Screening and Evaluation of Cytotoxicity and Antiviral Effects of Secondary Metabolites from Water Extracts of Bersama abyssinica against SARS-CoV-2 Delta

Never Zekeya (nmwambela@yahoo.com)
College of African Wildlife Management
Humphrey Ndossi
Tanzania Industrial Research and Development Organization
Bertha Mamiro
Tanzania Industrial Research and Development Organization
Rehema Chande
Muhimbili University of Health and Allied Sciences
Alex Kisingo
College of African Wildlife Management
Madundo Mtambo
Tanzania Industrial Research and Development Organization
Jafari Kideghesho
College of African Wildlife Management
Jaffu Chilongola
Kilimanjaro Christian Medical Centre
Mhuji Kilonzo
University of Dodoma

Research Article

Keywords: Coviba dawa, Covid-19, Taditional medicine; Tanzania

Posted Date: January 31st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1217677/v1

Abstract

Background

Bersama abyssinica is widely distributed herb in Africa with varying medicinal uses in different countries. In Tanzania, the plant is famous for treatment of respiratory diseases including tuberculosis, tonsillitis, bronchitis, asthma and recently used for treatment Covid-19 symptoms. *B. abyssinica* is rich in several groups of active compounds including, phenolic acids, coumarins, flavonoids and tannins with potential pharmacological activities. Due to their broad medicinal uses, the water extract of leaf and stem bark has been registered as herbal medicine known as 'Coviba Dawa' for the treatment of viral and bacterial respiratory infections.

Objective

The aim of this study was to extract active compounds from *B.abyssinica*, and analyse them for their effectiveness against *Corona virus* (SARS-CoV-2), the Delta variant obtained from Basel University thas is reported to be highly virulent.

Methods

Bersama abyssinica stembark and leaves were air dried, sequentially extracted in various solvent on increase of polarity to yield extracts and fractions. The extracts were tested for presence of several metabolites and antioxidant activity. The most active water extract was analyzed by LC-MS/MC for identification of active compounds and all extracts were shipped to Basel university for anti SARS-CoV-2 Delta B1screening and antiproliferation assay.

Results

The LC-MS/MS analysis of *B. abyssinica* water extract reveled the presence of four phenolic compounds namely; 2,4-di-tert-butylphenol, 4-formyl-2-methoxyphenyl propionate; 7,8-Dihydroxy-4-methylcoumarin and 2,3, 6-trimethoxyflavone. These compounds were revealed to have antioxidant activity and futher invitro analysis against SARS-CoV-2 Delta B1 revealed the antiviral activity against coronavirus.

Conclusion

This study revealed a wide variety of active metabolites in *B. abyssinica* water extract with high antioxidant and antiviral activity which points out at *B. abyssinica* as a potential source of effective anti viral agents including anti SARS Cov2. We recommend further pre-clinical and clinical evaluation of *B. abyssinica* metabolites as potential antiviral and antibacterial agents.

Introduction

Corona virus (SARS-CoV-2) causes COVID-19, a serious viral infectious disease of global concern without a reliable cure to-date [1]. This disease has greatly impacted global economy, mobility, socio-economy and health systems [2, 3]. To date, the world is attempting to tackle the epidemic, with each country responding in its own unique way to mitigate the disease's effects in order to save lives and livelihoods. Traditional medicines, according to recent studies, have a lot of potential for curing the disease [4, 5]. Other countries, such as India, China, and Nepal, have developed potent chemicals derived from medicinal plants to treat a variety of viral infections, including Covid-19 infection. [6, 7, 8, 9].

However, African medicinal plants have depicted high potential against *Corona virus* in some countries including Morocco [10, 11]. In East Africa region, several responses and measures to combat COVID-19 have been taken but one of the most promising solution is through application medicinal plant concoctions with unknown active compounds. *B. abyssica* is among most studied plants in Africa with a wide range of antimicrobial activities including viral diseases [12]. A study by [13] revealed antiviral activity of *B. abyssinica* roots against HIV-1 and HIV-2 against HIV-1 and HIV-2 in Ethiopia. Phytochemical studies revealed a wide range of active compounds from several medicinal plants treat microbial infections including viral ones [14, 15]. Studies in West Africa including Benin and Ivory coast revealed the antiviral of plants [16]. However, studies reported that despite the use of medicinal plants for treatment of emerging diseases screening for safety is crucial for validating their uses [17].

