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Research paper

A phylogenetic perspective of antiviral species of the genus *Artemisia* (Asteraceae-Anthemideae): A proposal of anti SARS-CoV-2 (COVID-19) candidate taxa

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ABSTRACT

Introduction: Different classes of disease-causing viruses are widely distributed universally. Plant-based medicines are anticipated to be effective cures for viral diseases including the COVID-19, instigated by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). This study displays the phylogenetic perspective of *Artemisia* and proposes some candidate taxa against different viral diseases, including SARS-CoV-2.

Methods: Data of *Artemisia* with antiviral activity were obtained from different published sources and electronic searches. A phylogenetic analysis of the nrDNA ITS sequences of reported antiviral *Artemisia* species, along with the reference species retrieved from the NCBI GenBank database, was performed using the maximum likelihood (ML) approach.

Results: In total, 23 *Artemisia* species have been documented so far with antiviral activity for 17 different types of viral diseases. 17 out of 23 antiviral *Artemisia* species were included in the ITS phylogeny, which presented the distribution of these antiviral *Artemisia* species in clades corresponding to different subgenera of the genus *Artemisia*. In the resultant ML tree, 10 antiviral *Artemisia* species appeared within the subgenus *Artemisia* clade, 2 species appeared within the subgenus *Absinthium* clade, 3 species appeared within the subgenus *Dracunculus* clade, and 2 species appeared within the subgenus *Seriphidium* clade.

Discussion: Artemisia species from different subgenera with antiviral activity are prevalent in the genus, with most antiviral species belonging to the subgenus *Artemisia*. A detailed analysis of taxa from all subgenera, particularly the subgenus *Artemisia*, is therefore proposed in order to discover compounds with potential anti-SARS-CoV-2 activity.

1. Introduction

Asteraceae is the largest eudicot angiosperm family and includes many species with medicinal and economic significance. *Artemisia* L. is the leading genus of this family, with ~500 species occurring commonly in the north hemisphere (Oberprieler et al., 2009; Bora and Sharma, 2011). However recently in the Plant List and World Flora Online, almost 2200–2300 species of the genus *Artemisia* have been stated. Abundant secondary metabolites retrieved from *Artemisia* extracts are used to treat certain health problems, such as stress, anxiety, depression, epilepsy, irritability, insomnia, and psychoneurosis (Walter et al., 2003). Many *Artemisia* species are reported with antimalarial, antibacterial, antirheumatic, antiseptic, antispasmodic, hepato-protective, antitumor (Terra et al., 2007; Koul and Taak, 2017; Hussain et al., 2017, 2022;

Abbreviations: BVD, Bovine viral diarrhea virus; DEN 2, Dengue virus type 2; FCV, Feline calci virus; FIV, Feline immunodeficiency virus; HBV, Hepatitis B virus; HeLa, Henrietta Lacks cells; HCV, Hepatitis C virus; HHV (HSV), Human alphaherpesvirus (Herpes simplex virus); HHV-4 (EBV), Human gammaherpesvirus type 4 (Epstein-Barr virus); HIV-, Human immunodeficiency virus; HBeAg, Hepatitis B e-antigen; HBsAg, Hepatitis B surface antigen; IV, Influenza virus; JUN V, Junin virus; MDBK, Madin-Darby bovine kidney cells; MDCK, Madin-Darby canine kidney cells; MTTA, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay; MNV, Murine norovirus; NDV, Newcastle disease virus; SARS CoV2, Severe acute respiratory syndrome corona virus 2; SV, Sindbis virus; PV, Polio virus; VERO, Verda reno cells; YFV, Yellow fever virus.

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Pandey and Singh, 2017; Mohammed et al., 2021 and references therein), antidiabetic (Dabe and Kefale, 2017), antioxidant, cytotoxic (Madhav et al., 2018; Cheraif et al., 2020; Jakovljevíc et al., 2020; Melguizo-Melguizo et al., 2020) and antiviral activities (Li et al., 2005; Romero et al., 2006; Efferth et al., 2008; Kim et al., 2018; Nie et al., 2021 and references therein). It is believed that compounds extracted from the plants are responsible for antimicrobial activity and, in the former inquiries, various other biological activities have also been validated (Mathlouthi et al., 2018, 2021). In the past, the majority of these plants have been utilized against microbial infections. Nevertheless, a preliminary documented awareness in plant utilization as antiviral candidates was made in Nottingham, England by a drug company (Boots Drug Company), where 288 plants were screened against the influenza virus (Chantrill et al., 1952). After that, further investigations on the inhibitory properties of alcoholic or water-soluble plant extracts against replication of numerous viruses were acknowledged. Predominantly, the effect on emerging viral diseases linked with SARS and pox virus (Kotwal et al., 2005) was reported. The effects of plant extracts on other viruses, including Human immunodeficiency virus type 1 (HIV-I) and type II (HIV-II) (Asres and Bucar, 2005), Human alphaherpesvirus (Herpes simplex virus type 2) (HHV-HSV-2) (Debiaggi et al., 1988) and Hepatitis B virus (HBV) (Kwon et al., 2005) were also accredited. Current inquiries have shown that plant extracts have promising antiviral results, even against those viruses which resist synthetic or conventional antiviral drugs (Tolo et al., 2006), thus challenging the contemporary drug discovery patterns and turning researches attention towards the search of natural antiviral constituents from plant sources.

