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Review Article

Potential of *Orthosiphon aristatus* Blume Miq as Antiviral: A Review

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ABSTRACT

Orthosiphon aristatus Blume Miq is one of the commonly used medicinal plants known to have many benefits, including antiviral activity. Components of the main secondary metabolites of *O. aristatus* are sinensetin, rosmarinic acid, and eupatorin. The development of plants or drugs that have the potential to act as antiviral agent during the Covid-19 pandemic continues. Based on previous research reports, the main secondary metabolite content in *O. aristatus* could have antiviral activity. The present review was done by searching and analyzing research journals on the potential for active content of *O. aristatus* in inhibiting the growth or replication of viruses. There has been no previous review regarding the potential for *O. aristatus* as an antiviral agent, therefore this review is expected to provide information about potential sources of antivirals originating from the *O. aristatus* plant.

Keywords: Medicinal plants, Antiviral, Covid-19, Anti-influenza virus, Antiherpetic, Anti HIV.

Introduction

O. aristatus has been widely used traditionally for treating several diseases and has been used as antiviral,[1] antihypertensive,[2] antioxidants,[3,4] prevention and treatment of cancer,[5-8] rheumatoid treatment and osteoarthritis arthritis,[9] antiobesity,[10] treating cardiovascular disorders,[11] anti-epilepsy,[12] antidiabetic,[13-15] enhancing memory,[16] antimicrobial activity,[17-19] hepatoprotective effect,[20,21] diuretics,[22-24] treatment of gastric disorders.[10,25]

Some studies also report that *O. aristatus* have passed clinical trials.[26,27] Safety testing of *O. aristatus* extracts during 60 days administration in male rats showed that all animals survived and showed no signs of toxicity. There were no significant food and water intake changes.[28]

During the Covid-19 pandemic, researchers searched for sources of plants and drugs with antiviral properties, one of which was the *O. aristatus* plant. Rosmarinic acid can be developed as a therapy for enterovirus 71 (EV71) infection.[29,30] Sinensetin has the potential as a drug for influenza H1N1 virus infection.[31] Based on an in-silico study, rosmarinic acid is a potent inhibitor of COVID-19;[32,33] rosmarinic acid reduces the mortality of mice infected with the Japanese encephalitis virus (JEV),[34] Water extract of the leaves, flowers, and all the plants in addition to the root of the *O. aristatus* (0.39 mg/mL) had antiviral activity with a 100% reduction in herpes simplex virus type 1 (HSV-1) plaque,[1] *O. aristatus* can be developed as an antiherpetic.[35] Rosmarinic acid can inhibit hepatitis B virus replication.[36]

The main SecondaryMetabolites in *Orthosiphon aristatus* (Blume) Miq are sinensetin, eupatorin, and rosmarinic acid (Figure 2).[37] Sinensetin and eupatorin are included in the class of flavonoid compounds and they are classified more specifically as polymethoxy compounds produced by secretory tissue and stored inside or outside the oil glands in plants. Flavone polymethoxy compounds have several pharmacological activities *in vitro* and *in vivo* and are part of the plant chemical defense mechanism.[38]

Flavone polymethoxy has an influence on biochemical processes and plant physiology, has activities as antioxidants, enzyme inhibitors, anti-allergic, anticarcinogenic, antiviral, antiproliferative, and anti-inflammatory. Sinensetin is a very important polymethoxy and bioactive product with various biological activities such as reduces pain, anticancer, antifungal, antitumor and antibacterial.[39] Eupatorin has pharmacological activity inducing apoptosis, vasodilators, antiinflammation, inhibitor P450, and antineoplastic.[40]

Rosmarinic acid was isolated from many species of Lamiaceae and Boraginaceae and identified as one of the active components of several medicinal plants.[41] In 1958 Scarpati and Oriente began to isolate rosmarinic acid from *Rosmarinus officinalis* (*Lamiaceae*). Rosmarinic acid has various biological activities that make it an attractive material for the pharmaceutical, food, and cosmetics industries. Rosmarinic acid pharmacological activities tested *in vitro* are antioxidant, anti-inflammatory, antimutagenic, antigenotoxic, cytotoxic, and antimetastatic antiangiogenic, antimicrobial, and immunomodulatory.[42]