Recently, studies show that *B. abyssinica* possess several active secondary metabolites with antioxidant, anti-respiratory and antimicrobial activities [18]. Another study revealed the potential of phenolic compounds from plant with high activity against viral infections by intrerferering the activity of protein or RNA in Corona virus [19]. *B. abyssinica* also possess several phenollic compounds including gallic acid that could be potential source of COVID -19 drug ([20]. Gallic acids has revealed several medicinal activity including antioxidant that has high correlation with antiviral activity in several cases [21]. Despite wide use of *B. abyssinica* for treatment of several microbial and viral illness, none of the study established and validated the application of the plant as antiCOVID-19 in East Africa. A study by [12] and indigenous knowledge from Southern of Tanzania show that, people in different communities use medicinal plants including *B. abyssinica* for relief against COVID-19. The water extract of *B. abyssinica* stem bark and leaf is commercially registered as COVIBA DAWA by the Traditional Medicine Authority of Tanzania (TAHPC) and currently used for treatment of Covid-19 and other viral and bacterial respiratory infections. Despite observed clinical effect of *B. abyssinica* on COVID-19, its cytotoxic and antiviral effects have not been quantified. In addition, the individual effects of the bioactive secondary metabolites on both host cells and the viruses have not be established. Hence, this study was designed to evaluate the in vitro efficacy and cytotocity effect of *B. abyssinica* stem and leaf extracts and fractions against *Corona virus* as a validation process of ‘Coviba Dawa’ for treatment of COVID-19 and other viral infections.

Material And Methods

Collection of plant

Bersama abyssinica plant materials were collected in Dry season of 2021 at Isongole area, at riverline forest patch with *Rauvolffia caffra* near pine plantation in Rungwe district, Mbeya region (9°34'60".9 S 33°62'84 E).The collection of leaves and stem bark was done by Dr. Never Zekeya with the assistance of of an experienced Curator, Mr. Frank .M Mbago with collection No. FMM 4052 from University of Dar es salaam. The plant was identified by Mr. Frank Mbago, and the voucher specimen number ND. Zekeya Nos.01 was deposited in the Herbarium (DSM) of Department of Botany at University of Dar es salaam. After collection, leaves and stem bark were dried under shade in room temperature and then pulverized to obtain small pieces before soaking into different solvents. All methods were performed in accordance with relevant guidelines and regulations, and the plant is recently considered as not of conservation concern.

Study site

Extraction and phytochemical analysis bioassay was conducted at Institute of Traditional Medicine at Muhimbili Institute of Health and Allied Sciences and The Governmnt Chemist Laboratory Authority. Active compounds with high phenolic compound and antioxidant activity were shipped to an advanced lab in Basel University for for antiviral assays against SARS-CoV-2 and prolifilation test.

Chemicals, Standard strains and Culture Media

Extraction Chemicals, Standard strains and Culture Media

Methanol (absolute) was bought from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands) and Dimethyl sulfoxide (DMSO) was purchased from RFCL Limited, Hayana, India. Dichololomethane, ethyl acetate and Ethanol will be purchased from Loba Chemie Pvt Ltd, Mumbai, India). Coronavirus SARS-CoV-2 –Delta B1 isolate was obtained from Basel University.

Preparation of Plant Materials and Extraction

Leaves and stem bark of *B. abyssinica* collected from Isongole of Rungwe district in Mbeya, Tanzania were selected based on the current use of herbal medicine named Coviba Dawa that is made from two parts and for conservation purpose, roots were not collected for this study. Leaves and stem bark were separately air dried under shade and then pulverized into fine particles by using electric blender (WESTPOINT M012). Then 100 g of leaves and 100g of stem bark were separately sequentially extracted in 1000ml of petroleum ether, dichloromethane, ethyl acetate and ethanol for 48h twice for each solvent. The respective extracts were filtered through muslin cloth on a plug of glass wool in a glass column and solvents were evaporated in vacuum using a rotary evaporator. Water extacts was obtained by decoction of 100g of stem bark into 1L of water by boiling at 100^0C for 10 minutes and infusion of

50g of leaves in stembark decoction. Thereafter, the concoction was filted using muslin cloth followed by lyophilization to obtain dry extract. All extract were stored in the refridgerator at 4^0C before further use.