In recent times, the viral disease Severe acute respiratory syndrome corona virus type 2 (SARS-CoV-2) (Phan et al., 2020) has instigated a COVID-19 pandemic (Huang et al., 2020), with increasing death tolls and grave societal and economic troubles that call for immediate availability of potential antiviral treatments to keep the population safe (Mitjà and Clotet, 2020).

From the 2002–2003 outbreak of SARS-CoV in China, some pertinent evidence exists concerning anti SARS-CoV treatments. Particularly, Li et al. (2005) tested an *in vitro* antiviral activity of *Artemisia annua* L. whole plant ethanolic extracts and found them to be 50 % effective against SARS-CoV. Their outcomes strongly support the utilization of *A. annua* for the treatment of SARS-CoV disease. More evidence from China has confirmed that the natural compounds from traditional herbs are operative for the treatment of SARS-CoV (Lin et al., 2003). Considering these results, it is urgently needed to check the safety profiles and then again approval of *A. annua* based therapies against COVID-19. Natural products present in *A. annua* plant are useful against various types of viruses, including Bovine viral diarrhea virus (BVDV), Human gammaherpesvirus type 4 (Epstein–Barr virus) (HHV-4 (EBV), Hepatitis B virus, Human alphaherpesvirus (herpes simplex virus type 1) HHV (HSV-1) and Hepatitis C virus (HCV) (Efferth et al., 2008) Moreover, research on antiviral activities of different *Artemisia* species including *A. annua* is continuing globally with promising results. Therefore, the foremost objective of this study was to compile data on reported antiviral *Artemisia* species and determine their phylogenetic relationships, which could be helpful for identifying species with possible antiviral bioactivity for further consideration. The number of *Artemisia* taxa with antiviral activities was highlighted on a comprehensive phylogeny of the genus to deliver essential baseline data for the utilization of *Artemisia* taxa against viral diseases including the SARS-CoV2.

2. Materials and methods

2.1. Data collection of Artemisia plants with antiviral activity

Data of *Artemisia* species with reported antiviral activity was attained from published sources including scientific journals, reports, books, theses, conference papers, and an electronic search of Biological Abstracts, BIOSIS, BioOne Previews, CabDirect, Cochrane Library, Pubmed/Medline, GeoRef, Google Scholar, JSTOR, Journal Citation Reports, Mendeley, Publons, Researchgate, Scopus, SciELO, Springer Link, Science Direct, Web of Science, Taylor and Francis. The keywords used to search in the aforementioned databases include "*Artemisia* plants, antiviral activity, Artemisinin, *A. annua*, Antiviral compounds in *Artemisia*". For plant synonyms and accepted names, The Plant List (www.theplantlist.org) database was searched. About 125 articles were looked over and some were designated as providing wide-ranging data of antiviral activity of *Artemisia* species. The compiled data of antiviral activity of *Artemisia* species is provided in Table 1, including region, extraction solvent and plant part used, investigated cell lines, virus type or stain, viral assay used against specific virus and active compounds tested against viral strains.

2.2. Phylogenetic analysis of Artemisia with ITS sequences

For the phylogenetic analysis, ITS sequences of nuclear ribosomal DNA (nrDNA) for a total of 147 *Artemisia* species, including the antiviral *Artemisia* species compiled (Supplementary file 1) for this study, were retrieved from GenBank, signifying all subgenera of the genus *Artemisia* revealed in earlier studies on the phylogeny of *Artemisia* by Torrell et al. (1999), Vallès et al. (2003), Tkach et al. (2008), Pellicer et al. (2010), Garcia et al. (2011), Pellicer et al. (2011), Riggins and Seigler (2012), Hobbs and Baldwin (2013), Malik et al. (2017), Pellicer et al. (2018) and Hussain et al. (2019). The raw data of sequences retrieved were assembled with MEGA-7 software (Kumar et al., 2016). A multiple sequence alignment (MSA) (n = 147) was generated from ITS sequences of *Artemisia* species. *Chrysanthemum dichroum* (C.Shih) H. Ohashi & Yonek., *Chrysanthemum indicum* L., and *Ajania fastijiata* (C.Winkl.) Poljakov were used as outgroup species from the same tribe using their ITS sequences (Hussain et al., 2019). The ITS sequences were edited with the BioEdit v.7.0.9 software (Hall, 1999) and then CLUSTAL X (Thompson et al., 1997) program in MEGA-7 software (Kumar et al., 2016) was used to align the sequences with some manual modifications for gaps. Using MEGA-7 software (Kumar et al., 2016), the maximum likelihood (mL) analysis was generated for the MSA (n = 147) with 1000 bootstrap (BS) replicates. The resultant tree was visualized in the software FigTree 1.4.3. (2018).