Qualitative Analysis of Compounds in Orthosiphon aristatus (Blume) Miq

Qualitative of the *Orthosiphon stamineus* with UHPLC – ESI-QTOF-MS detected 61 compounds, 52 chemical structures can be identified to provide reference data for efficient separation and the idea of identifying secondary metabolites in other plants of the same genus. The compounds are six diterpenes, 26 phenolic acids, five tanshinones, four fatty acids and 11 flavonoids. Among these compounds, the sinensetin, rosmarinic acid, danshensu, caffeic acid and eupatorin are the main secondary metabolites.[43] Rosmarinic acid and sinensetin which are the main componen of *O. aristatus* have potential as antiviral agent.[43] The *O. aristatus* are divided into three varieties, namely white, white-purple and purple, based on the flower colour (Figure 1).[44]

While nine compounds that have been successfully separated but have not been successfully identified, further studies are needed by comparing spectrum data and fragmentation patterns with literature. Guo and the team (2019) also reported compounds that have never been published in previous research or reviews.[26,37,44,45] The newly

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reported compounds are three flavonoid compounds, namely 3 ', 4', 5,7- tetrahydroxy -3 ', 4', 5 tribenzoate, isorhamnetin-3-O-hexoside, and sinensetin derivatives (Figure 3). Fifteen phenolic compounds, namely protocatechuic acid methyl ester, caffeic acid isomers, protocatechuic pentoxide acid, protocatechuic acid derivative, cleroden F, orthosiphoic acid A, cleroden I, yunaneic acid D / isomer, salvianolic acid A, cleroden J, salvianolic acid E, rosmarinic acid dimer, cleroden D, N-feruloyltyramine and caffeic acid tetramer (Figure 3). Hossain and Rahman (2015) reported one flavonoid compound that was successfully isolated for the first time, 5,6,7,30-tetrametoxy-40- hydroxy -8-C-prenilflavone where the compound has not been listed in the previous review and research on *Orthosiphon aristatus* (Blume) Miq.[46] The essential oils in *O. aristatus* are eugenol, limonene, *β*-pinene, and *β*-caryophyllene.[37]

Orthosiphon aristatus (Blume) Miq. toxicity study

Toxicity study standardized water extract of *O. aristatus* at doses of 250 - 2000 mg/kg BW /day, during the administration of 60 days in male rats, demonstrated that all animals survived and showed no signs of toxicity. There were no significant water intake and food changes, organs and body weight in all groups. The number of implants, sperm concentration, morphology, resorption, and live fetus was not statistically significant. The levels of red blood cells, hematocrit, hemoglobin, and platelets were significantly higher in mice that received extracts of 2000 mg/kg BW. Standardized water extract *O. aristatus* do not cause toxic effects but instead stimulates erythropoiesis in mice, so further investigation is needed.[47]

Acute toxicity studies of standard *O. aristatus* the extract showed no signs of toxicity in Sprague Dawley (SD) mice.[37] Chronic toxicity water extract also reported at high dose, it reduced serum sodium levels but increased alkaline phosphatase levels.[48] Administration in oral 14 days of the methanol extract of leaves *O. aristatus* on rat Sprague-Dawley (SD) female with a dose of 0.5 - 5 g / kg body weight showed there is no death or adverse toxic signs were seen during the trial period or caused adverse effects on water intake body weight, relative organ weight and food consumption.[49]

The water extract of *O. aristatus* plant at doses up to 5000 µg/plat non-toxic strains of *Salmonella* and does not increase the number of colonies normal phenotype during the incident. In a test in the bone marrow of mice, the extract does not change the ratio of erythrocytes polychromatic normochromic, which does not increase erythrocytes incidence polychromatic micronuclear. There is no open toxicity and no changes in CYP1A (EROD) and 2B9 / 10 (BROD). Therefore *O. aristatus* in traditional medicine does not cause genotoxic risk.[50]

