Biomedical Communication

Bupleurum turcicum: A Rich Source of Saikosaponin A and D with Potential Use as Adjunct for the Management of Acute Respiratory Diseases: A Meta Review

Hani A. Alfheeaid,,[1,2] Bassam I. Alkhalifah,[3] Abdulrahman A. Alsayegh[4] Md Faruque Ahmad,[4] Mohammed Idreesh Khan[5] and Faiyaz Ahmed[5]

[1]Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia

[2]Human Nutrition, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, New Lister Building, Glasgow Royal Infirmary, Glasgow, United Kingdom

[3]Department of Radiology College of Medicine and Medical Sciences, Qassim University, Unaizah, Saudi Arabia

[4]Department of Clinical Nutrition, College of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia

[5]Department of Clinical Nutrition, College of Applied Health Sciences in Ar Rass, Qassim University, Ar Rass, Saudi Arabia

ABSTRACT

COVID-19 pandemic caused by SARS-CoV-2 has resulted in unprecedented havoc worldwide with significant morbidity and mortality. Till now, no effective antivirals are at disposal prompting researchers to explore potential lead molecules including from bioactive phytochemicals. An extensive literature search was carried out utilizing online resources; Google Scholar and PubMed to collect published reports on pharmacological potential of saikosaponin particularly in underexplored *Bupleurum* species. A number of molecular docking studies have reported promising antiviral effects of saikosaponins particularly of saikosaponin A, D, U and V with tremendous potential to be developed as anti-SARS-CoV-2 therapy. The search for potential sources of saikosaponin A and D led to the identification of *Bupleurum turcicum*; an unexplored, underutilized and endemic *Bupleurum* species. The observation that *B. turcicum* root extract contains highest amount of SSa and SSd among endemic Bupleurum species found in Turkey (Bupleurum sulphureum, *Bupleurum lycaonicum*, *Bupleurum turcicum*, *Bupleurum heldreichii, Bupleurum pauciradiatum*) and presence of significant amounts of antioxidant compounds led to the proposition of using *B. turcicum* extracts as adjust therapy in the management of COVID-19. The proposal also relies on the evidence of SSa and SSd being effective against a number of viruses including SARS-CoV. This review discusses phytochemical composition of *B. turcicum* root, antiviral, immunomodulatory and anti-inflammatory potential of saikosaponins in view of its plausible usefulness in the management of COVID-19. *B. turcicum* is an underutilized species rich in saikosaponin A and D with potential antiviral properties which could be effective alternative therapy in COVID-19 management..

KEY WORDS: ANTIVIRAL, BUPLEURUM TURCICUM, COVID-19, SAIKOSAPONINS, SARS-COV.

INTRODUCTION

SARS-CoV-2 (COVID-19) infection started as an outbreak of pneumonia of unknown origin in Wuhan City, China during late December 2019 and soon declared as a global epidemic by February 2020 (Lu et al. 2020; WHO 2020). Soon the sequencing of virus genome from patient samples and human to human transmission were confirmed by researchers (Zhu et al. 2020; Chan et al. 2020). Researchers began to explore novel therapeutic approaches to treat COVID-19 in view of its potential to rapidly develop into acute respiratory distress syndrome which can in some cases possibly cause multiple organ failure in the absence of effective drugs/vaccines (Patel and Jernigan 2020; Sahin et al. 2020; Gralinski and Menachery 2020).

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It is reported that during the outbreak of SARS-CoV in 2003, traditional herbal medicines used in conjunction with conventional medicine were effective in alleviating the symptoms of SARS (Lin et al. 2003; Xiao et al. 2003; Zhao et al. 2003; Zhong et al. 2003). Though the mechanism of action of those herbal products was not fully understood, the general consensus was that they contain antiviral molecules which might be acting by inhibiting viral replication (Vlietinck et al. 1991; McCutcheon et al. 1995; Jassim and Naji 2003). Therefore, exploring natural products with demonstrated antiviral efficacy against other coronaviruses is one of the therapeutic approaches. Since SARS-CoV-2 is a betacoronavirus which invades the host cell through angiotensin converting enzyme 2 (ACE2) receptor similar to SARS-CoV, it is assumed that antiviral biomolecules that are active against SARS-CoV can be repurposed to be used in COVID-19 patients (Cheng et al. 2006; Huanga et al. 2020; Shahrajabian et al. 2020).

