magnetic stirrer and equilibrated (10 min, 40 °C). Extraction was performed at 40 °C for 10 min employing a pre-conditioned (250 °C, 5min) 50/30 µm polydimethylsiloxane carboxen/divinyl benzene solid phase micro extraction fiber. Extracted volatiles were injected in GC/MS by desorbing the fibre on the injection port kept at 250 °C for 2 min. The analytical procedure was performed on GC/MS having GC

capillary column Rxi-5 ms (l: 20 m, inner diameter: 0.18 mm, film thickness: 0.18 μm). Helium gas served as a carrier gas. The injector port was equipped with inlet liner (0.75 mm ID, Supelco) compatible for solid phase micro-extraction analysis. With a split ratio 5, the injections were done. Initial temperature setting of GC was 40 °C (hold time 3.2 min) which was further raised to 200 °C (rate of increase: 6.2 °C/min) and subsequently to 280 °C (15 °C/min) which was further maintained for 7 min. The mass–spectrometry parameters were: ionization voltage (70 eV), electron multiplier voltage (1 kV), and m/z 35–500. The identification of the detected peaks was performed by comparing the Kovat retention indices based on a homologous series of n-alkanes (C5–C24) with that of standards and also from the MS data accessible in the mass spectral library (NIST/EPA/NIH, 2014 version). Internal standard was used for evaluating the peak areas of the targeted volatile compounds.

2.5. Representative molecular docking study using α -pinene, eugenol and SARS-CoV spike protein

A representative molecular docking study was performed using Auto-dock Vina wizard. Ligands (α -pinene and eugenol) were downloaded from ChemDB Chemoinformatics Portal. The SARS CoV-2 spike protein structure file (PDB ID 6xr8) was accessed and downloaded from the website of protein data bank. The protein molecule was processed using AutoDock tool. Water molecules and other associated ions were selectively eliminated whereas polar hydrogens were added to the spike protein molecule. Processed file was saved in *.pdbqt format. Region specific docking of α -pinene as well as eugenol was performed with SARS-CoV-2 spike protein. AutoDock Vina parameters for spike protein (PDB ID 6xr8) were: center_x = 198.0, center_y = 198.0, center_z = 191.9, size_x = 28.0, size_y = 27.8, size_z = 28.0. The interaction was viewed in PMV viewer ver.1.5.6.

3. Results

3.1. Composition of herbal mixture 'BhAVI-23'

'BhAVI-23' infusion was prepared using 23 different spice & herbs based upon the reported scientific evidences as detailed in Table S1 [39,40]. Their relative proportion was properly selected based upon their pharmacological properties and recommended dosage for an adult individual as shown in Tables S1 and S2 [12–36,39,40]. Ingredients were grouped in 4 different categories: major (4–6 g), medium (2–3 g), minor (1–1.5 g) and trace ingredients (0.1–0.5 g). Besides, the major pharmacological benefits of 'BhAVI-23' ingredients (based upon earlier scientific findings) are summarized in Table S2.

3.2. 'BhAVI-23' infusion possesses broad spectrum anti-viral property

3.2.1. Anti-retroviral potential

This assay was performed with major (A), medium (B), minor (C), trace (D) and total (T1: 1.4% and T2: 2.8%) components of 'BhAVI-23' mix (Table 1). The extracts displayed prominent RT inhibition and the extent of RT inhibition was found to be 30, 37, 36, 30, 47, 56%, respectively (Fig. 1 A, B). These observations also indicated about the possibility of synergism being shown at the level of HIV1-RT inhibition by the components of the 'BhAVI-23'.

The retroviruses such as HIV, HTLV-1, HTLV-II are also implicated in several life threatening human diseases and the retroviral family member has a characteristic reverse transcriptase [41].

Involvement of this reverse transcriptase is a prerequisite in the initial stages of propagation including proviral DNA synthesis and thus many anti-retroviral therapeutic regimes target the reverse transcriptase enzyme.[42, 43] Therefore assaying the extract for the anti-reverse transcriptase activity provided evidence ascertaining its anti-retroviral property.