Determination of bioactive metabolites

Determination of phenol

Two (2) ml of Iron III chloride solution were added to the 2ml of 100mg/ml of each extracts and fraction, the appearance of deep bluish-green solution indicated the presence of phenolic compounds.

Determination of flavonoid

The presence of flavonoid was determined by addition of 5ml of dilute ammonia solution into 2ml of 100mg/ml of extracts followed by addition of few drops of concentrated Sulphuric acid. Thereafter, a yellow coloration indicated the positive result for the presence of flavonoid compounds.

Test for tannin

Test for tannin was done by boiling 100mg of each extracts/fraction in 2ml of water in a test tube and then filter, this was followed by addition of few drops of 0.1% Ferric chloride solution. A brownish green, blue black coloration indicated the present of tannin compounds.

Determination of saponin

100mg of each extracts/fraction was dissolved into 2ml of distilled water in the test tube and warmed, this was followed by vigorously shaken. The formation of froth for at least a minute indicated the present of Saponin compounds.

Determination for antioxidant

100mg of each sample was dissolved in the 1ml of extractor solvents, filter and divide equally into two different test tubes. This was followed by the addition of 0.5ml of pre prepared from 0.1mM DPPH in one of the test tube and the second test tube as control and the mixture was shaken and allowed to stand for 1 minute. The formation of discoloration in comparison to control indicated the present of antioxidant compounds in the extract.

LC-MS/MS analysis of water extract of bark stem bark and leaves

The selection for this analysis was based on phytochemica screening and the most polar extact with potential pharmacological activity was selected for this aanlysis.Analysis of polar extracts was done by LC-MS/MS (Q-orbitrap-Ultra High Performance Thermofisher Company) at the Government Chemist Laboratory Authority. The extract was re-dried by using Rotavap under reducing pressure with flowing of Nitrogen gas 15psi at 45℃ then the Liquid Chromatography was eluted by mobile phases of ((A)0.1% formic acid in water followed by 0.1% Formic acid in Acetonitrile). The column conditions were 35℃ and 1.9µ of oven temperature and particle size respectively. The coupled MS was scanned in range of 150 – 2000m/z with resolution 140000, AGC Target1e6. The maximum IT setting was 200ms with ionization mode (HESI) collision Energy 45v.

Determination of antiviral and cytotocity activity

All antiviral screenining and cytotoxicity experiments were conducted at Basel University Laboratory according to the previously established Standard operating procedures (SOPs). All infections with live SARS-CoV-2 were strictly performed in the BSL-3 facility of the institute in accordance with the official authorization for work with SARS-CoV-2 by the Federal Government of Switzerland (BAG), permit #A202850/3.

Detemination of antiviral activity

Compounds were pre-diluted in a deep-well plate according to the dilution scheme (top graph below) in a way that afterwards the addition of a volume of 50µL will provide the final test concentration on the cells. Remdesivir (RDV) was included as established and validated activity control. After compound addition, cultures were transferred to the BSL-3 facility. After about 30 minutes of preincubation of cells and compound, 100 pfu of the DELTA strain (BS-01) of SARS-CoV-2 virus were added to each culture well.Subsequently, after an adsorption period of 15-30 minutes, every well was overlayed with low-melting agarose according to the corresponding SOP.Cultures were incubated at 37°C as described below to allow virus-induced plaques to form.After 48 h of incubation at 34°C. As cytopathic changes (CPE) develop within hours, the optimal time of harvest was determined by microscopic inspection, before paraformaldehyde (PFA) was added as fixative. Quantitative plaque formation served as proof for viral replication in the infectivity range (number of plaques = ca. 100 / well). The inhibitory potency was judged as plaque reduction at a given compound concentration. The IC_{50} for the RDV was at ca. 2.5µM, correlating with the reported activity.The plate on the right is a replicate plate with the same compound concentrations but with no virus added. The plate on the left (Fig. 1) displays the fixed and stained culture plate. Viral plaques were visualized as small white spots. Compound dilutions were from top to

bottom, and red lines indicate the respective compound concentration of the 50%-inhibition of plaque formation (IC_{50}).