3. Results

Results showed that a total of 23 species of *Artemisia* were reported so far for the treatment and management of viral diseases and have potential antiviral activity. The antiviral activity is due to the presence of biologically active compounds with known mechanisms of action (Table 1). Among the reported *Artemisia* species, methanol, ethanol and aqueous extracts of *A. annua* have proven to be extensively used against different viruses including SARS-CoV-2, HBV, BVDV, HHV (HSV-1), HIV-1 and Influenza virus type A (IV-A), due to artemisinin, an active antimalarial as well as an antiviral compound. Methanol and aqueous extracts and tea infusion of *Artemisia afra* Jacq. Ex Willd. leaves were used against HIV-1, HIV-2 and SARS-CoV-2. Methanol extracts of *Artemisia abyssinica* Schultz Bip ex Richard. were tested against HIV-1 and HIV-2 with better results. Methanol and aqueous extracts of *Artemisia absinthium* L. were used against HHV (HSV-1), Sindbis virus (SV), Polio virus (PV), HIV-1 and HBV. Aqueous extracts of *Artemisia arborescens Vaill. L.* leaves and aerial parts were reported with promising antiviral activity against HHV (HSV). *Artemisia campestris* L. and its sub species' methanol, ethanol and aqueous extracts were described with anti-HHV (HSV-1) and anti-HIV-1 activities. The extracts from whole plant, buds and aerial parts of *Artemisia capillaris* Thunb. were recognized with potential anti-humanherpes virus type 4 (HHV-4), HIV and HBV activities.

Moreover, the methanol extracts of aerial parts of *Artemisia chamaemelifolia* Vill. were found to be active against HHV (HSV-1). Methanol extracts from whole plant and aerial parts of *Artemisia caruifolia* Roxb. were active antiviral agents against HHV (HSV-1) and IV-A

Table 1
Reported *Artemisia* species used against different viral diseases in different regions of the world.