In view of this, an extensive literature search was carried out utilizing online resources; Google Scholar and PubMed to collect published reports on herbal medicines and natural products with antiviral activity. The search was then tapered down to the natural compounds active against SARS-CoV wherein, saikosaponins widely distributed in *Bupleurum* spp., *Heteromorpha* spp. and *Scrophularia* spp were selected to explore further as saikosaponin A, B2, C and D have been reported to show significant antiviral activity against human coronavirus 229E *in vitro* (Cheng et al. 2006). The search was further narrowed down to *Bupleurum* spp. and *Bupleurum turcicum* was selected for this study since it contained highest levels of saikosaponin A and saikosaponin D amongst *Bupleurum sulphureum, Bupleurum lycaonicum, Bupleurum heldreichii*, and *Bupleurum pauciradiatum* (Kars et al. 2012; Huanga et al. 2020).

Bupleurum L. genus belongs to Apiaceae family which consists of around 200 species distributed across Northern Hemisphere, Eurasia, and North Africa. The plants are often annual or perennial herbs/shrubs up to 2 m tall with simple, entire and alternate leaves and the flowers are small, radical and yellowish (Xie et al. 2009). Many plants particularly roots from *Bupleurum* genus are used in traditional systems of medicine across China, Japan, Korea and Taiwan in the treatment of fevers associated with common cold and malaria, inflammation, hepatitis, diabetes, cancer and for wound healing, while the essential oils are used as anti-inflammatory and antiseptic agents (Nose et al. 1989; Motoo et al. 1994; Benito et al. 1998; Van-Wyk and Wink 2004; Wu 2005). *Bupleurum* species are used as analgesics in amenorrhea and cholecystitis. They also find their use in nephrotic syndrome, autoimmune diseases deafness, dizziness, dry throat, vomiting, diarrhea and hemorrhoids (Ashour and Wink 2011). These plants reportedly contain diverse classes of bioactive components including essential oils, alkaloids, flavonoids, coumarins, polysaccharides, lignans, triterpene saponins, phytosterols and polyacetylenes. WHO monographs list *Bupleurum* species as commonly used medicinal plants of China and Korea and also officially listed in Japanese and Chinese Pharmacopoeias (Ashour and Wink 2011; Huanga et al. 2020).

Bupleurum Bioactive Saikosaponins: Saikosaponins are triterpene oleanane saponin glycosides widely distributed bioactive compounds in *Bupleurum* spp amounting to up to 7% of the total dry weight in roots. Though saikosaponins are classified into seven types based on the type of aglycone (closely related oxygenated pentacyclic triterpenoidal structures), four different types namely saikosaponin-A (SSa), saikosaponin-B (SSb), saikosaponin-C (SSc) and saikosaponin-D (SSd) are considered as most commonly occurring and biologically active saikosaponins (Yuan et al. 2017). SSa, SSd and SSc are epoxy-ether saikosaponins designated as type I and SSb2 is a heterocyclic diene saikosaponin designated as type II (Lin et al. 2013; Huanga et al. 2020).

Plant extracts/herbal medicines containing saikosaponins are commonly prescribed as anti-inflammatory and anti-infectious medicine in Asian countries including China, Taiwan and Japan. More than 120 types of saikosaponins have been isolated from *Bupleurum* species till date and some of them have significant bioactivity both *in vitro* and in vivo (Kim 2018). Previous studies have reported antioxidant, antidepressant, anti-inflammatory, antimicrobial, antiviral, anticancer, anti-tumor and immunomodulatory effects of saikosaponins (Wu et al. 2008; Wu et al. 2010; Jin et al. 2013; Sui et al. 2011; Wu et al. 2011; Ying et al. 2014; Zhang et al. 2014; Huanga et al. 2020).

Bupleurum turcicum: *B. turcicum* belongs to Apiaceae family which mostly has flower bearing shrubs (Davis 1972). The essential oils from flowers, fruits and roots contain 39 distinctive compounds (Table 1). Heptanal, pentadecane and undecane were major compounds of flower and fruit essential oils. The flowers contained 33.2% heptanal, 19.6% pentadecane, 6.6% undecane, while fruits contained 23.5% heptanal, 13.4% pentadecane and 8.9% undecane. On an interesting note, essential oil from root did not contain heptanal, pentadecane or undecane which were major compounds of flowers and fruits. The major compounds of root essential oil were pentacosane (9%), 1-undecanol (8.8%) and hexacosane (8.0%) (Huanga et al. 2020).