3.2.2. P1 (virulent) phage infectivity lowering potential

P1 virulent phage is a temperate bacteriophage that is known to infect *E. coli* and some other bacteria [44]. A novel P1 virulent bacteriophage based qualitative screening assay was employed to further assess the antiviral potential of the 'BhAVI-23' infusion and the observations are shown in Fig. 2. Almost complete absence of growth of bacteria *E. coli* was observed when phage and bacteria were co-incubated together indicative of complete lysis of host bacteria (Fig. 2B). Interestingly, 'BhAVI-23' extract inhibited the infectivity of P1 (vir) bacteriophage as manifested by reduced plaque forming ability at the non-cytotoxic concentration of 1.75% of the infusion resulting in almost comparable bacterial growth (Fig. 2C). The negative control (*E. coli* co-incubated with BhAVI-23 extract) is displayed in Fig. 2D. These observations further corroborated the anti-retroviral activity as mentioned above.

3.3. Anti-glycation potential of 'BhAVI-23' extract and its relevance

Anti-glycation property of the infusion is shown in Fig. 3. The extract displayed significant anti-glycation property against glucose mediated glycation of haemoglobin thus conferring anti-diabetic property to 'BhAVI-23'. Anti-glycation was found to be 22% and 64% at extract concentration of 250 and 1250 $\mu\text{g/ml}$, respectively.

3.4. Head space gas analysis confirmed the presence of antiviral components in 'BhAVI-23'

Around 47 different compounds were detected and identified in the 'BhAVI-23' infusion upon head space gas analysis (Table 1). The concentration of eugenol was observed to be prominently higher as compared to other compounds. Many of these compounds have been reported for their potent anti-viral activities against wide variety of viruses [45–55].

3.5. Molecular docking analysis

Compounds showing antiviral activity were further selected for molecular docking analysis. Fig. 4 (A, B) shows binding pose of α -pinene and eugenol with the spike protein of SARS-CoV-2 and the predicted binding energy was found to be -6.4 & -6.3 kcal/mol, respectively. Different proteins of SARS CoV-2 have been discovered that could act as potentially important targets for the management of COVID-19. Some of the key protein targets of SARS-CoV-2 are main protease (Mpro), ADP-ribose-1-phosphatase (ADRP), endoribonuclease (Nsp 15/NendoU), the binding domain of the spike protein (SARS-CoV-2rS), RNA-dependent RNA polymerase (RdRp), and hACE2 [56]. Docking study of both the ligands was also carried out with main protease (Mpro) (PDB ID 6wqf), Endoribonuclease (PDB ID 6x41) and RNA dependent RNA Polymerase (PDB ID 6m71). However, the docking score values for α pinene with Mpro, Endoribonuclease and RdRP were -1.3 , -4.2 and -4.1 kcal/mol, respectively while that of eugenol were -3.7 , -3.8 and -3.2 kcal/mol, respectively. In both the ligands, spike protein showed the lowest binding energy (indicating highest affinity) with the spike protein.

Table 1
Head space GC–MS analysis of the 'BhAVI-23' infusion and the identified compounds and their concentrations.

