Pharmacological Effects Study and Safety *of Andrographis Paniculata* Fever Medicine Recipe

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ABSTRACT

Fever medicine recipe G531/64 is an herbal recipe containing *Andrographis paniculata* formulated for treating COVID-19. The capacity of the recipe was evaluated by chemical and pharmaceutical analysis. Total phenolic content of the recipe was 137.70 mg GAE/g. The extract of the recipe at 1 mg/mL showed slightly lower ORAC and FRAP antioxidant activity than L-ascorbic acid at the same concentration. The concentration that reduces 50% of cell number (IC_{50}) was 60.29 µg/mL. G531/64 at 100 µg/mL exhibited 77.65% anti-inflammation which was higher than those of Lopinavir (65.29%) and Ritonavir (60.35%) with the same concentration. The results from this study indicated the feasibility of using G531/64 as an alternative drug for treating COVID-19.

Introduction

COVID-19, caused by SARS-CoV-2, emerged in 2019 and still lacks a direct cure. Current antiviral drugs, such as Nirmatrelvir/Ritonavir, Remdesivir, and Molnupiravir, have limitations, side effects, and high costs. COVID-19 affects the respiratory system and can cause complications like pneumonia and organ failure (Channuwong & Ruksat, 2022). Thai traditional medicine suggests herbal remedies, including Andrographis paniculata (Fah Talai Jone), which is the only herbal medicine listed in the National Essential Medicines List for COVID-19 treatment. Instead of using single herbs, traditional medicine combines multiple herbs for a holistic treatment approach.G531/64, a registered herbal remedy, contains eight ingredients: Andrographis paniculata, Tinospora crispa, Phyllanthus amarus, Coriander seeds, Indian gooseberry, Terminalia bellirica, Terminalia chebula, and Licorice. These herbs help reduce fever, ease respiratory symptoms, and protect the lungs.

The studies suggest that G531/64 has strong antioxidant properties that reduce inflammation and inhibit the SARS-CoV-2 main protease (Mpro), It is essential for viral replication. This research aims to evaluate G531/64's effectiveness by analyzing its chemical properties, anti-inflammatory effects (compared to Diclofenac), and enzyme inhibition potential (compared to Lopinavir and Ritonavir).

Research Methodology

The G531/64 herbal medicine formula, which contains *Andrographis paniculata* (commonly known as Fah Talai Jone), was manufactured by Thepruksa Thai Herbal Co., Ltd., located in Saraburi, Thailand. The production complies with Pharmaceutical Inspection Co-operation Scheme (PIC/S) standards and is certified under GMP/PICs and GHP (Good Hygiene

Practice). The formula consists of 400 mg capsules, packaged in plastic bottles, with 60 capsules per bottle. Samples were taken from Lot No. 2, using three bottles for the study. Preparation of the Test Solution are as following First, sampling: Four capsules were randomly selected. Second, weighing: 1 gram of G531/64 powder was measured and placed into a 15 mL centrifuge tube. Third, the Solvent Addition: 10 mL of Dimethyl sulfoxide (DMSO) was added. Next, Mixing & Extraction: The solution was vortex-mixed for 3 minutes, followed by 1-hour continuous shaking at room temperature for extraction. Then, the filtration: The extract was filtered using a 0.45-micrometer syringe filter. Finally, the concentration adjustment: The solution was adjusted to 100 mg/mL concentration for further analysis.

Analysis of Total Phenolic Content and Antioxidant Activity

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method based on Lawag et al. (2023). The process was involved: First, mix 10 µL of the G531/64 extract (in DMSO) with 40 µL of Folin-Ciocalteu reagent in a 96-well plate. Second, incubate the mixture for 5 minutes, then adding 200 µL of 75% sodium carbonate (Na_2CO_3). Third, allow the reaction to proceed in the dark for 2 hours. Forth, measure absorbance at 760 nm using a microplate reader. Lastly, calculate TPC using a gallic acid standard curve, expressing results in mg gallic acid equivalent per gram of extract (mg GAE/g).