The oils were evaluated for their antibacterial activity against *Staphylococcus aureus, Staphylococcus aureus, Escherichia coli, Escherichia coli, Bacillus cereus, Streptococcus salivarius, Pseudomonas aeruginosa, Pseudomonas aeruginosa* and *Proteus mirabilis*. It was found that the essential oils of flowers and roots did not show any activity against tested bacteria, while the oil obtained from roots exhibited good antibacterial activity which was comparable to that of chloramphenicol against *E. coli, B. cereus, S. salivarius* and *P. aeruginosa*. The observed phenomenon is attributable their chemical composition wherein, both fruits and flowers contained heptanal, pentadecane or undecane as major components which were not found in root samples. It was concluded that the essential oils from the roots of *B. turcicum* could be used as a potential source of novel antibacterial agents (Cheng et al. 2006; Kars et al. 2012; Saraçoğlu et al. 2012; Tykheev et al. 2020).

Table 1. Photochemical composition of *Bupleurum turcicum* essential oils

Compounds	Flowers (%)	Fruits (%)	Roots (%)	Compounds	Flowers (%)	Fruits (%)	Roots (%)
(E)-2-Decanal	0.4	0.5	0.9	Dodecanoic acid	0.4	0.8	
(E)-2-Nonenal	1.5	2.3	-	Farnesyl acetone	0.1	0.4	1.4
(E)-2-Octenal	0.5	0.2	-	Heneicosane	-	-	1.1
(E)-2-Undecanal	-	-	0.8	Heptacosane	0.3		3.6
(E)-Geranyl acetone	3.1	7.7	0.8	Heptadecane	0.8	0.8	0.6
(E)-Neralidol	-	0.5	-	Heptanal	33.2	23.5	-
(E)-β-Ionone	0.8	0.8		Heptanoic acid	1.0	0.7	-
(E,E)-2,4-Decadienal	0.8	0.7	0.8	Hexacosane	0.2	-	8.0
(Z)-Geranyl acetone	1.4	2.5	-	Hexadecane	0.2	0.2	0.4
1-Decanol	-	-	0.8	Hexadecanoic acid	1.9	2.8	0.2
1-Dodecanol	-	-	6.3	Hexahydrofarnesyl acetone	1.4	3.6	3.7
1-Heptadecene	0.5	0.5	-	Hexanal	0.9	0.5	-
1-Hexadecanol	0.3	0.7	-	Limonene	1.5	1.8	-
1-Octadecene	0.3	0.7	-	Nonacosane	0.2	-	2.4
1-Octen-3-ol	0.2	-	-	Nonadecane	-	-	0.5
1-Tetradecanol	-	-	2.2	Nonanal	0.3	0.4	0.2
1-Undecanol	-	-	8.8	Octacosane	0.1	-	3.9
2-Decyl acetate	-	-	4.5	Octadecane	0.2	-	0.5
2-Hexyl furan	0.3	0.3	-	Octanal	0.5	0.5	-
2-Octanone	0.3	-	-	Pentacosane	0.2	-	9.0
2-Pentyl furan	1.3	1.0	-	Pentadecane	19.6	13.4	3.0
2-Undecanone	0.3	0.7	0.5	Phytol	-	1.0	-
3,4-Dimethyl-5-Pentyl-5 HFuran-2-one	0.4	-	-	Spathulenol	3.5	5.9	6.3
4,8-Dimethyl-1,3,7-nonatriene	0.2	-	-	Tetracosane	0.2	-	5.4
6-Methyl-5-hepten-2-one	-	0.3	-	Tetradecane	0.3	-	-
Aristolene	-	0.2	-	Tetradecane	-	0.2	0.5
Benzyl salicylate	-	-	1.8	Tetradecanoic acid	0.6	2.6	-
Bornyl acetate	-	-	0.2	Tricosane	-	-	3.3
Caryophyllene oxide	3.5	2.4	-	Tridecane	1.8	1.9	1.8
Cuparene	-	-	0.5	Undecanal	-	-	0.5
Decanal	-	-	0.4	Undecane	0.6	8.9	5.5
Decane	-	-	0.1	α-Pinene	0.8	2.0	-
Dihydroedulan II	-	0.2	-	β-Caryophyllene	0.5	-	-
Docosane	-	-	1.4	β-Elemene	0.2	0.8	-
Dodecanal	-	-	0.4	γ-Muurolene	1.6	-	-