Artemisia taxa	Region	Extract used	Part used	Culture cells	Virus typpe/ strain	Assay applied	Active compound	Effective concentration (IC_{50}/ ED_{50}/EC_{50}/CC_{50})	Reference
A. annua L.	Korea	Methanol	ND	T-lymphocytes	HIV-1	Syncytium inhibition assay	ND	100 μg/mL	Chang and Woo (2003)
	China	Ethanol	Whole plant	Vero E6 /HEPG2	SARS-CoV/ BJOO1, BJOO6	CPE/MTS assay	ND	1053.0 μg/mL	Li et al. (2005)
	Spain	ND	ND	EBTr	BVDV	Cytopathic assay	Artemisinin with mixture of interferon-α and ribavirin	100 mmol/L	Romero et al. (2006)
	ND	ND	Plant powder	Cardiac	HBV, HCV and BVDV	ND	Artemisinin	ND	Efferth et al. (2008)
	Iran	Methanol	Aerial	HeLa	HHV (HSV-1)	MTT assay	ND	12.5 μg/mL	Karamoddini et al. (2011)
	Africa	Tea infusion	Leaves	FIGS- and deCIPhR	HIV-1	Cellular toxicity assay	Artemisinin	ND	Lubbe et al. (2012)
	China	Aqueous	Whole plant	HeLa	IV-A (FM1)	Toxicity test	ND	3.90 mg/mL	Tao et al. (2020)
	Germany	Aqueous	Leaves	Vero E6	SARS-CoV-2	Plaque reduction and cell viable assay	ND	0.01–10 mg/mL	Nie et al. (2021)
A. afra Jacq. ex Willd.	Ethiopia	Methanol	Aerial	MT-4	HIV-1 (III_B), HIV-2 (ROD)	Anti-HIV cytotoxic assay	ND	> 123.5 mg/mL	Asres et al. (2001)
	Africa	Tea infusion	Leaves	ND	HIV-1	Cellular toxicity assay	ND	ND	Lubbe et al. (2012)
	Germany	Aqueous	Leaves	VeroE6 cells	SARS-CoV-2	Plaque reduction and cell viable assay	ND	0.01–10 mg/mL	Nie et al. (2021)
A. abyssinica Schtz. Bip ex A. Richard.	Ethiopia	Methanol	Aerial	MT-4	HIV-1 (III_B), HIV-2 (ROD)	Anti-HIV cytotoxic assay	ND	> 103 mg/mL	Asres et al. (2001)
A. absinthium L.	ND	ND	ND	ND	HIV-1	ND	ND		
	Morocco	Methanol	Aerial	Vero	HHV (HSV-1), SV and PV	Antiviral photosensitizers activity	ND	100 μg/mL for SINV and HSV and 200 μg/mL for PV	Mouhajir et al. (2001)
	India	Aqueous	Whole plant	HBsAg and HBeAg, Plasma	HBV	Loss of HBsAg and HBeAg, plasma HBV DNA level	ND	ND	Ansari et al. (2018)
A. arborescens (Vaill.) L.	Italy	ND	Leaves	Vero	HHV (HSV-1)	Tetrazolium-based colorimetric assay	Essential oil	ND	Sinico et al. (2005)
	Italy	Aqueous	Leaves	Vero	HHV (HSV-1) and HSV-2	Plaque reduction assay, MTT assay	ND	2.4 and 5.6 μg/mL for HSV-1 and 4.1 and 7.3 μg/mL for HSV-2	Saddi et al. (2007)
	Italy	Aqueous	Aerial	Vero	HHV (HSV-1)	MTT assay	ND	100 μg/mL	Lai et al. (2007)
A. campestris L.	Iran	Methanol	Aerial	HeLa	HHV (HSV-1)	MTT assay	ND	6. 25 μg/mL	Karamoddini et al. (2011)
A. campestris subsp. *glutinosa* (Besser) Batt.	Spain	Ethanol and aqueous	Aerial	Lymphoblastoid	HIV-1	Transcriptional activity test	Damsin, canrenone, 6, 2, 4–trimethoxyflavon, acerosin, cardamonin and xanthomicrol	14.62 μg/mL	Ticona et al. (2020)
A. capillaris Thunb.	Japan	ND	Buds	Rat hepatocytes	Hepatitis (anti-hepatotoxicity activity)	Cytotoxicity assay	Eupatolitin, arcapillin, chrysoeriol, esculin, scopolin, isoscopoletin *O*-glucoside	ND	Kiso et al. (1984)
	ND	ND	ND	ND	HHV-4 (EBV)	ND	Capillin	ND	Dembitsky and Levitsky (2006)
	China	ND	Aerial	H9 lymphocytic	HIV	Anti HIV replication assay	Isorhamnetin, arcapillin, aesculetin	ND	Wu et al. (2001)
	Korea	Ethanol	Whole plant mixture (KCT01)	HepG2.2.15	HBV	Hydrodynamic injection model	ND	500 μg /kg and 200 μg /kg	Kim et al. (2018)
A. chamaemelifolia Vill.	Iran	Methanol	Aerial	HeLa	HHV (HSV-1)	MTT assay	ND	12.5 μg/mL	

(*continued on next page*)

Table 1 (*continued*)