Detemination of cytotoxicity

All compounds were pre-diluted in DMSO (research grade) to obtain stock concentrations of 20mg/mL. Further dilutions were done in culture medium (DMEM/2%FBS) until the final compound concentration as indicated.Cells were pre-seeded on day -1 as detailed in Fig.1 to allow adherence to the culture plate.

Compound dilution and dispensing was as described in Fig. 1 to obtain serial dilutions of each compound. This was to cover the entire anticipated biological activity range. DMSO concentrations on the cells were always below 0.5% final concentration to ensure full cell viability.A cell viability plate, using identical compound concentrations and cell count but without viral infection was included for each compound as control. The cytotoxicity was also assessed for the compound exposure for 48hrs.

Result

Results show that *B. abyssinica* extracts possess phenolic compouns, tannins, flavonoids and most extracts and fractions exhibit antioxidant activity. The methanolic and water extract showed high activity of active metabolites that are potential for pharmacological activity (Table 1).

However, phytochemical analysis of water extract by LC-MS/MS analysis revealed the presence of active metabolites ranging from phenols, coumarin and flavonoids (Table 2).

Investigations into the antiviral activity demonstrated that *B. abyssinica* has active metabolites with a high potential for inhibiting coronavirus, including the Delta variant, which shows higher virulence than its predecessors variants. Except for P2 and P5, all compounds demonstrated anti-SARS-CoV-2 (Delta) activity, with B2 showing plaque reduction only at the highest concentration of 50g/mL, whereas P1 and P3 showing plaque reduction at 16g/mL.

The water extract, B3 exhibited high inhibitory activity against Delta B1 by causing 75% viral death with no cytotoxicity effect on the cell, followed by the methanolic fraction of leaves (P4) where high activity was observed at a concentration of 16µg/mL than 50 µg/mL. The methanolic stembark and leaf fractions;B1 and P4 respectively showed a dose-dependent inhibition with significant activity in the absence of cytotoxicity also at 5 µg/mL(Fig. 2). The ethylacetate fraction of leaves (P1) showed activity against the virus by inhibiting at 50% compared to the petroleum ether extract of stem bark and leaves that showed no inhibition rate on Delta virus (Fig. 2). However, the cytotoxicity assay revealed no proliferation in all wells but sight toxicity was observed in ethylacetate stem bark fraction (B4) at a concentration of 50µg/mL(Fig. 1)

Table 1
Qualitative results of Selected Group of Secondary metabolites present in *B. abyssinica* extracts and fractions

Solvent	Plant part					
		Tannin	Phenol	Flavonoid	Saponin	Antioxidant
Petroleum ether	Stem bark	+	+	-	+	+
	Leaf	-	-	-	-	+
Dichloromethane	Stem bark	+	-	-	-	+
	Leaf	-	-	-	-	+
Ethyl acetate	Stem bark	-	+	+	+	+
	Leaf	-	+	+	+	+
Methanol	Stem bark	+	+	+	-	+
Water	Leaf	+	+	+	+	+
	Stembark+Leaf	+	+	+	+	+
Key: + = indicates presence of bioactive metabolites and - = indicates absence of bioactive metabolite						