Antioxidant Activity – ORAC Assay

The Oxygen Radical Absorbance Capacity (ORAC) assay followed the method of Thaipong et al. (2006)Boonprakob, Crosby, Cisneros-Zevallos, & Byrne (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne, 2006) .The G531/64 extract (1 mg/mL in DMSO) was tested, with L-ascorbic acid as the control. Trolox (1.56, 3.12, 6.25, and 12.5 µM) was used as the standard. Fluorescein served as the reaction probe in a 96-well plate. Fluorescence was measured at 485 nm (excitation) and 535 nm (emission). Results were expressed in µmole of Trolox Equivalent (TE) per gram of extract.

Antioxidant Activity – FRAP Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was conducted based on Thaipong et al. (2006): The G531/64 extract (0.1 mg/mL in DMSO) was tested, with L-ascorbic acid (10 µg/mL) as the control. Ferrous Ammonium Sulfate (4, 8, 12, 16, and 20 nM) was used to create a standard curve respectively. The reaction occurred in a 96-well plate, where the FRAP Assay Probe reacted with $FeCl_3$.The absorbance at 594 nm was measured after 1 hour. The results were expressed in µmol of Fe(II) per gram of extract.

Cellular Toxicity and Anti-Inflammatory Activity Tests

Cytotoxicity Assay with (MTT Assay) was performed following Al-Sheddi et al. (2019) to assess the cytotoxicity of the G531/64 extract. The G531/64 extract (1 mg/mL) was prepared in cell culture medium containing: Dulbecco's Modified Eagle's Medium (DMEM), 10% Fetal Bovine Serum (FBS),1% Sodium Pyruvate,1% HEPES buffer and 1% Antibiotic-Antimycotic solution. As for Cells (100,000 cells/well) were seeded into a 96-well plate and incubated at 37°C for 24 hours. The culture medium was replaced with various concentrations of the G531/64 extract (2–500 µg/mL) and incubated for 72 hours. After incubation, MTT reagent (0.5 mg/mL) was added and incubated for 3 hours. The MTT solution was removed, and 50 µL of DMSO was added to

dissolve the formazan crystals. Next, the absorbance was measured at 570 nm using a microplate reader. The IC_{50} value (concentration reducing cell viability by 50%) was calculated using GraphPad Software.

Anti-Inflammatory Activity (Nitric Oxide Inhibition Assay)

The anti-inflammatory activity was assessed by measuring the inhibition of Nitric Oxide (NO) production in RAW 264.7 macrophages, following Inkanuwat et al. (2019):RAW 264.7 cells (1.0×10^5 cells/well) were cultured in a 96-well plate using DMEM medium supplemented with: 10% FBS, 1% Sodium Pyruvate, 1% HEPES buffer and 1% Antibiotic-Antimycotic solution. Cells were incubated at 37°C with 5% CO_2 for 24 hours. The medium was replaced with G531/64 extract at concentrations of 100, 200, 400, 2500, 5000, and 10,000 µg/mL respectively, followed by 1 hour of incubation. Lipopolysaccharide (LPS, 1 µg/mL) was added to induce inflammation, and the cells were incubated for another 24 hours.25 µL of the culture medium was transferred to a 96-well plate for no quantification using the Griess reagent assay:50 µL of Sulfanilamide solution was added and incubated in the dark for 10 minutes.50 µL of NED solution was added and incubated in the dark for another 10 minutes. The absorbance was measured at 540 nm using a microplate reader. There was no concentration was compared between: LPS-treated control group (without extract). Positive control (LPS + Diclofenac, an anti-inflammatory drug).Lastly, test groups (LPS + G531/64 extract) was evaluated its anti-inflammatory effect.