Artemisia taxa	Region	Extract used	Part used	Culture cells	Virus typpe/ strain	Assay applied	Active compound	Effective concentration (IC_{50}/ ED_{50}/EC_{50}/CC_{50})	Reference
									Karamoddini et al. (2011)
	Bulgaria	Aqueous	Aerial	MDBK	HHV (HSV-2) strain BA	MTT assay	ND	0.562 mg/mL	Angelova et al. (2019)
A. caruifolia Roxb.	Nepal	Methanol	Whole plant	Vero cells/ MDCK	HHV (HSV-1), IV-A	Cytotoxicity Assay/ dye uptake assay	ND	92 mg/mL for HSV-1 and 22 mg/mL for IV-A	
	China	Methanol	Aerial	HIV-1 protease	HIV-1	HIV PR assay	Tri-*p*-coumaroyl spermidine and dicaffeoylquinic acids	100 μg/mL	Ma et al. (2001)
A. douglasiana Bess.	Argentina	Aqueous	Leaves	Vero	HHV (HSV-1), JUNV, DEN-2	Plaque formation assay	α-thujone β-thujone, borneol, *p*-cymene,1.8-cineole, isocaryophylene-epoxide	65–125 ppm for HHV (HSV-1) and 60 and 150 ppm for DEN-2	García et al. (2003)
A. fragrans Willd.	Iran	Methanol	Aerial	HeLa	HHV (HSV-1)	MTT assay	ND	12.5 μg/mL	Karamoddini et al. (2011)
A. glabella Kar. et Kir.	Kazakhstan	ND	Aerial	Chicken embryonic	A/FPV/ Rostok 34 strain of IV, LaSota strain of NDVs	ND	Essential oil	5–100 μM for A/FPV/ Rostok 34 IV and LaSota NDVs strain	Seidakhmetova et al. (2002)
A. herba-alba Asso.	Morocco	Methanol	Aerial	Vero	HHV (HSV-1), SV and PV	Antiviral photosensitizers activity	ND	50 μg/mL for SINV and HSV and 100 μg/mL for PV	Mouhajir et al. (2001)
	Morocco	Aqueous	Aerial	ND	SARS-CoV	ND	Chrysanthenone	ND	Asdadi et al. (2020)
A. incana (L.) Druce	Iran	Methanol	Aerial	HeLa	HHV (HSV-1)	MTT assay	ND	12.5 μg/mL	Karamoddini et al. (2011)
A. kermanensis Podl.	Iran	Aqueous	Aerial	Vero	HHV (HSV-1)	MTT/Plaque reduction assay	ND	0.2–0.6 μg/mL	Gavanji et al. (2015)
A. mendozana D.C. (v. n. ajenjo)	Argentina	Aqueous	Leaves	Vero	HHV (HSV-1), DENV-2, JUNV	Virucidal test	Camphor, artemisole, artemisia alcohol, borneol	298.61 ppm	Duschatzky et al. (2005)
A. morrisonensis Hayata.	China	ND	ND	Huh-7	HBV	Promoter activity analysis/ Cell cytotoxicity assay	*p*-hydroxy Acetophenone (PHAP)	ND	Huang et al. (2014)
A. persica Boiss.	Iran	Methanol	Aerial	HeLa	HHV (HSV-1)	MTT assay	ND	12.5 μg/mL	Karamoddini et al. (2011)
A. princeps var. *orientalis*	Seoul Korea	Aqueous	Aerial	RAW 264.7, CRFK, FCV-F9, MNV-1	MNV-1 and FCV-F9	Plaque assays	α -thujone	0.01 and 0.1 μg/mL	Chung (2017)
A. scoparia Waldst. & Kit.	China	Ethanol	ND	MDCK	IV	Neuraminidase (NA) activity	Cirsimaritin	ND	Wang et al. (2017)
A. verlotiorum Lamotte.	Pisa Italy	Aqueous	Leaves	CrFK	FIV	Virus-induced syncytia, Viral reverse transcriptase activity, Viral capsid protein P24 expression	ND	10^{-3} mg/mL	Calderone et al. (1998)
A. vulgaris L.	Armenia	Aqueous	Whole plant	Vero	YFV (17D strain)	Plaque and cytotoxicity assay	α-thujone, β-thujone, 1,8-cineole, *trans*-carveol, sabineno	100 μg/mL	Meneses et al. (2009)
	Iran	Methanol	Aerial	HeLa	HHV (HSV-1)	MTT assay	ND	25 μg/mL	Karamoddini et al. (2011)

BVDV = Bovine viral diarrhea virus, COVID-19 = Coronavirus disease of 2019, DEN 2 = Dengue virus type 2, FCV = Feline calci virus, FIV = Feline immunodeficiency virus, HBV = Hepatitis B virus, HeLa = Henrietta Lacks cells, HCV = Hepatitis C virus, HHV (HSV-1) = Human alphaherpesvirus (Herpes simplex virus type 1), HHV (HSV-2) = Human alphaherpesvirus (Herpes simplex virus type 2), HHV-4 (EBV) = Human gammaherpesvirus type 4 (Epstein-Barr virus), HIV − 1 = Human immunodeficiency virus type 1, HIV -II= Human immunodeficiency virus type 2, HBeAg = Hepatitis B e-antigen, HBsAg = Hepatitis B surface antigen, IV = Influenza virus, IV-A = Influenza virus type A, JUN V = Junin virus, MDBK = Madin-Darby bovine kidney cells MDCK = Madin-Darby canine kidney cells, MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2 H-tetrazolium bromide assay, MNV 1 = Murine norovirus type1, ND = Not defined, NDV = Newcastle disease virus, SARS-Cov 2 = Severe acute respiratory syndrome corona virus 2, SV = Sindbis virus, PV = Polio virus, Vero = Verda reno cells, YFV = Yellow fever virus