*Source: (Cheng et al. 2006; Kars et al. 2012; Saraçoğlu et al. 2012; Tykheev et al. 2020)

In an important study, the levels of phenolic compounds (catechin, quercetin, isoquercitrin), SSa, SSd and podohyllotoxin were determined in the root extracts of *B. sulphureum, B. lycaonicum, B. turcicum, B. heldreichii* and *B. pauciradiatum* using HPLC. *B. turcicum* was found to contain significant amounts of total phenolics (34.48 mg GAE/g extract), catechin (0.11 mg/g extract), quercetin (0.21 mg/g extract) and isoquercitrin (1.85 mg/g extract). Furthermore, *B. turcicum* root extract contained highest amounts of SSa (12.99 mg/g extract) and SSd (17.96 mg/g extract) among all the other extracts investigated (Huanga et al. 2020; Shahrajabian et al. 2020).

B. turcicum root extract exhibited significant free radical scavenging activity with lowest IC50 value (57.3 µg/mL) amongst other extracts in DPPH radical scavenging assay.

In antiproliferative assay, *B. turcicum* root extract exhibited potent activity against sensitive and drug resistant MCF-7 cells (Kars et al. 2012). The observation that *B. turcicum* root extract contains highest amount of SSa and SSd among endemic *Bupleurum* species also found in Turkey (*B. sulphureum, B. lycaonicum, B. turcicum, B. heldreichii, B. pauciradiatum*) and presence of significant amounts of antioxidant compounds led to the proposition of using *B. turcicum* extracts as adjust therapy in the management of COVID-19. The proposal also relies on the evidence of SSa and SSd being effective against a number of viruses including SARS-CoV (Huanga et al. 2020; Shahrajabian et al. 2020).

Antiviral properties of saikosaponins: Recent studies have evaluated the anti-SARS-CoV-2 effects of different saikosaponins found in *Bupleurum* Spp. using advanced molecular docking techniques and have found promising results with potential to be studied further with respect to saikosaponins A, D, U and V. Similarly, in the experimental setup saikosaponins isolated from Bupleurum spp. have been shown to possess potent antiviral activity against a number of viruses including herpes simplex virus, influenza virus (IAV), hepatitis B virus (HBV), hepatitis C virus (HCV), measles and varicella zoster viruses (Ashour and Wink 2011). Based on these findings, saikosaponins from *Bupleurum* Spp. have been proposed to be repurposed for the treatment of COVID-19 (Bahbah et al. 2020).

In a molecular docking study, SSa has been shown to possess significant affinity towards binding ACE 2 receptors through which SARS-CoV-2 infect lung epithelial cells (Yan et al. 2020). Another molecular docking simulation study showed that Saikosaponins exhibit high affinity towards RBD region of the spike glycoprotein of SARS-CoV (Goswami and Bagchi 2020). Another molecular docking study conducted to evaluate the affinity of Saikosaponins towards SARS-CoV-2 binding protein showed, Saikosaponins U and V to exhibit strong affinity towards SARS-CoV-2 binding protein. The study concluded that Saikosaponins U and V could be future research molecules for SARS-CoV-2 research (Sinha et al. 2020).

A study conducted to evaluate the antiviral and immunoregulatory activities of SSa and SSd against Porcine Coronavirus 2 (PCV2) showed that saikosaponins reduced the incidence and severity of PCV2-induced immunopathological damage in terms of pyrexia, weight loss, anemia, and internal organ oedema in mice. Immunoglobulin and protein absorption levels were also affected suggesting immunoregulatory effect of saponins (Yang et al. 2017). In another study, antiviral activity of saikosaponins against herpes simplex type I (HSV-1), vesicular stomatitis virus (VSV) and poliovirus type 1 was evaluated *in vitro* at non-cytotoxic concentrations. Buddlejasaponin 4 was found to be potent against vesicular stomatitis virus (Bermejo et al. 2002; Shahrajabian et al. 2020).