Inhibition of SARS-CoV-2 Main Protease

The SARS-CoV-2 main protease (Gen Script Biotech, USA) is an enzyme responsible for processing viral proteins necessary for COVID-19 replication. Inhibiting this enzyme can prevent viral replication. The study followed the protocol of Ihssen et al. (2021) using the G531/64 extract at concentrations of 1, 10, and 100 µg/mL in a buffer solution containing:20 mM Tris-HCl (pH 7.5),100 mM Sodium chloride (NaCl) ,2 mM DL-Dithiothreitol (DTT) , 0.05 mM Ethylenediaminetetraacetic acid (EDTA). There are Control Groups namely; Lopinavir 98%, Pharmaceutical grade, Thermo Fisher Scientific, United State) and Ritonavir 98%, Pharmaceutical grade, Thermo Fisher Scientific, United State) .The concentrations is 100 µg/mL in DMSO.. Experimental Procedure:

The reaction was initiated by adding Dabcyl-KTSAVLQSGFRKM-E(Edans) substrate (40 µM, MedChemExpress, USA). Fluorescence intensity was measured using TECAN fluorescence microplate reader at : Excitation wavelength: 340 nm, Emission wavelength: 430 nm respectively. Fluorescence intensity was recorded every 1 minute for 30 minutes. The percentage of enzyme inhibition (% relative inhibition) was calculated based on the initial reaction rate.%

$$\text{Relative Inhibition} = \frac{[(V_0 \text{ Enzyme} - V_0 \text{ Blank}) - (V_0 \text{ Sample} - V_0 \text{ Blank})] \times 100}{[(V_0 \text{ Enzyme} - V_0 \text{Blank})]}$$

Where V_0 is initial reaction rate of the enzyme in the absence of the test sample without inhibitor (V_0 Enzyme) and V_0 Blank

Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD) from at least three independent replicates. Statistical analysis was performed using statistical software, and data variability was assessed through: Analysis of Variance (ANOVA), Duncan's New Multiple Range Test – Applied for post-hoc comparisons to identify significant differences between mean values and Paired sample *t*-test (2-tailed). A confidence level of 95% ($p < 0.05$) was used to determine statistical significance.

Research Findings

Total Phenolic Content

The total phenolic content (TPC) of formulation G531/64 was measured at three different concentrations: 0.01, 0.1, and 1 mg/mL, with values of 133.56, 159.05, and 120.48 mg GAE/g, respectively, as shown in Table 1.The average TPC for formulation G531/64 was 137.70 mg GAE/g. The variation in TPC values suggests differences in the types and amounts of phenolic compounds present in the herbal ingredients of the formulation.

Table 1 Total phenolic content (TPC) in G531/64

Concentration of G531/64 (mg/mL)	TPC (mg GAE/g)	Average TPC (mg GAE/g)
0.01	133.56±17.51	
0.1	159.05±0.74	137.70±11.32
1	120.48±0.21	

Results are shown as mean±SD (n=3)

Antioxidant Activity (ORAC Assay)

The oxygen radical absorbance capacity (ORAC) of G531/64 extract at a concentration of 1 mg/mL was 11,420.44 µMoles TE/g, while that of vitamin C was 13,571.11 µMoles TE/g, as shown in Figure 1(a). The ORAC value of vitamin C was significantly higher than that of G531/64 ($p < 0.05$). This difference may be attributed to the fact that vitamin C is a pure compound, whereas G531/64 is an herbal formulation containing multiple plant extracts with varying antioxidant properties.

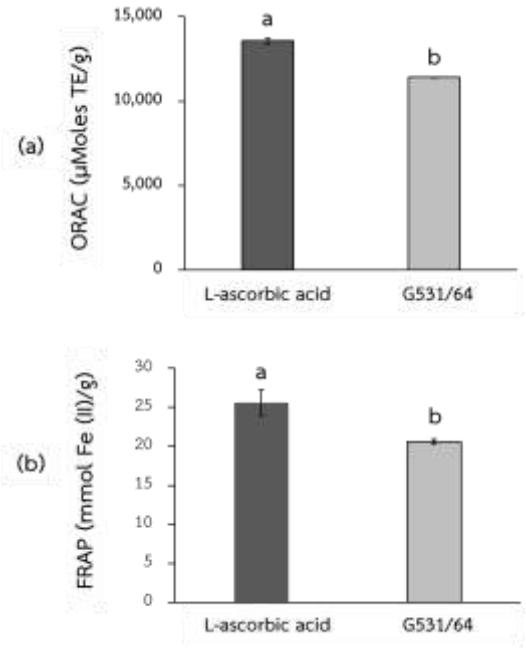

Figure 1 ORAC (a) and GRAP (b) antioxidant activities of G531/64 and L-ascorbic acid. [a-b] indicate significantly different of the values ($p<0.05$)

Antioxidant Activity (FRAP Assay)

The ferric reducing antioxidant power (FRAP) of G531/64 extract was 20.61 mmol Fe (II)/g, which was slightly lower than the FRAP value of vitamin C (25.60 mmol Fe (II)/g). The results showed that vitamin C had significantly higher FRAP antioxidant activity than G531/64 ($p < 0.05$), as shown in Figure 1(b).