viruses. Aqueous leaves extracts of *Artemisia douglasiana* Bess. were reported to have active antiviral compounds against HHV (HSV-1), Junin virus (JUNV) and Dengue virus type 2 (DEN-2). Methanol extracts of aerial parts of *Artemisia fragrans* Willd. were shown to have antiviral activity against Human HHV (HSV-1), JUNV and DEN-2 viruses. Antiviral potentials of extracts from the aerial *Artemisia glabella* Kar. et Kir. were demonstrated against Newcastle disease virus (NDV) LaSota strain (vaccine strain) and A/FPV/ Rostok 34 IV strain. Aqueous extracts from the aerial parts of *Artemisia herba-alba* Asso. were documented with antiviral activity against HHV (HSV-1), SV, PV and SARS-CoV-2. Methanol extracts of aerial parts of *Artemisia incana* L. Druce were found to be active against HHV (HSV-1). Aqueous extracts from aerial parts of *Artemisia kermanensis* Podl. were documented with potential anti-HHV (HSV-1) activity. *Artemisia morrisonensis* Hayata. extracts have proven antiviral activity against HBV. *Artemisia princeps* var. *orientalis* aqueous extracts from aerial parts were documented with antiviral activity against Murine norovirus-1 (MNV-1) and Feline calci virus (FCV). Methanol extracts of *Artemisia persica* Boiss. were reported as active antiviral candidates against HHV (HSV-1). Ethanol extracts of *Artemisia scoparia* Waldst & Kit. were documented with potential anti-influenza virus (IV) activities. *Artemisia verlotiorum* Lamotte. aqueous leaves extracts were reported with anti-feline calci virus (FIV) activities. Methanol extracts from aerial parts of *A. vulgaris* were documented with antiviral activity against HHV (HSV-1) (Table 1). Based on the collected data of this study, *Artemisia* species were documented with antiviral activity for 17 different types of viral diseases where most species were described in more than one category of viral diseases (Table 2).

A phylogeny based on ITS sequences was used to determine the phylogenetic relationships and subgeneric placements of *Artemisia* species with reported anti-viral activity. Only 17 out of 23 reported *Artemisia* species with antiviral activity were included in the ITS phylogeny and 6 *Artemisia* species (*A. abyssinica, A. campestris* subsp. *glutinosa, A. caruifolia, A. kermanensis, A. morrisonensis, A. glabella*) with antiviral activity were not included, due to the unavailability of their ITS sequences from GenBank. The multiple sequence alignment (MSA) for ITS phylogeny comprised of 17 sequences of antiviral *Artemisia* species of this study, 127 reference sequences of other *Artemisia* species, and 3 outgroup species sequences from the GenBank (n = 147). The resulting consensus phylogram following 50 % majority rule mL tree attained from the ITS dataset (MSA = 147) is given in Fig. 1, where the monophyly of the genus is strongly supported (mL BS = 99 %). Overall, the subgeneric classification based on molecular data was resolved with the exception of some lineages. The mL tree based on ITS sequences attained largely corresponds to current understanding of the evolutionary relationships as given in the most recent phylogenetic studies, with some exceptions; for example, the placement of some *Artemisia* species are indicated in the ITS phylogeny of this study which were not previously addressed in the phylogenetic studies of the genus *Artemisia.* Outcomes of the ITS phylogeny displayed the dispersion of 17 antiviral *Artemisia* species in clades belonging to different subgenera of the genus *Artemisia*. In the resulting mL tree, 10 antiviral *Artemisia* species appeared within the clades corresponding to the subgenus *Artemisia*. Two species appeared within the subgenus *Absinthium* clade. Three species were appeared within the subgenus *Dracunculus* clade. Two species appeared within the subgenus *Seriphidium* clade.

Table 2
Major viral disease categories and number of *Artemisia* species reported against viral diseases.

S/ No	Viral disease category	No of *Artemisia* species
1	Bovine viral diarrhea virus (BVDV)	01
2	Dengue virus type 2 (DEN 2)	02
3	Feline calci virus (FCV)	01
4	Feline immunodeficiency virus (FIV)	01
5	Hepatitis B virus (HBV)	03
6	Hepatitis C virus (HCV)	01
7	Human immune virus (HIV)	07
8	Human alphaherpesvirus (Herpes simplex virus) or HHV (HSV)	16
9	Human gammaherpesvirus type 4 (Epstein-Barr virus) or HHV-4 (EBV)	01
10	Influenza virus (IV)	04
11	Junin virus (JUN V)	02
12	Murine noro virus-1 (MNV 1)	01
13	Newcastle disease virus (NDV)	01
14	Severe acute respiratory syndrome corona virus 2 (SARS-Cov 2)	03
15	Sindbis virus (SV)	02
16	Polio virus (PV)	02
17	Yellow fever virus (YFV)	01

4. Discussion

Details in Table 1 provide baseline data on *Artemisia* species used against viral diseases universally. This record could be a vital starting point for evaluating the effectiveness of these *Artemisia* species to treat viral diseases, specifically SARS-CoV-2, which causes Covid-19 disease, and the expansion of operative drugs to treat such pandemic diseases. In order to discover novel anti-SARS-CoV-2 therapeutic representatives, screening of these antiviral *Artemisia* species is imperative because of their widespread utilization around the world in treatment of fatal viral infections. On the basis of data collected in this study, *Artemisia* species were recognized with potential antiviral activity for 17 different types of viral diseases where most species were described against more than one virus, as shown in Table 2.