Discussion

Herbal medicines have for long time been used for treatment of infectious diseases. *Bersama abyssinica* has been used in many African countries in traditional healing system. This plant possess a wide range of active metabolites including antioxidant [22] and some with antiviral activities [23]. In Tanzania, *B. abyssinica* is widely used for treatment of various infectious diseases including Covid-19. The plant concoction of stembark and leaves has been registered as Coviba Dawa in traditional medicine of Tanzania. This study revealed high amount of phenolic compounds in the dry water extract of stem bark and leaves (Coviba dawa), all with reported antioxidant activity that is potetential for viral inhibibion [24]. The antiviral screening revealed that *B. abyssinica* water extracts of stem and leaves exhibited high inhibitory activity against SARS-CoV-2 Beta B1 compared to methanolic, ethyl acetate and petroleum ether extract. High activity of water extract could be to high amount of polar compounds specially phenolic compounds with high antioxidant and antiviral activities [25]. The water extract of *B. abyssinica* yielded polyphenols especially the coumarin and flavonoids which are reported to have high antiviral activity against Corona virus [26]. High antiviral activity from water extract could be due to presence of 2,4-di-tert-butylphenol that is well studied with antiviral activity [27, 28] and strong antioxidant properties of 7,8-Dihydroxy-4-methylcoumarin and 2,3, 6-trimethoxyflavone [29, 30], enhancing viral inhibition. In addition, 4-formyl-2-methoxyphenyl propionate identified in water extract has various pharmacological uses including anticardiovasular and anti-inflammatory [31] that would enhance inhibition in SARS-CoV-2

Beta. Other studies revealed that plants and foods with antioxidants are used for treatment of early stages of Covid-19 [32, 33]. It wa also revealed that antioxidant combat viral infection through boosting immune system for fighting against SARS-CoV-2 [34]. Recent study in Egypt revealed that medicinal plants have possess high active metabolites for inhibition of Coronavirus [35]. More interestingly, the cytotoxicity screening of all extracts and fractions from this study revealed no proliferation on cell that justify the use of plant for medicinal purpose in Tanzania.

Conclusion

This study revealed the antiviral activity of *B. abyssinica* water extracts with no cytotocity effect on cell. However, isolation of compounds for further profiling and development, should be repeated with an independent preparation of the substance for activity verification, and possibly covering a broader concentration range with 2-fold dilutions. We also recommend further studies on other viral isolates of clinical concern such as SARS-CoV-2 Beta B1 and newer emerging viruses including SARS-CoV-2 Omicron that has recently been discovered in many countries.

Abbreviation

MUHAS- Muhimbili University of Health and Allied Sciences

SARS-Cov-2- Severe acute respiratory syndrome coronavirus-2

B1- *Bersama abyssinica* methanolic stembark fraction

P4- *Bersama abyssinica* methanolic leaf fraction

B2- *Bersama abyssinica* dichloromethane leaf fraction

P3- *Bersama abyssinica* dichloromethane stembark extract

B3- *Bersama abyssinica* water stem bark and leaf extract

B4- *Bersama abyssinica* ethylacetate stembark fraction

P1- *Bersama abyssinica* ethylacetate leaf fraction

P2- *Bersama abyssinica* petroleum ether fraction

P5- *Bersama abyssinica* petroleum ether leaf extract

Declarations

Ethics approval and consent to participate

-Not applicable in this study

Consent for publication

-Not applicable

Availability of data and materials

-Raw data are submitted with this manuscript as sumplementary materials

Competing interests

Authors declared that no competing interest exist.

Funding

-This study was funded by Bioinnovate Project through small Grant Evaluation and Validation of African Medicinal Plant: Bersama abyssinica for treatment of Covid-19 “ BA CI2017 10.”

Authors’ Contribution

NZ- drafted the proposal,won the funding project, designed the study and compiled data, BM and HN participated in proposal write up, extraction process and wrote first draft, RC and MK worked on bioassay analysis and revised the first draft, AK and JK advised the study design and revised the second draft of manuscript whereas MM and JC suggested the journal for submission and revised the third draft. However, all authors reviewed the manuscript..

Aknowldgement

Authors are thankful to BioInnovate Africa For funding this study. We are also thankful to technicians at MUHAS and Prof. Thomas Klimkait from Basel University is acknowledged for accepting to conduct bioassay activities in his lab.

Author’s Information

Never Zekeya (PhD), an expert in botany and traditional medicine, developed and commercialized herbal medicine namely; Coviba Dawa for treatment of Covid-19 symptoms.