Cytotoxicity on HepG2 Cells (MTT Assay)

The cytotoxicity of G531/64 extract was evaluated using the MTT assay on HepG2 liver cells. The survival rate of cells treated with G531/64 extract at concentrations ranging from 1.95 to 500.00 µg/mL was between 21.24% and 102.86%. The IC50 value of G531/64 was determined to be 60.29 µg/mL, as shown in Table 2. For the controls, the negative control (distilled water) showed no cytotoxicity, with a cell survival rate of 100.28%.The positive control (10% DMSO) was highly toxic, resulting in 0.00% cell survival.

Table 2 Cell viability as shown by IC_{50} of G531/64

Treatment	Concentration (µg/mL)	Cell viability (%)	IC_{50} (µg/mL)
H_2O	-	100.28±3.38	-
DMSO	-	0.00±0.00	-
G531/64	1.95	102.86±1.15	60.29±1.22
	3.91	100.13±0.42	
	7.81	104.41±2.60	
	15.63	99.75±0.48	
	31.25	95.61±3.22	
	62.50	60.27±0.69	
	125.00	32.93±1.00	
	250.00	27.83±0.62	
	500.00	21.24±0.09	

Values are shown as mean±SD (n=3)

Anti-inflammatory Activity (Cell Level)

The anti-inflammatory activity of G531/64 was evaluated by measuring its ability to inhibit nitric oxide (NO) release from RAW 264.7 cells stimulated with LPS. The results are shown in Table 3. The extract at concentrations of 100, 200, and 400 µg/mL exhibited anti-inflammatory activity at 77.65%, 83.71%, and 92.43%, respectively. These values were higher than the positive control (diclofenac), which showed 74.24% inhibition.This suggests that G531/64, a combination of herbs including Andrographis paniculata, has anti-inflammatory properties comparable to the standard anti-inflammatory drug, diclofenac shown in Table 3

Table 3 Concentration of released nitric oxide and percentage inhibition

Treatment	Concentration (µg/mL)	Concentration of released NO (µM)	Inhibition of NO (%)
H_2O	-	$50.77±1.46^{a}$	0.00
Diclofenac	75	$25.64±0.56^{b}$	$74.24±1.65^{c}$
G531/64	100	$24.49±0.34^{b}$	$77.65±1.01^{c}$
	200	$22.44±0.26^{c}$	$83.71±0.76^{b}$
	400	$19.49±0.46^{d}$	$92.43±1.37^{a}$

Values were presented as mean±SD (n=3), Values in the same column followed by the same letters are not significantly different ($p<0.05$) as tested by Duncan' new multiple range test.

Inhibition of SARS-CoV-2 Main Protease Activity

The study evaluated the antiviral activity of the G531/64 formulation by analyzing its ability to inhibit the SARS-CoV-2 main protease. The experiment tested the extract at concentrations of 100, 10, and 1 µg/mL, comparing it with the antiviral drugs Lopinavir and

Ritonavir at a concentration of 100 µg/mL. Control groups were included an untreated enzyme group (normal activity) and a positive control group (complete enzyme inhibition).Results showed that G531/64 at 100 and 10 µg/mL exhibited inhibition rates of 100% and 96.87%, respectively—significantly higher than Lopinavir (65.29%) and Ritonavir (60.35%) ($p<0.05$), as shown in Table 4.

Table 4 Percentage relative inhibition of G531/64 at different concentrations, Lopinavir, and Ritronavir against SARS-CoV-2 main protease.