Compounds detected	Rt	RI cal	RI actual	Amount (µg/L)
1-Penten-3-ol	1.773	735	684	9.2 ± 2.32
3-Methyl butanal	1.905	740	652	4.93 ± 1
3-Methyl-1-butanol	2.411	758	736	0.65 ± 0.03
Dimethyl disulfide	2.493	761	746	1.4 ± 0.38
1-Pentanol	2.971	778	768	3.83 ± 1.02
2Z-Penten-1-ol	3.037	780	770	4.15 ± 0
Hexanal	3.609	800	800	9.79 ± 1.65
2-Hexenal	4.967	849	851	29.41 ± 7.51
3Z-Hexen-1-ol	5.105	854	856	22.15 ± 6.16
2 E-Hexen-1-ol	5.417	865	862	32.66 ± 11.6
1-Hexanol	5.492	868	868	34.28 ± 5.9
α-Pinene	7.199	929	937	2.4 ± 0.87
Benzaldehyde	7.967	956	962	2.23 ± 0.19
β-Pinene	8.418	972	979	4.12 ± 1.65
1-Octen-3-one	8.594	978	979	0.99 ± 0.04
1-Octen-3-ol	8.647	980	980	1.86 ± 0.24
6-Methyl-5-hepten-2-one	8.856	988	986	3.35 ± 0.31
β-Myrcene	8.933	991	991	3.58 ± 1.42
Eucalyptol	9.996	1029	1032	112.24 ± 3.43
Benzyl alcohol	10.138	1034	1036	1.03 ± 0.15
Benzeneacetaldehyde	10.34	1042	1045	7.45 ± 0.29
γ-Terpinene	10.774	1058	1060	5.64 ± 2.3
Fenchone	11.548	1086	1096	22.37 ± 0.84
cis-β-Terpineol	11.849	1097	1144	2.3 ± 0.08
Linalool	11.926	1100	1099	63.59 ± 3.78
Benzenepropanal	13.498	1161	1162	32.02 ± 1.58
endo-Borneol	13.593	1165	1166	36.37 ± 1.95
Terpinen-4-ol	13.888	1177	1182	22.87 ± 0.63
α-Terpineol	14.24	1190	1185	70.97 ± 3.17
3-p-Menthen-7-al	14.309	1193	1196	7.45 ± 0.46
3-(1-Methylethyl)-phenol	15.214	1231	1228	2.59 ± 0.26
3-Phenylpropanol	15.262	1233	1232	1.94 ± 0.23
4-(1-Methylethyl)-benzaldehyde	15.515	1240	1239	384.09 ± 48.82
Thymoquinone	15.726	1252	1250	86.73 ± 10.66
E-Cinnamaldehyde	16.211	1272	1274	182.53 ± 12.9
2-Caren-10-al	16.522	1285	1289	123.63 ± 9.45
1,4-p-Menthadien-7-al	16.66	1291	1288	38.6 ± 3.74
2-Methyl-5-(1-methylethyl)-phenol	16.917	1302	1299	4.07 ± 0.27
3-phenyl-2E-propen-1-ol	17.041	1308	1313	1.43 ± 0.09
Eugenol	18.365	1367	1392	507.02 ± 80.26
Methyleugenol	19.329	1410	1402	222.61 ± 29.51
Coumarin	19.953	1440	1441	18.18 ± 1.56
Acetic acid cinnamyl ester	20.084	1446	1445	4.41 ± 0.36
Eugenol acetate	21.801	1529	1524	19.78 ± 2.24
Ar-turmerone	24.514	1668	1660	6.69 ± 0.63
Hexadecanal	27.203	1817	1817	1.46 ± 0.01
Dimethyl palmitamine	28.731	1905	1894	83.9 ± 0

4. Discussion

Large scale drug and phytomedicine repurposing is being currently investigated as it is plausible that anti-retroviral therapy may also be effective against SARS coronavirus [57–60]. 'BhAVI-23' displayed prominent inhibition of HIV-1 RT activity as well as infectivity of P1 (vir) phage thus ascertaining its anti-viral potential. The reverse transcriptase assay based upon HIV-1 RT has been reported earlier as potentially beneficial for the assessment of RT activity from different retroviruses as well as screening tool for the prospective RT inhibitors [61].

Besides, assessment of anti-glycation potential has significance due to growing evidence strongly suggesting that the evaluation of hemoglobin glycation is relatively more advantageous in forecasting the risk of developing diabetes [62]. 'BhAVI-23' has been conceptualized to also confer additional potential benefits to people suffering from diabetes because such people belong to high risk categories and can have serious illness if they get infected to the coronavirus. Herbals and spices are known for their prophylactic anti-diabetic and immunomodulatory attributes [63,64]. Acute hyperglycemia may be alarming because significantly glycosylated

ACE2 receptor is conducive for cellular intrusion of the coronavirus subsequently leading to a pronounced COVID-19 infection with increased disease seriousness [65]. Hyperglycemia may escalate viral proliferation, inhibit & weaken the anti-viral immune response and may also affect pulmonary function [66]. In a recently conducted clinical study at the United States, COVID-19 patients suffering from diabetes had a longer stay in hospital and prominently higher mortality (28%) as compared to non-diabetic patients (6.2%). Therefore, it was further recommended and advised that inpatient hyperglycemia is effectively and safely treated by the medical management [67].