Potential of O. aristatus as Antiviral

New antiviral sources are from synthetic compounds and natural products such as traditional medicinal plants that contain several secondary metabolites that can inhibit viral replication without affecting normal cells or host cells. [51] Medicinal plants can also increase the body's immunity against viruses to reduce adverse effects and reduce mortality. The host protease, polymerase, influences viral growth, and enzymes, understanding these three aspects, can control the virus's growth or replication. Study and analysis of virus structure, reverse transcription, and replication can also find new antivirals.[52]

Preclinical tests *in vitro* and *in vivo* are essential to determine the level of secondary metabolites' safety, influence on genes, and study metabolism, absorption, and excretion. The acute toxicity safety test must be tested on at least two experimental animal species over a while. Traditional medicinal plants that have potential as antiviral must have good preclinical test results for further clinical testing to be mass produced.[53] The strategy for developing the O. aristatus plant as a new antiviral agent can be seen in Figure 4. Several previous studies have reported that the extract and content of the *O. aristatus* plant have antiviral potential. Secondary metabolites in the *O. aristatus* plant that antiviral activities can be seen in Table 1.

Antiherpetic activity

Water extract of leaves, flowers, and all the plants in addition to the root of the *O. aristatus* (0.39 mg/mL) had strong antiviral activity observed after normal cells (Vero cells) were inoculated with HSV-1 (post-treatment) with a 100% reduction in HSV-1 plaque. In the pre-treatment test, leaf water extract, flowers, and all parts of plants other than roots showed HSV-1 plaque reduction activity 79%, 84%, and 97% using the same concentration. Therefore water extract of *O. aristatus* has promising potential to be extracted as an anti-HSV-1 agent.[1] Extract of *O. aristatus* reduces viral replication by blocking the virus's adsorption to the target cell surface. This mechanism occurs by deactivating the surface binding of glycoproteins from the virus or inhibiting viral cell fusion at the initial replication stage and ultimately preventing the early stages of viral reproduction. At a concentration of 0.05 mg/mL, the extract tested could kill more than 70% of the virus.[35] Several secondary metabolites from the *O. aristatus* plant are reported to have antiherpetic potential, including caffeic acid mainly inhibits HSV-1 multiplication before viral DNA replication is completed, but not afterwards,[54] N-trans-feruloyltyramine has medium HSV-1 activity,[55] β-caryophyllene has an excellent potential to deactivate the infectious force of the herpes virus and provides a high selectivity index with a value of 140.[56] Limonene and β-Pinene showed strong anti-HSV-1 activity by direct contact with free virus particles. The two drugs tested were dose-dependent in HSV-1 interacting to inactivate viral infection,[57] eugenol provides good protection from HSV-2 infection in experimental animals,[58] eugenol was tested for antiviral activity against the HSV-1 and HSV-2 viruses. *In vitro*, replication of these viruses was found to be inhibited in the presence of this compound with IC50 values for eugenol anti-HSV effects were 25.6 µg/mL (HSV-1) and 16.2 µg/mL (HSV-2)[59] and p-cymene has anti-HSV-1 activity (IC50 >0.1%).[60]

Anti-influenza virus

The formation of pro-inflammatory mediators above normal values is associated with symptoms of viral disease.[61] Sinensetin, a major secondary metabolite content in *O. aristatus*, can reduce the release of LPS-mediated pro-inflammatory cytokines by inactivating the NF-κB pathway.[62] Sinensetin can decrease pro-inflammatory mediators' production stimulated by the influenza A virus (IAV), including PGE2, IL-6, IL-8 IP-10, TNF-α, COX-2, and MCP-1.[31] The increased production of IL-6 in influenza A H1N1 patients can affect the severity of the disease.[63] TNF-α can stimulate the expression of other inflammatory mediators.[64,65,66] Sinensetin compounds can reduce the signal activation of p38 MAPK, ERK1 / 2 MAPK, NF-κB mediated by IAV. Therefore, it can be speculated that sinensetin exerts an anti-inflammatory effect on IAV-mediated inflammation, which may be due to its inhibitory action on the intracellular signaling cascade, making it a potential drug candidate for the treatment of influenza disease with excessive inflammation.[31] Other compounds in the *O.aristatus* plant that have the potential to be anti-influenza virus are caffeic acid suppresses the degeneration of virus-infected cells, at least temporarily, and the antiviral activity observed is not likely to be a secondary effect of the cytotoxic effects of the reagent. These findings demonstrate the possible pharmacological application of caffeic acid or its derivatives as influenza A antiviral medication.[67] Limonene is a potential anti avian influenza A (H5N1) agent,[68] 1,8-cineol may increase protection against influenza A virus infection in mice by reducing the pulmonary inflammatory reaction,[69] linalool has potential as an anti-influenza, further research is needed to study the mechanism of linalool antiviral activity against influenza A/WS/33 viruses,[70] Eugenol was able to inhibit autophagy and IAV replication, inhibiting the activation of the signal pathways ERK, p38MAPK and IKK/NF-µB[71] and aurantiamide acetate has powerful anti IAV-infected cells by NF-µB inhibition.[72]