References

1. Gangal N, Nagle V, Pawar Y, Dasgupta S. Reconsidering traditional medicinal plants to combat COVID-19. AIJR Preprints. 2020;34:1–6.
2. Chinazzi M, Davis JT, Ajelli M, Gioannini C, Litvinova M, Merler S, y Piontti AP, Mu K, Rossi L, Sun K, Viboud C. The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID-19) outbreak. Science. 2020 Apr 24;368(6489):395–400.
3. Hadjidemetriou GM, Sasidharan M, Kouyialis G, Parlikad AK. The impact of government measures and human mobility trend on COVID-19 related deaths in the UK. Transportation research interdisciplinary perspectives. 2020 Jul 1;6:100167.
4. Ren JL, Zhang AH, Wang XJ. Traditional Chinese medicine for COVID-19 treatment. Pharmacological research. 2020;155:104743.
5. Lim XY, Teh BP, Tan TY. Medicinal Plants in COVID-19: Potential and Limitations. Frontiers in pharmacology. 2021;12:355.
6. Ahmad S, Zahiruddin S, Parveen B, Basist P, Parveen A, Parveen R, Ahmad M. Indian medicinal plants and formulations and their potential against COVID-19–preclinical and clinical research. Frontiers in pharmacology. 2021;11:2470.
7. Khadka D, Dhamala MK, Li F, Aryal PC, Magar PR, Bhatta S, Thakur MS, Basnet A, Cui D, Shi S. The use of medicinal plants to prevent COVID-19 in Nepal. Journal of ethnobiology and ethnomedicine. 2021;17(1):1–7.
8. ul Qamar MT, Alqahtani SM, Alamri MA, Chen LL. Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. Journal of pharmaceutical analysis. 2020;10(4):313–9.
9. Vimalanathan S, Ignacimuthu S, Hudson JB. Medicinal plants of Tamil Nadu (Southern India) are a rich source of antiviral activities. Pharmaceutical Biology. 2009;47(5):422–9.
10. Aanouz I, Belhassan A, El-Khatabi K, Lakhlifi T, El-Ldrissi M, Bouachrine M. Moroccan Medicinal plants as inhibitors against SARS-CoV-2 main protease: Computational investigations. Journal of Biomolecular Structure and Dynamics. 202;39(8):2971-9.
11. Gyebi GA, Ogunro OB, Adegunloye AP, Ogunyemi OM, Afolabi SO. Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CLpro): an in silico screening of alkaloids and terpenoids from African medicinal plants. Journal of Biomolecular Structure and Dynamics. 2021;39(9):3396–408.
12. Zekeya N, Chacha M, Shahada F, Kidukuli A. Analysis of phytochemical composition of Bersama abyssinica by gas chromatography–mass spectrometry
13. Asres K, Bucar F, Kartnig T, Witvrouw M, Pannecouque C, De Clercq E. Antiviral activity against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) of ethnobotanically selected Ethiopian medicinal plants. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2001;15(1):62–9.