Different compounds detected during head-space analysis have been reported for their anti-viral activities. Tumerones detected have been reported to have activity against H5N1 influenza virus, whereas, coumarins have been shown to possess activity against RNA viruses like HCV, HIV and influenza virus. Eugenol has been found to display direct inhibitory activity against both intracellular and extracellular viruses including Herpes Simplex virus [45]. Carvacrol has been reported to be active against HSV-1 by direct inhibition of virus particle. This compound possessed anti-murine norovirus activity too [46,47]. The α and β-pinene detected are

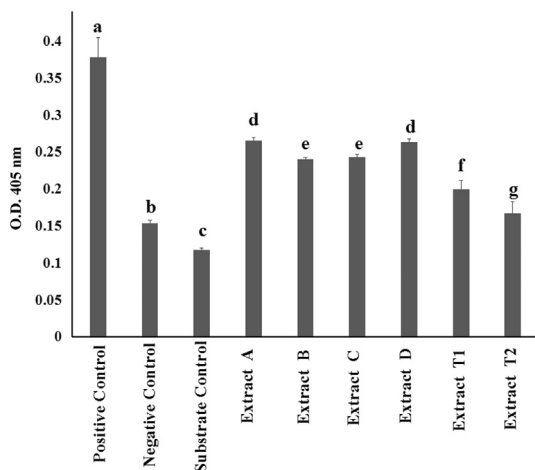


Fig. 1. Anti-viral activity based upon HIV-1 Reverse Transcriptase inhibition. * Reaction mixture having RT without extract, # Reaction mixture without RT, ^{a-i}Different letters across the columns indicate the mean values are significantly different, [level of significance ($p \leq 0.05$) as analyzed by ANOVA].

naturally found in black cumin and clove, respectively. The α -pinene has been reported to display prominent antiviral activity against IBV, SARS-CoV as well as HSV-1 [48]. The β -myrcene detected possessed anti HSV-1 activity [48]. Besides, linalool which was detected in the infusion has been reported earlier for its activity against Influenza A virus [49]. Recently, antiviral activities of borneol and isoborneol derivatives against Influenza A virus has been reported [50]. Terpinen-4-ol found has been reported for its significant activity against Influenza A virus [51]. Thymoquinones and curcumin have been reported for their antiviral activity and could boost immune response [52,53]. Curcumin has also been reported for its potential health importance in severe pneumonia [53]. Cinnamaldehyde was one of the major compounds detected in 'BhAVI-23' infusion and shown to inhibit adenovirus type 3 & influenza A/PR/8 virus in earlier reports [54,55]. Selection of target proteins of virus for docking study is an important step during evaluation of compounds for antiviral activity. Different viral proteins are responsible for crucial functions in viral entry, replication and its assembly and spread to other host cells. Main protease (Mpro) is required to cleave the polyprotein into different nonstructural proteins required for viral activity. Spike protein is required for recognition and high affinity binding with the functional ACE-2 receptor of the host cell while endoribonuclease degrades polyuridine sequence of viral RNA to prevent detection of virus by host immune cells [68]. RNA dependent RNA polymerase is required to generate multiple copies of viral genome. Different researchers have carried out *in-silico* docking analysis of number of

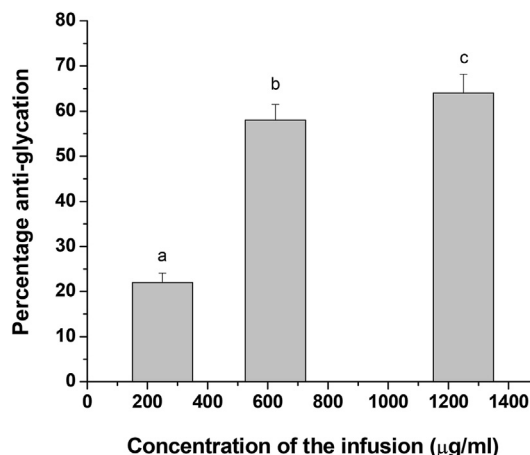


Fig. 3. Anti-glycation property of 'BhAVI-23' extract. ^{a-c}Different letters across the columns indicate the mean values are significantly different ($p \leq 0.05$) as analyzed by ANOVA.

essential oil compounds for the SARS CoV-2 proteins mentioned above [69,70,71]. These include the major compounds detected in BhAVI-23 extract. β -myrcene showed highest docking score (-98.7 kJ/mol) normalized for molecular weight with main protease. In case of endoribonuclease, endo-borneol showed highest docking score (-91.8 kJ/mol), while for rest of the 4 proteins, eugenol showed highest docking score [69]. Docking study exclusively for eucalyptol with main protease has been reported earlier [70]. They obtained full fitness as well as binding affinity values of -2291.07 and -6.04 kcal/mol, respectively. They concluded that eucalyptol showed significantly high binding affinity, however, further validation is required to confirm the interaction of eucalyptol with main protease [70]. Screening of different natural derivatives of coumarin for the selective inhibition of Mpro has also been performed earlier and the ΔG values ranged from -6.80 to -8.57 kcal/mol [72]. Besides, in another docking study thymoquinones displayed binding affinity values for COVID-19 Mpro and ACE-2 as -4.7 and -5.5 kcal/mol, respectively [72].