Inhibitor of COVID-19

In an *in silico* (molecular docking) study, rosmarinic acid as a COVID-19 inhibitor using Auto Dock 4.0 (AD4.0) showed promising results, where protein and ligand interactions produced binding energy equivalent to COVID-19 inhibitors hydroxychloroquine and

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remdesivir.[32] Another In-silico study report states that rosmarinic acid has an excellent binding affinity for the 6LU7 and 6Y2E proteases.[33,73] 6LU7 and 6Y2E are the Main Protease Proteins (MPP) structures of SARS-Cov-2. This information is obtained from the genomic information of NCBI and RCSB Protein Data Bank.[74,75,76] Another compound in the *O. aristatus* plant that has the potential to act as a COVID-19 inhibitor is sinensetin, based on in silico study sinensetin has the potential to inhibit enzyme responsible for the capping of SARS-CoV-2 mRNA (SARS-CoV-2 Guanine-N7 methyltransferase),[77] cirsimaritin demonstrated more significant active inhibition than COVID-19 primary protease active site Hydroxy-Chloroquine and ACE2,[78] Caffeic acid derivatives possessing more binding energies than nelfinavir against COVID-19 Mpro, Nsp15, SARS-CoV-2 spike S2 subunit, spike open state and closed state structure respectively,[79] sagerinic acid has a better binding score against the critical targets of COVID-19, RNA polymerase (PDB ID: 7bV2) than remdesivir,[80] based on in-silico studies β-caryophyllene can inhibit COVID-19 Main Protease (Mpro,)[81]. Results of molecular docking show that 1,8-cineole can bind to main Protease so that it can inhibit virus reproduction and 1,8-cineole complexes have been shown to form strong hydrophobic, hydrogen bonding and ionic interactions.[82]

Anti-Hepatitis virus

Rosmarinic acid administration can reduce the HBV part and extracellular HBV DNA with little cytotoxicity. The combination of rosmarinic acid and lamivudine can increase the anti-HBV activity of lamivudine slightly. Two phenolic hydroxyl groups at both ends of the rosmarinic acid compound and its derivatives play a role in inhibiting ε-Pol binding. Rosmarinic acid inhibits HBV replication in HBV-infected cells by explicitly targeting ε-Pol binding,[36] ladanein inhibits post- attachment entry-stage but does not inhibit RNA replication. The inhibition concentration of 50% was 2.5 μm. Ladanein is active against all major hepatitis C virus genotypes, including variants resistant to the entry inhibitor. The synergistic effect of inhibiting hepatitis C virus infection is produced by the combination of ladaneine and cyclosporine A,[83] danshensu can decrease HBV reverse transcriptase activity so that it can affect hepatitis B virus (HBV) DNA replication,[84] oleanolic acid and ursolic acid has potential as anti-HCV with the suspected mechanism of action is to suppress the activity of HCV NS5B RdRp as a noncompetitive inhibitor,[85] and ursolic acid inhibits the activation of the Ras homolog gene family member A (RhoA) which is mediated by Hepatitis B virus X (HBx), activation of the beclin-1 promoter and subsequent induction of autophagy.[86]

Anti-Japanese ensefalitis Virus (JEV)

Rosmarinic acid, which is the main component in *O. aristatus* has good antiviral activity in inhibiting Japanese encephalitis growth. *In vivo* studies revealed that rosmarinic acid could inhibit viral replication in the brain and secondary inflammation due to microglial activation. Therefore rosmarinic acid plays an essential role in reducing the disease's severity caused by the Japanese Encephalitis Virus. Rosmarinic acid is highly recommended to reduce neurological complications in Japanese encephalitis patients.[34]