Ushio and Abe evaluated the virus neutralizing effect of SSd against measles virus and herpes simplex virus *in vitro* (Ushio and Abe 1992). SSd at above 5 µM concentration rendered measles virus and herpes simplex virus (HSV) completely non-infective when incubated together for 10 min at room temperature. Lin et al (2015) evaluated the antiviral activity of SSa, SSb_2, SSc, and SSd against cultured hepatitis C virus (HCV) *in vitro*. The effect of saikosaponins on virus entry, RNA replication/translation and particle production were studied using different HCV genotypes, clinical isolates and infection of primary human hepatocytes. All saikosaponins were found to exhibit potent inhibition of HCV infections even at non-cytotoxic concentrations by targeting early steps of the viral life cycle. SSb_2 was found to significantly prevent virus entry by neutralizing virus particles, preventing their attachment and inhibiting viral entry/fusion.

SSb_2 also inhibited other genotypic strains and prevented HCV binding onto hepatoma cells thereby blocking HCV infection of primary human hepatocytes. The study proposed further research into SSb_2 to develop it as an HCV entry antagonist. These finding were reiterated by another study in 2019, wherein SSb2 was found to inhibit viral entry, replication, and translation of hepatitis C virus (HCV) in a cell culture-derived HCV system *in vitro*. SSb_2 also inhibited daclatasvir-resistant mutant strains of HCV when used in combination with daclatasvir indicating potential antiviral effects of SSb_2. Antiviral activity of SSa, SSc and SSd against anti-hepatitis B virus (HBV) was evaluated *in vitro* using HBV-transfected human hepatoma cells (Lee et al. 2019; Shahrajabian et al. 2020).

SSc was found to inhibit DNA replication of HBV which was higher than that of lamivudine- a known antiviral drug in clinical use (Chiang et al. 2003; Chang et al. 2007). In another study SSd showed significant antiviral activity against HBV through inhibition of HBV-DNA replication (Yin et al. 2008). The anti-viral activity of SSa was evaluated against influenza A virus (IAV) infections *in vitro* and *in vivo*. SSa reduced replication of three different influenza A virus strains, including a H5N1 strain, in human alveolar epithelial A549 cells by downregulating NF-κB signaling and caspase 3-dependent virus ribonucleoprotein nuclear export. SSa decreased viral replication, production of pro-inflammatory cytokines in H1N1 PR8 model of influenza A virus lethality in C57BL/6 mice. SSa also attenuated lung neutrophil and monocyte recruitment during the early stages of immune response to PR8 infection (Chen et al. 2015; Shahrajabian et al. 2020).

The antiviral activities of saikosaponins against human coronavirus (HCoV), which cause severe acute respiratory syndrome (SARS), were also studied using 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino) carbonyl-2H-tetrazolium hydroxide] (XTT) assay *in vitro*. Results indicated both SSa and SSb2 showed significant antiviral activity against HCoV at concentrations of 0.25-25 mmol/L. Both saikosaponins exhibited no cytotoxic effects on target cells at tested concentrations. The antiviral activity of SSb2 was more significant than that of SSa and mediated by inhibition of attachment and penetration of the virus to target cells (Cheng et al. 2006; Shahrajabian et al. 2020).

Immunomodulatory and anti-inflammatory activity of saikosaponins in view of COVID-19: SARS-CoV-2 much like Influenza A virus (IAV) can cause severe pneumonia resulting in morbidities and mortalities (Chen et al. 2015). Generally, infection starts in the upper respiratory tract epithelial cells and spreads aggressively into deeper regions of the lung parenchyma and might also enter into macrophages and dendritic cells (Spiegel et al. 2006; Manicassamy et al. 2010; Shahrajabian et al. 2020).

The infected cells release pro-inflammatory cytokines (IL2, IL7, IL10, IP10 and TNFα) and chemokines (MIP-1a and MCP1). Although these responses are important for controlling viral replication in the initial phase of infection through recruitment of immune cell into lungs, excessive pro-inflammatory components raise the levels of cytotoxic and pro-apoptotic products which damage lung tissue (Herold et al. 2008). Thus, natural products with anti-inflammatory and immunomodulatory properties could be useful in the management of COVID-19 (Huanga et al. 2020). Kumazawa and coworkers investigated macrophage activation potential of SSa and SSd in mice. Intraperitoneal injection of saikosaponins induced a dose dependent activation of peritoneal macrophages leading to enhanced phagocytic activity, increased cellular lysosomal levels, induction of cytostatic activity and expression of Ia antigen on the cell surface. Authors opined that SSd could be a potent macrophage activator as it showed significantly higher activity compared to ginsenoside Rg1 and glycyrrhizin. Furthermore, SSd also modulates lymphocyte activity through suppression of T-cell and induction of B-cell response to different mitogens and up-regulates IL-2, IL-4 production in thymocytes through post-receptor signal transduction (Kumazawa et al. 1989; Ushio and Abe 1991; Kato et al. 1995; Lin et al. 2015; Huanga et al. 2020).