Representative docking result is depicted in Fig. 4 where α -pinene and eugenol were used as a ligand for spike protein. α -pinene was selected as this molecule has been shown to possess anti-SARS CoV activity while eugenol was selected since it was the most abundant molecule present in BhAVI extract. As seen from the figure, the ligand is binding into the pocket formed by 3D assembly of three spike protein polypeptides. However, the binding affinity score reflects slightly weak affinity of α -pinene with the spike protein assembly. Besides, eugenol also displayed significant binding with the spike protein. Likewise, other ligands present in

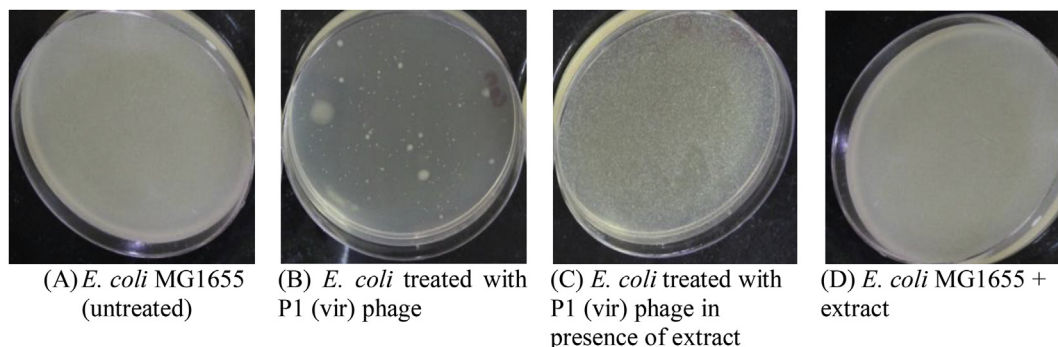


Fig. 2. Anti-viral activity based upon P1 (vir) bacteriophage assay.