Treatment	Concentration (µg/mL)	Relative inhibition (%)
Control	-	0.01±0.01
Blank	-	100.00±0.00[a]
Lopinavir	100	65.29±3.73[b]
Ritronavir	100	60.35±0.75[b]
G531/64	100	100.03±0.00[a]
	10	96.87±1.11[a]
	1	44.26±2.36[c]

Values were presented as mean±SD (n=3), Values in the same column followed by the same letters are not significantly different ($p<0.05$) as tested by Duncan' new multiple range test.

Total Phenolic Content Discussion

The main ingredient in the G531/64 formulation is *Andrographis paniculata* (Fah Talai Jone), but its phenolic content varies depending on the plant part and extraction method. Studies have reported total phenolic content (TPC) in *Andrographis paniculata* leaves ranging from 1.5–1.7 mg GAE/g (Kurzawa et al., 2015) to 7.78 mg GAE/g (Md Salleh et al., 2014). Extraction with different solvents such as water, ethanol, and acetone results in TPC values between 1.8–7.5 mg GAE/g (Salleh et al., 2014).

In the G531/64 formulation, herbs with low total phenolic content include *Tinospora crispa* (Borapet) and *Coriandrum sativum* seeds. Studies indicate that methanol and ethanol extracts of *Tinospora crispa* have TPC values of 17.52–24.84 mg GAE/g (Sharma & Joshi, 2011), while *Coriandrum sativum* seeds range from 2.24 mg GAE/g (Martins et al., 2016) to 13.72 mg GAE/g (Derouich et al., 2020). Water and ethanol extracts of *Coriandrum sativum* seeds showed TPC values of approximately 5 mg GAE/g and 2 mg GAE/g, respectively (Saleem et al., 2017).

On the other hand, herbs with high phenolic content in the formulation include *Phyllanthus niruri* (Luk Tai Bai), *Phyllanthus emblica* (Makham Pom), *Terminalia bellirica* (Samo Pibeg), *Terminalia chebula* (Samo Thai), and *Glycyrrhiza glabra* (Licorice). Studies report methanol and water extracts of *Phyllanthus niruri* have TPC values of 159.13 mg GAE/g and 107.09 mg GAE/g (Zain & Omar, 2018). Different parts of the plant show varied TPC levels: 97.4–360 mg GAE/g in water extracts and 31.8–105 mg GAE/g in methanol extracts (Harish & Shivanandappa, 2006).For *Phyllanthus emblica*, ethanol extracts have TPC values ranging from 90 mg GAE/g (Sani Nurlaela et al., 2018) to 274.6 mg GAE/g (Halim et al., 2022). Ethanol extracts of its fruit range between 63.39–188.71 mg GAE/g (Dharmaratne et al., 2018).Meanwhile, *Terminalia*

chebula shows extremely high TPC values: 925.5 mg GAE/g (methanol), 1,041.8 mg GAE/g (water), and 867.2 mg GAE/g (ethanol) (Chang & Lin, 2012).For *Glycyrrhiza glabra* (Licorice), TPC ranges from 150–300 mg GAE/g, depending on root size and age (Behdad et al., 2020).The high total phenolic content of the G531/64 formulation, as seen in Table 1, is likely due to the presence of *Phyllanthus niruri*, *Coriandrum sativum*, *Phyllanthus emblica*, *Terminalia bellirica*, *Terminalia chebula*, and *Glycyrrhiza glabra*, which have significant phenolic compound levels. In contrast, *Andrographis paniculata* and *Tinospora crispa* may not contribute significantly due to their lower phenolic content.