Anti Enterovirus

Rosmarinic acid provides strong protection against EV71 infection when the multiplicity of infection (IC50 4.33 ± 0.18 μM) and the therapeutic index are high (340). Rosmarinic acid inhibitory activity is highest in the early stages of viral infection. *In silico* study predicts that rosmarinic acid can replace the natural receptacle factor in the VP1 capsid-binding hydrophobic vessel.[29] Rosmarinic acid has an excellent opportunity as an antiviral candidate to inhibit replication and reduce the adverse effects caused by EV71 infection because rosmarinic acid is effective in reducing EV71 viral infection in the early stages. Rosmarinic acid acts in the early stages of viral infection and can target viral particles directly to affect the interaction between virus-P-selectin glycoprotein ligand-1 (PSGL1) and virus-heparan sulfate.

Anti-human immunodeficiency virus (HIV)

The compound that can be anti-HIV in *O. aristatus* is lithospermic acid, which can inhibit the activity of 3'-processing by HIV-1 integrase with an IC50 value of 0.83 microM and inhibits the catalytic activity of HIV-1 integrase (IC50 0.48 microM). lithospermic acid does not prevent HIV entry into H9 cells and suppresses acute HIV-1 infection in H9 cells with IC50 2 and 6.9 microM values. Lithospermic acid needs to be developed as a new anti-HIV agent,[87] Chicoric acid can protect HIV-infected cells and exhibits antiviral activity against MT-4 cells infected with HIV-1 and HIV-2 (EC50 1.7 - 20 microM),[88] 2,3-Dicaffeoyltartaric acid has high specificity for HIV integration, and their activity against integrase in biochemical tests is consistent with the anti-HIV activity they observed in tissue culture. 2,3-Dicaffeoyltartaric acid has a very promising potential to be developed into an anti-HIV agent because it has a different mechanism from the anti-HIV drugs currently circulating.[89] Tanshinone ⅡA inhibits Tat-induced Transactivation of LTR HIV-1 depending on the AMPK-Nampt pathway so that it can be developed into a new anti-HIV agent,[90] oleanolic acid inhibits HIV-1 replication by inhibiting HIV-1 protease activity *in vitro*,[91,92] and maslinic acid has potent inhibitory activity against HIV-1 proteases.[93]

Table 1: Potential chemical content in *O. aristatus* as an antiviral

Group	Compound	Antivirus potential
Flavonoid[94,43]	Ladanein	Anti-HCV hepatitis C virus (HCV) activity[83]
	Sinensetin	- Treatment of influenza disease with excessive inflammation[31] - Inhibitors of SARS-CoV-2 Guanine-N7 Methyltransferase (in silico study)[77]
	Cirsimaritin	- Inhibitors of COVID-19 main protease (in silico study)[78] - Anti-influenza virus[95]
	Rhamnazin	Anti-HCV hepatitis C virus (HCV) activity[99]
Phenolic Acid[96,43, 97,98]	Rosmarinic acid	- Anti enterovirus 71 (EV71)[29,30] - Potential inhibitor of COVID-19 (in silico study)[32,33,73] - Reduces the mortality of mice infected with the Japanese encephalitis virus (JEV)[34] - Inhibit hepatitis B virus replication[36]
	Caffeic acid	- Anti-herpes simplex virus type 1[54] - Inhibition influenza A virus multiplication in vitro[67]