14. Amit L, Vikas G, Vaibhav T, Vikash K, Siddhartha G, Lather A. Phytochemistry and pharmacological activities of Bersama engleriana Guerke-An overview. International Research Journal of Pharmacy. 2010;1(1):89–10.
15. Zekeya N, Shahada F, Chacha M. In vitro antibacterial and antifungal activity of Tanzanian Bersama abyssinica. International Journal of Science and Research. 2014;3(7):1150–3.
16. Asian J. In-vitro antihelmentic evaluaion of leaf extract of bersama abyssinica (mellanthaceae) on Haemonchus contortus. Asian J Med Pharm Res. 2018;8(2):05–14
17. Mbaveng AT, Zhao Q, Kuete V. Harmful and protective effects of phenolic compounds from African medicinal plants. InToxicological Survey of African Medicinal Plants. Elsevier.2014;. 577–609.
18. Sinan KI, Chiavaroli A, Orlando G, Bene K, Zengin G, Cziáky Z, Jekő J, Fawzi Mahomoodally M, Picot-Allain MC, Menghini L, Recinella L. Biopotential of bersama abyssinica fresen stem bark extracts: UHPLC profiles, antioxidant, enzyme inhibitory, and antiproliferative propensities. Antioxidants. 2020;9(2):163.
19. Mansour MA, AboulMagd AM, Abdel-Rahman HM. Quinazoline-Schiff base conjugates: in silico study and ADMET predictions as multi-target inhibitors of coronavirus (SARS-CoV-2) proteins. RSC advances. 2020;10(56):34033–45.
20. Genwali GR, Acharya PP, Rajbhandari M. Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity. Nepal journal of science and technology. 2013;14(1):95–102.
21. GoveaSalas M, RivasEstilla AM, RodríguezHerrera R, LozanoSepúlveda SA, AguilarGonzalez CN, ZugastiCruz A, SalasVillalobos TB, MorlettChávez JA. Gallic acid decreases hepatitis C virus expression through its antioxidant capacity. Experimental and therapeutic medicine. 2016;11(2):619–24.
22. Bene K, Camara D, Kanga Y, Zirihi GN. Antiscavenging potential of leaves extracts from Bersama abyssinica Fresen.(Melianthaceae). International Journal of Biological and Chemical Sciences.11(6):2962–70.
23. Rotich W, Sadgrove NJ, Mas-Claret E, Padilla-González GF, Guantai A, Langat MK. HIV-1 Reverse Transcriptase Inhibition by Major Compounds in a Kenyan Multi-Herbal Composition (CareVid™): In Vitro and In Silico Contrast. Pharmaceuticals. 2021;14(10):1009.
24. Hosakote YM, Jantzi PD, Esham DL, Spratt H, Kurosky A, Casola A, Garofalo RP. Viral-mediated inhibition of antioxidant enzymes contributes to the pathogenesis of severe respiratory syncytial virus bronchiolitis. American journal of respiratory and critical care medicine. 2011;183(11):1550–60.
25. Gligorijevic N, Radomirovic M, Nedic O, Stojadinovic M, Khulal U, Stanic-Vucinic D, Cirkovic Velickovic T. Molecular Mechanisms of Possible Action of Phenolic Compounds in COVID-19 Protection and Prevention. International Journal of Molecular Sciences. 2021;22(22):12385.
26. El-Missiry MA, Fekri A, Kesar LA, Othman AI. Polyphenols are potential nutritional adjuvants for targeting COVID-19. Phytotherapy Research. 2021;35(6):2879–89.

27. Zhao F, Wang P, Lucardi RD, Su Z, Li S. Natural sources and bioactivities of 2, 4-di-tert-butylphenol and its analogs. Toxins. 2020; 12(1):35.
28. Leila A, Lamjed B, Roudaina B, Najla T, Taamalli A, Jellouli S, Mokhtar Z. Isolation of an antiviral compound from Tunisian olive twig cultivars. Microbial pathogenesis. 2019;128:245–9.
29. Kabeya LM, Fuzissaki CN, Andrade MF, Azzolini AE, Taleb-Contini SH, Vermelho RB, Lopes JL, Lucisano-Valim YM. 4-methylcoumarin derivatives inhibit human neutrophil oxidative metabolism and elastase activity. Journal of medicinal food. 2013;16(8):692–700.
30. Mohamed AA, Rateb ME, Jasapars M. Potency of Antioxidant Properties of Major Secondary Metabolite from Stevia rebaudiana in a Comparison to Synthetic Antioxidants. The Egyptian Journal Of Experimental Biology (Botany). 2017;13:151–8.
31. Gabriele E. Synthesis of New Sulfurated Derivatives of Natural and Synthetic Systems as Multitarget Anticancer Agents and Development of New Drug Discovery Methodologies.2017.PhD Thesis-AIR Unimi
32. Qin M, Cao Z, Wen J, Yu Q, Liu C, Wang F, Zhang J, Yang F, Li Y, Fishbein G, Yan S. An Antioxidant Enzyme Therapeutic for COVID-19. Advanced Materials. 2020 Oct;32(43):2004901.
33. Lapenna D. Antioxidant therapy in COVID-19: The crucial role of early treatment and antioxidant typology. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. 2021.
34. Trujillo-Mayol I, Guerra-Valle M, Casas-Forero N, Sobral MM, Viegas O, Alarcón-Enos J, Ferreira IM, Pinho O. Western dietary pattern antioxidant intakes and oxidative stress: importance during the SARS-CoV-2/COVID-19 pandemic. Advances in Nutrition. 2021;12(3):670–81.
35. Abdo N, Moheyeldin O, Shehata MG, El Sohaimy S. Inhibition of COVID-19 RNA-Dependent RNA Polymerase by Natural Bioactive Compounds: Molecular Docking Analysis. Egyptian Journal of Chemistry. 2021;64(4):1989–2001.