Many inquiries have acknowledged the inhibitory actions of medicinal plants extracts worldwide on the replication of numerous viruses. Predominantly, HHV (HSV-2) (Debiaggi et al., 1988), HIV-I and HIV-II (Asres and Bucar, 2005), Hepatitis B virus (HBV) (Kwon et al., 2005; Huang et al., 2006), and developing viral contagions linked with Poxvirus (PV) and SARS virus (Kotwal et al., 2005), were powerfully impeded by many plant extracts. Numerous investigations have reported the inhibitory effects of extracts from *Artemisia* species on several types of viruses (Chang and Woo, 2003; Li et al., 2005; Romero et al., 2006; Efferth et al., 2008; Karamoddini et al., 2011; Lubbe et al., 2012; Tao et al., 2020; Nie et al., 2021 and references therein). This effect could be due to the presence of terpenoids and flavonoids, which are group of active antiviral compounds present in the *Artemisia* species extracts. A promising terpenoid compound, artemisinin (Tu et al., 1981; Tu, 2016, 2017), is among those antiviral agents obtained from *Artemisia* species widely used for the treatment and management of malaria (Daddy et al., 2017; Pellicer et al., 2018; Zeb et al., 2018 and references therein) and some deadly viruses (Romero et al., 2005, 2006; Paeshuyse et al., 2006; Efferth et al., 2008; Lubbe et al., 2012; Cao et al., 2020 and references therein). Artemisinin is a sesquiterpenoid lactone present in the extracts of different *Artemisia* species, including *A. annua* (Covello, 2008; Ikram and Simonsen, 2017; Pellicer et al., 2018; Nganthoi and Sanatombi, 2019) *A. apiacea*, *A. macrocephala* and *A. thuscula* (Pellicer et al., 2018), with 1, 2, 3-trioxane structure and an endo-peroxide bridge (Mannan et al., 2010). A number of *in vitro* studies exhibited that lower concentrations of artemisinin has antiviral properties on IV-A (Krishna et al., 2008), HBV and HCV (Romero et al., 2005; Paeshuyse et al., 2006), bovine viral diarrhea virus (Romero et al., 2006) different human herpes viruses, including Human gammaherpesvirus type 4 (Epstein-Barr virus) (HHV-4 (EBV) and Human betaherpesvirus type 5 (human cytomegalovirus) (HHV-5 (HCMV) (Efferth et al., 2008). Cao et al. (2020) emphasized the anti-SARS-CoV-2 potential of artemisinin and provided leading candidates for anti SARS-CoV-2 drug research and development. Artesunate is another promising compound obtained from *Artemisia* species with demonstrating efficacy in decreasing HHV-5 (HCMV) in an immunosuppressed child with no toxic effects (Shapira et al., 2008).

Together with terpenoids like artemisinin and artesunate in natural plant materials, flavonoids are also of increasing interest because of