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	Lithospermic acid	Inhibitors of HIV-187
	Chicoric acid	HIV-1 integrase inhibitors[88]
	N-feruloyltyramine	Anti-simplex virus type 1 (HSV-1)[55]
	Caffeic acid derivative	Inhibitors of SARS-CoV-2 (in silico study) [79]
	Danshensu	Anti hepatitis B virus[84]
	Salvionolic acid B	Anti Enterovirus 71 Replication[100]
	Sagerinic acid	Inhibitor of COVID-19 Main Protease and RNA Polymerase (In-Silico study)[80]
	2,3-Dicaffeoyltartaric acid	Selective Inhibitors of Human Immunodeficiency Virus Type 1 Integrase[89]
	β-Caryophyllene	- Reduced the virus serotype-2 replication in HepG-2 cells[101] - Herpes simplex virus type 1 (HSV-1)[56] - Exhibiting anti-viral activity against SARS-CoV-2[81] - Protection against virulent Newcastle disease virus NDV challenge[102]
	β-Pinene	Anti herpes simplex virus type 1 (HSV-1)[57]
	Limonene	- Anti herpes simplex virus type 1 (HSV-1)[57] - Influenza virus type A H1N1[68]
	α-Pinene	Anti-Infectious Bronchitis Virus (IBV) Activity[103]
Essential oil fragments[19]	1,8-Cineol	- Protect Against Influenza-Virus[69] - Against SARC-CoV-2 proteinase[82,104,105] - HSV-2 virus[58] - Against influenza A virus (H3N2)[106]
	Linalool	Possessing Anti-Influenza A/WS/33 Virus Activity[70]
	Camphor	Inhibition of the bovine viral diarrhoea virus[107]
	Eugenol	- Antiviral activity against HSV-1 and HSV-2 viruses[59] - Promising inhibitor for autophagy and influenza A virus (IAV) infection[71] - Rapid anti- influenza virus IFV actions[108] - Against various proteins targets of SARC-CoV-2 (In silico study)[109] - Inhibitor of the Ebola Virus[110]
	p-Cymene	Against herpes simplex virus[60]
	Tanshinone IIA	Inhibits tat-induced HIV-1 transactivation[90]
Tanshinones[43]	Cryptotanshinone	Inhibits the infection of various strains of Porcine reproductive and respiratory syndrome virus (PRRSV)[111]
	Ursolic acid	- Inhibiting rotavirus infection[113] - Inhibitors against porcine reproductive and respiratory syndrome virus[114] - Novel hepatitis C virus antivirals[85] - Suppresses Hepatitis B Virus X Protein-mediated Autophagy[86]
Triterpene[94,112]	Oleanolic acid	- Novel hepatitis C virus[85] - Anti-HIV activity[91,92]
	Betulinic acid	- Suppressed ECHO 6 virus reproduction[115] - Anti SARS-CoV[116] - Competitive inhibitors of SARS-CoV 3CL protease[117]
	Maslinic acid	Inhibitory activity against HIV-1 protease[93]
Dipeptide[94]	Aurantiamide acetate	Anti influenza A virus[72]

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Figure 1: *O. aristatus* purple variety (a) and white purple variety (b)

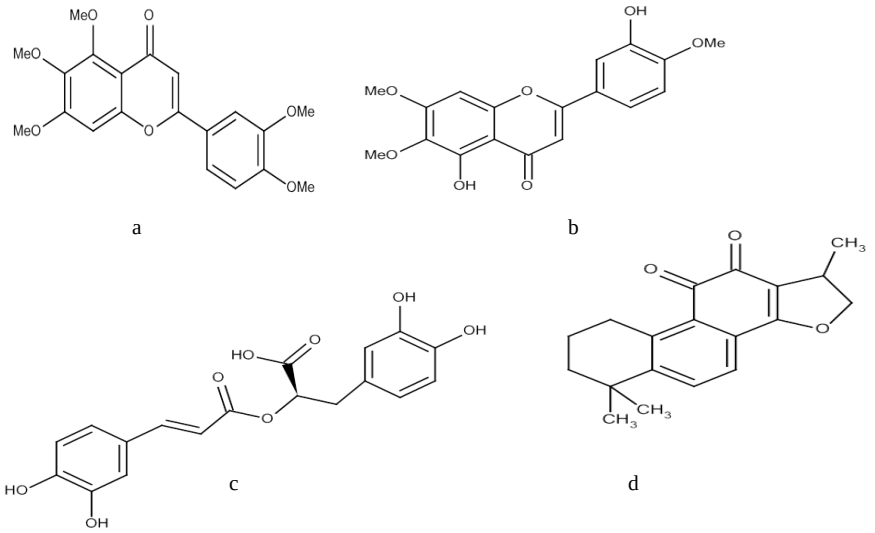
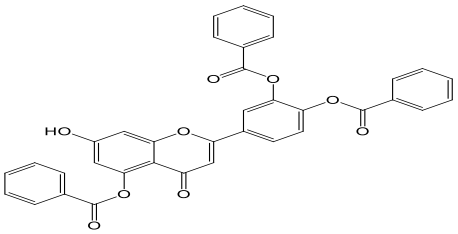
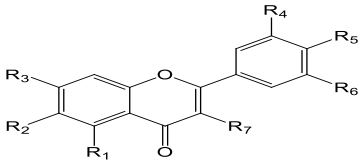