The Oxygen Radical Absorbance Capacity (ORAC) values of individual herbal ingredients in the G531/64 formulation have been reported as follows: Andrographis paniculata (*Fah Talai Jone*) shows 1.05 mmol TE/g (Low et al., 2015).Tinospora crispa (*Borapet*) shows 1,000–2,252 µmol TE/g (Sud et al., 2020; Jayawardena et al., 2015).Phyllanthus niruri (*Luk Tai Bai*) shows 6.50 mmol TE/g, which is relatively low (Navarro et al., 2017).Coriandrum sativum (*Coriander seeds*) shows 28.5 µmol TE/g (El-Zaeddi et al., 2017). EC50 value shows 2,069 µg/mL, indicating lower antioxidant activity compared to Trolox (EC50 = 41 µg/mL) (Martins et al., 2016). Phyllanthus emblica (*Makham Pom*) shows 90–140 µmol TE/g (Li et al., 2015) and 5,480 µmol TE/g (Kunchana et al., 2021).Terminalia bellirica (*Samo Pibeg*) shows 2.89 µM Trolox (Pfundstein et al., 2010) and 29.17 µM Trolox (Arya et al., 2012).Terminalia chebula (*Samo Thai*) shows 3.27 µM Trolox (Pfundstein et al., 2010) and 18.23 µM Trolox (Arya et al., 2012).Glycyrrhiza glabra (*Licorice*) shows 1,813 µmol TE/g (Dong et al., 2014) and 84.5 mg TE/g (Molan & Mahdy, 2016).

These values indicate that *Terminalia bellirica*, *Terminalia chebula*, and *Phyllanthus emblica* exhibit strong antioxidant properties, contributing significantly to the overall ORAC value of the G531/64 formulation. Conversely, *Andrographis paniculata*, *Phyllanthus niruri*, and *Coriandrum sativum* show relatively lower antioxidant activity in comparison.

Antioxidant Activity (FRAP)

The study in Figure 2 shows that the G531/64 formulation has a higher FRAP antioxidant activity compared to individual herbal components.Tinospora crispa (*Borapet*) shows 0.78–1.15 µmol Fe(II)/g (Manne et al., 2021).Terminalia chebula (*Samo Thai*) shows 243 µg/mL (Arya et al., 2012) and 21.50 mM $FeSO_4$/mg (Wetchakul et al., 2019).Terminalia bellirica (*Samo Pibeg*) shows 145–265 µg/mL (Pfundstein et al., 2010; Wetchakul et al., 2019).Phyllanthus emblica (*Makham Pom*) shows 18.50 mM $FeSO_4$/mg and 4,280 µmol TE/g (Kunchana et al., 2021).Coriandrum sativum (*Coriander seeds*) shows 46.0 µmol TE/g (El-Zaeddi et al., 2017).Glycyrrhiza glabra (*Licorice*) shows High FRAP: 15,000–35,000 µM (D'Angelo et al., 2009).Lower FRAP: 14.1–26.8 µM (Aiello et al., 2017); 200 µmol TE/g (Molan & Mahdy, 2016).These findings suggest that the strong antioxidant capacity of G531/64 is likely due to its combination of high-FRAP herbs like *Terminalia chebula*, *Phyllanthus emblica*, and *Glycyrrhiza glabra*, rather than lower-FRAP herbs like *Tinospora crispa*.

Cellular Toxicity of G531/64

The G531/64 formulation was tested for cytotoxicity at different concentrations. The result showed at 1.95–31.25 µg/mL, cell viability remained high (95.61–104.41%).Accordingly, higher concentrations (≥62.50 µg/mL), cell viability decreased sharply: The rate 62.50 µg/mL was decreased into 60.21%.The rate 0f 125.00 µg/mL was decreased into 32.93%The rate 0f 250.00 µg/mL was decreased into 27.83%.The rate 0f 500.00 µg/mL was decreased into 21.24%.This data indicates that G531/64 is non-toxic at lower concentrations but exhibits dose-dependent cytotoxicity at higher levels.

Anti-Inflammatory Activity at the Cellular Level

The inhibition of nitric oxide (NO) production is positively correlated with the antioxidant content of the tested substances, as indicated by the total phenolic content (TPC) and antioxidant capacity. Plant extracts with high TPC generally exhibit strong antioxidant activity and tend to have greater NO inhibition as well (Mazlan et al., 2013; Chen et al., 2017).A study by Perera, Samarasekera, Handunnetti, & Weerasena (2016) found a moderate positive correlation between NO inhibition and FRAP ($r = 0.623$) and ORAC ($r = 0.525$) but a weak correlation with TPC ($r = 0.183$). This suggests that the antioxidants responsible for NO inhibition may not belong to the phenolic compound group. Consequently, the NO inhibition effect of the G531/64 formulation .The result from other bioactive compounds present in its herbal components. This contributes to its anti-inflammatory effects comparable to diclofenac at similar concentrations, aligning with the formulation's objective of leveraging the benefits of individual herbs to alleviate COVID-19 symptoms.