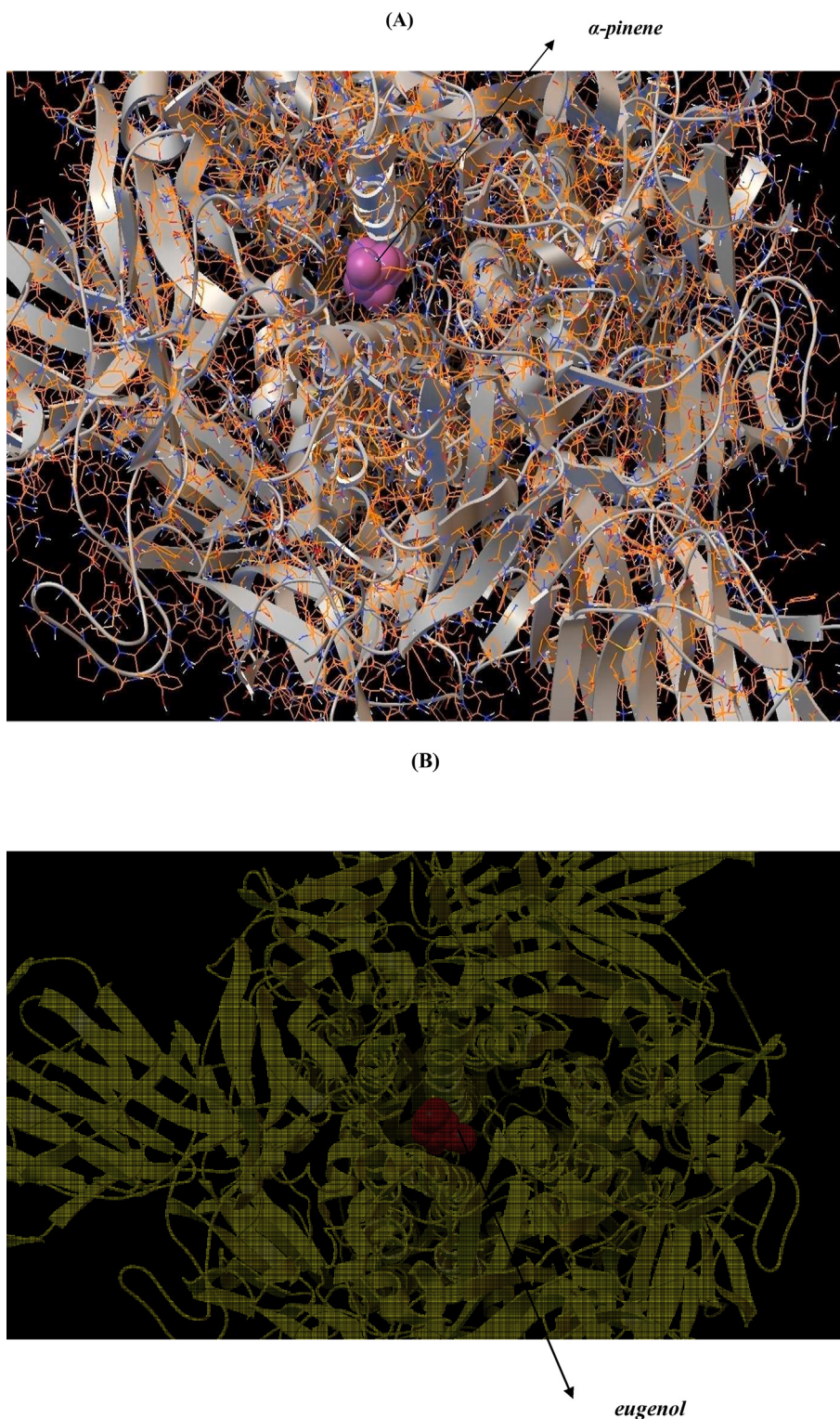


Fig. 4. Representative simulation of lowest energy docked pose of (A) α -pinene and (B) eugenol with SARS-CoV-2 spike protein.

the spice herbal infusion can have higher binding affinities with the spike protein assembly, exerting synergism in inactivating this protein.

In the absence of specific anti-COVID-19 therapeutics, the major treatment regime is directed on targeting the symptoms [73]. Immunomodulatory activity to several compounds detected in the

‘BhAVI-23’ infusion such as butanal, 3-methylhexanal, 2-hexenal, β -myrcene, gamma-terpinene, terpinen-4-ol and α -terpineol is also reported [74]. Immunity is known to play a vital role in COVID-19 pathogenesis in early non-severe as well as during the severe stage of the disease. Corona viruses are proficient in eluding immune detection and weakening the immune responses [75,76].

Thus, the treatments targeting immune system could potentially help COVID-19 affected patients.

In this context, traditional Indian herbs of prophylactic relevance could serve as effective strategy to counter viruses like SARS-CoV-2 and have potential to prevent disease deterioration into a critical state [8,77]. Recently *in silico* and experimental observations affirmed therapeutic potency of Qingfei Paidu Decoction in treating COVID-19 patients in China [78]. Many of the selected herbs and spices comprising 'BhAVI-23' have been reported for their prophylactic and therapeutic health benefits including immune boosting property (Table S2).

5. Conclusion

'BhAVI-23' comprising of 23 natural ingredients displayed *in-vitro* antiviral property against reverse transcriptase as well as infectivity of P1 (vir) phage. This infusion also displayed *in-vitro* anti-diabetic property as indicated by its anti-glycation activity. These attributes ascertain the potential broad-spectrum antiviral action of this 'BhAVI-23' mix besides having already reported immunomodulatory functions of the spice-herbal components comprising this infusion. GC-MS analysis confirmed the presence of many compounds of therapeutic potential that may have a role in conferring antiviral properties. The health benefiting effects including antiviral activity could potentially mitigate the severity of COVID-19 symptoms in the current pandemic. Further studies in higher systems including animal models and/or trial study in human volunteers could be proposed to be pursued upon due regulatory approvals to corroborate the initial *in-vitro* findings and affix an antiviral health claim to 'BhAVI-23'.

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None.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaim.2020.11.005>.

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