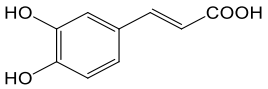
Figure 2: Chemical structure of sinensetin (a), eupatorin (b), rosmarinic acid (c) and tanshinone IIA (d)



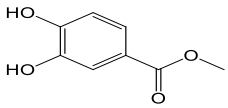
3 ', 4', 5,7- tetrahydroxy -3 ', 4', 5 tribenzoate



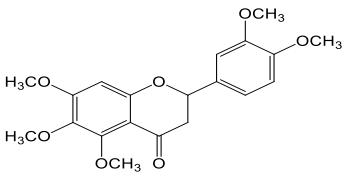
sorhamnetin-3-O-hexoside



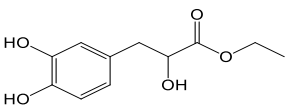
caffeic acid isomers



protocatechuic acid methyl ester



isorhamnetin-3-O-hexoside



protocatechuic acid derivative

orthosiphoic acid A

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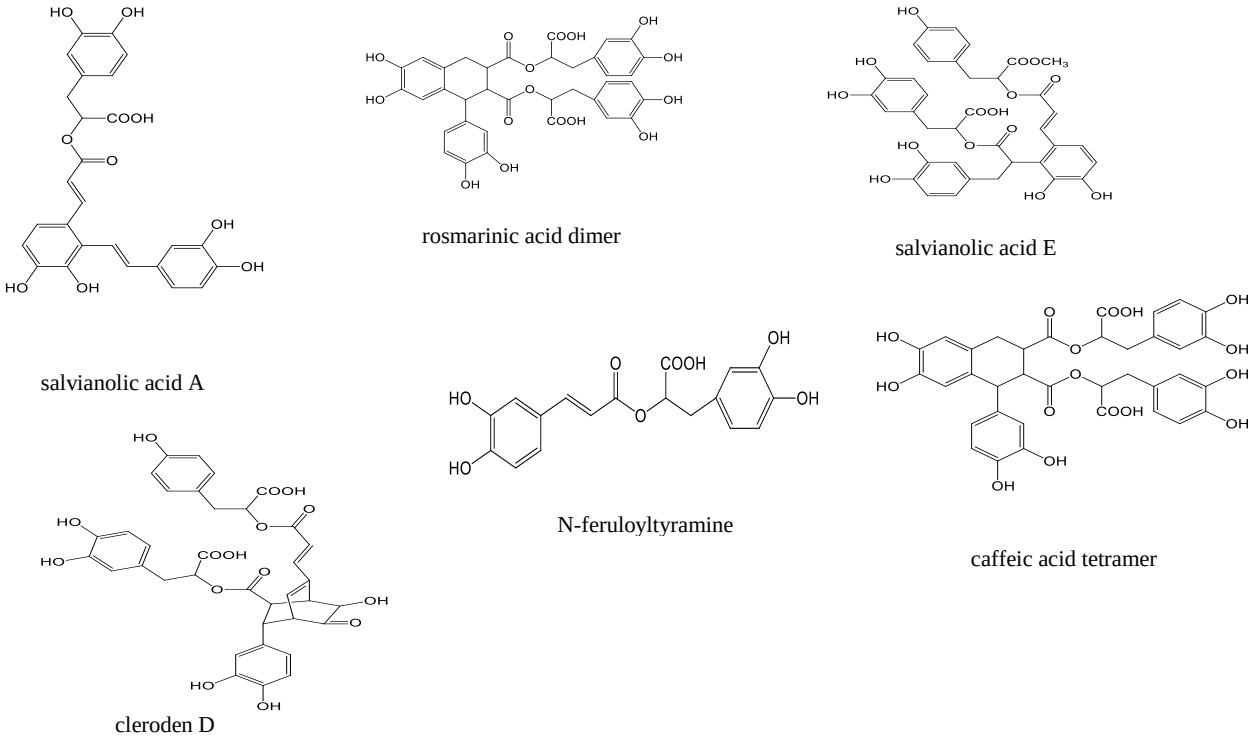