A study by Seetaha et al. (2022) examined another Andrographis paniculata-based herbal formulation for COVID-19 and found that at concentrations of 100, 250, 500, and 1,000 µg/mL, the NO inhibition percentages were 7.87%, 34.99%, 59.46%, and 79.66%, respectively. These values were lower compared to the G531/64 formulation at equivalent concentrations, indicating that G531/64 contains herbal components that effectively enhance its inhibition activity, resulting in stronger anti-inflammatory effects.

Inhibition of SARS-CoV-2 Main Protease Activity

The experimental results in Table 4 indicate that the G531/64 formulation exhibits higher inhibitory activity against the SARS-CoV-2 main protease than some COVID-19 antiviral drugs. This finding is consistent with Drakulich et al. (2021), who reported that favipiravir inhibits the SARS-CoV-2 main protease with an EC50 ranging from 10 µg/mL to over 78 µg/mL. In comparison, the G531/64 formulation at 1 µg/mL showed 44.26% inhibition, which is within the range of favipiravir's activity. Another study by Seetaha et al. (2022), the SARS-CoV-2 main protease inhibition of lopinavir and ritonavir at 10 µg/mL was approximately 18% and 50%, respectively. In contrast, G531/64 at the same concentration (10 µg/mL) exhibited significantly higher inhibition at 96.87% (Table 4). When tested at a higher concentration (100 µg/mL), lopinavir and ritonavir showed inhibition levels of 65% and 60%, respectively, which closely matched the results from this study (65.29% and 60.35%, respectively, at 100 µg/mL). However, both drugs still exhibited lower inhibitory effects than G531/64, which demonstrated 100% inhibition at 100 µg/mL.The G531/64 formulation also demonstrated superior anti-COVID-19

effects compared to single herbal extracts. Seetaha et al. (2022) reported that Andrographis paniculata (Fa Thalai Chon) extract at 10 µg/mL and 100 µg/mL inhibited SARS-CoV-2 main protease by 22% and 40%, respectively. In contrast, a multi-herbal formulation containing Andrographis paniculata exhibited 76.46% and 100% inhibition at 10 µg/mL and 100 µg/mL, respectively, the highlighting enhanced antiviral efficacy of the G531/64 formulation over single-herb extracts.

Conclusion and Recommendations

The G531/64 herbal formulation, a registered fever remedy containing Andrographis paniculata, exhibited antioxidant activity comparable to vitamin C at the same concentration. The cytotoxicity study on HepG2 liver cells showed an IC50 value of 60.29 µg/mL, indicating moderate toxicity. The anti-inflammatory activity, evaluated through nitrite inhibition in RAW264.7 cells, demonstrated that G531/64 at 100 µg/mL achieved 92.43% inhibition, surpassing diclofenac at 75 µg/mL (74.24% inhibition).The anti-COVID-19 efficacy of G531/64 was examined through its inhibition of SARS-CoV-2 main protease, showing a dose-dependent effect. At concentrations of 1, 10, and 100 µg/mL, the formulation inhibited the enzyme by 44.26%, 96.87%, and 100.03%, respectively. In comparison, lopinavir (100 µg/mL) and ritonavir (100 µg/mL) exhibited lower inhibition rates of 65.29% and 60.35%, respectively. These findings suggest that G531/64 has superior inhibitory activity against SARS-CoV-2 main protease compared to these commercial antiviral drugs and is more effective than single-herb extracts.

However, further studies should be conducted to evaluate the long-term toxicity of G531/64. Additionally, it is recommended to compare its SARS-CoV-2 main protease inhibitory activity with other antiviral drugs such as remdesivir, molnupiravir, and Paxlovid (a combination of nirmatrelvir and ritonavir), which are currently distributed by the Ministry of Public Health for COVID-19 treatment.

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