Wong and coworkers reported *in vitro* suppression of OKT3/CD28-costimulated human T cell proliferation and inhibition of PMA, PMA/Ionomycin and Con A-induced mouse T cell activation by saikosaponin D *in vitro*. Examination of T cell activation signaling pathways suggested that saikosaponin inhibits T cell activation downregulating CD69, CD71 expression and IL-2 production through modulation of PKC pathway via PKCh, JNK, and NF-kB transcription factors. It also downregulates CD25, IL-6, TNFα and IFNγ through NF-kB, NF-AT and AP-1 (c-Fos) signaling pathways (Wong et al. 2009). These observations indicate that SSd could be a potential molecule with immunomodulatory functions (Leung et al. 2005; Huanga et al. 2020).

SSa was found to inhibit inflammatory factors such as cyclooxygenase-2 (COX-2) and inducible nitric-oxide synthase (iNOS) and production of pro-inflammatory cytokines TNFα, IL1β and IL6 in an experimental model of inflammation (lipopolysaccharide (LPS)-stimulated RAW 264.7 cells). The study concluded that SSa exhibits significant anti-inflammatory activity through regulation of inflammatory mediators and suppression of MAPK and NF-κB signaling pathways (Zhu et al. 2013). These finding were reiterated in another study, wherein SSa significantly inhibited COX-2, iNOS, tumor necrosis factor-α, IL1β and IL6 in an obesity experimental model using mouse embryo fibroblast 3T3-L1 cells. The study also suggested SSa to be a potential therapeutic agent against obesity associated inflammation (Kim et al. 2015). SSa and SSd are reported to exhibit potent anti-inflammatory activity by inhibiting production of nitric oxide induced by lipopolysaccharide in BV-2 microglial cells. The inhibitory effect of both SSa and SSd were comparable to that of dexamethasone (Wang et al. 2017; Huanga et al. 2020).

In vivo anti-inflammatory activity of SSa and SSd were evaluated in female albino rats by granuloma pouch method and antigranulomatous action by cotton pellet method. Oral administration of SSa and SSd showed significant anti-inflammatory effect without affecting hematocrit and plasma-11-OH-corticosteroid levels (Yamamoto et al. 1975). The anti-inflammatory effect of saikosaponins were evaluated in mice paw oedema model, wherein oedema was induced by injecting formalin into hind par and paw oedema index was used as a measure of anti-inflammatory effect.

Saikosaponins were found not only to decrease paw oedema but also decreased inflammatory metabolites including nicotinate, niacinamide, arachidonic acid (AA), and 20-carboxy-leukotriene B4 as evidenced by HPLC metabolomic study. The study concluded that saikosaponins exert their anti-inflammatory effect through regulation of nicotinate and nicotinamide and arachidonic acid metabolism (Ma et al. 2016). The saikosaponins have also been reported to exhibit anti-inflammatory effect in TPP-induced ear oedema model in rats (Recio et al. 1995). In experimental model of cigarette smoke induced lung inflammation in mice, SSa was found to inhibit inflammatory cell infiltration, nitric oxide production, TNF-α, and IL-1β production, MPO and MDA levels in lung tissues (Chen et al. 2018; Huanga et al. 2020).

CONCLUSION

The findings of the present study suggests that *B. turcicu*m is an unexplored and underutilized species of genus *Bupleurum* L which is a rich source of saikosaponin A and D having potential antiviral properties against a number of virus including IAV, HBV, PCV-2, SARS-CoV and SARS-CoV-2. Further phytochemical characterization and standardization of *B. turcicum* root extract might be beneficial in finding safe and effective alternative therapy with respect to COVID-19.

Data Availability Statement: The database generated and /or analysed during the current study are not publicly available due to privacy, but are available from the corresponding author on reasonable request.

Conflict of Interests: Authors declare no conflicts of interests to disclose.

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