Figure 3: Chemical structure of flavonoid and phenolic acid compounds

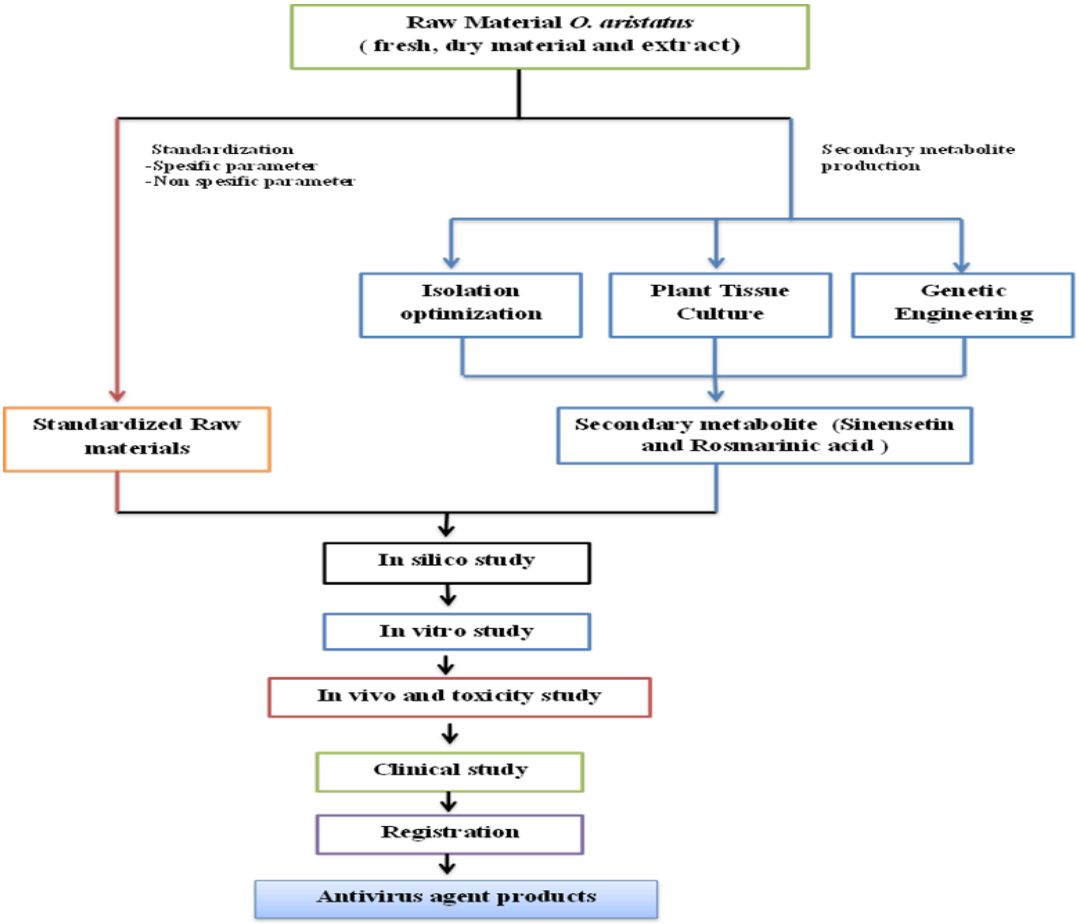

Figure 4: Strategy to develop *O. aristatus* as an antiviral agent

Conclusion

The *O. aristatus* plant has great potential to be developed into antiviral herbal medicines and a source for new antiviral agents such as sinensetin and rosmarinic acid through chemical synthesis or plant tissue culture. The results of the *in silico* study show that the main content of *O. aristatus* , namely rosmarinic acid, has the potential to inhibit the replication of COVID-19.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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