Table

Table 2 is only available as a download in the Supplemental Files section.

Figures

Figure 1

A 96-Well plate showing dilution and antiviral assay (left) and Cytotoxicity assay (right)

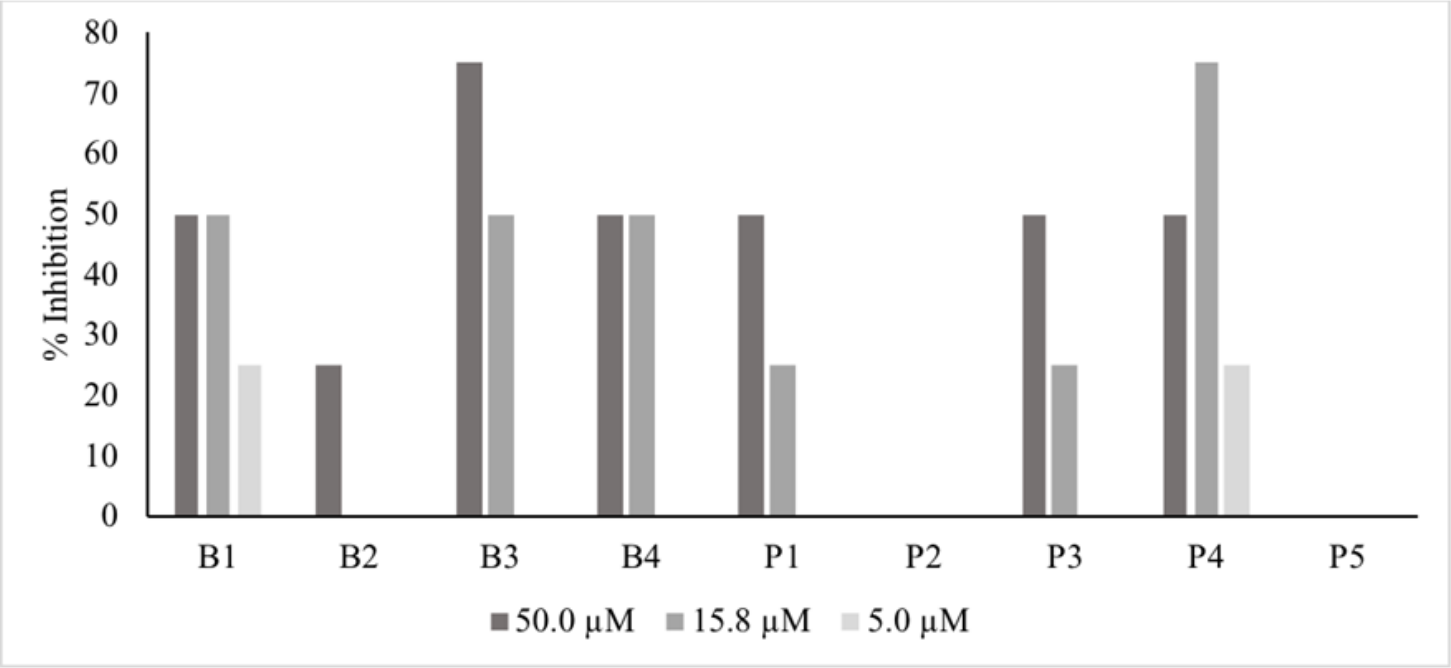

Figure 2

Effect of *B. abyssinica* extracts on inhibition of SARS-CoV-2 Delta B1

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- METABOLITESBIOASSAYSUPPLIMENTFILE.pdf
- SARCOV2BioassayNeverDaudi.pdf
- Table2.docx