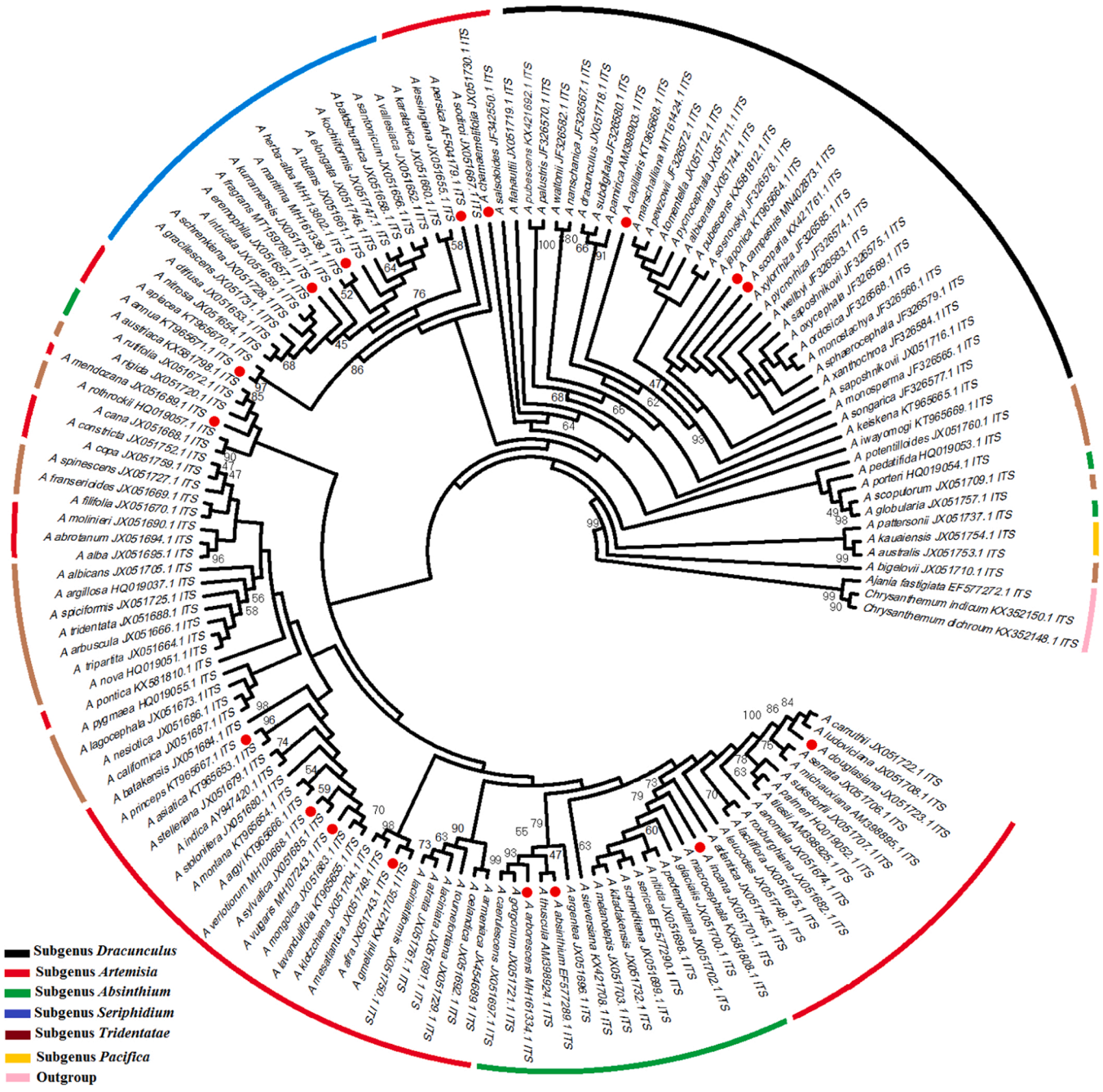

Fig. 1. Maximum likelihood consensus phylogram based on nrDNA ITS sequences of *Artemisia* species. Bootstrap values (>50) are indicated along branches. The red circled specifies sequences of corresponding antiviral *Artemisia* species. The colored lines specify subgeneric classification of the genus *Artemisia* following Bremer (1994), Torrell et al. (1999), Vallès et al. (2003), Sanz et al. (2008), Garcia et al. (2011), Pellicer et al. (2010), Riggins and Seigler (2012), Hobbs and Baldwin (2013), Malik et al. (2017), Pellicer et al. (2018), Hussain et al. (2019).

their extended biological benefits. Flavonoids are classified into various types according to their structure and possess different activities depending on this. Flavonoids are natural compounds linked by three carbon chains, usually C6-C3-C6, and consist of an oxygenated heterocyclic ring (Xiao, 2017). Among the different plant sources containing flavonoids, the genus *Artemisia* is very diverse and widely distributed (Hussain et al., 2022). In a study, the total number of phenols and flavonoids identified was 32 in *A. annua,* 37 in *Artemisia iwayomogi* Kitam., and 14 in *Artemisia argyi* H.Lév. & Vaniot., out of which flavonoids accounted for 16 in *A. annua*, 27 in *A. iwayomogi* and 7 in *A. argyi* (Kim et al., 2020). Similarly in this study, polymethoxyflavonols were found to be prevalent in *Artemisia* species, as shown in Table 1. It has been shown that more than 10 types of flavonoids, like apigenin, catechin, genkwanin, quercetin, kaempferol, malvidin, rhamnetin, diosmetin, luteolin and dimethoxyflavone, from *Artemisia* species possess potential antiviral activity (Kim et al., 2020). These findings clearly indicate that terpenoids and flavonoids are a group of compounds found in different *Artemisia* species with potential antiviral activity. This study further validated the subgeneric grouping of *Artemisia* and its species with antiviral activity in the mL ITS phylogeny of the genus (Fig. 1). Current research possibilities anticipate that plant species selection for analysis could be ensured through phylogenetic analysis particularly, by plotting bioactivity and photochemistry data (Larsen et al., 2010; Zhu et al., 2011) with ethnobotanical uses (Forest et al., 2007; Saslis-Lagoudakis

et al., 2012; Grace et al., 2015; Ernst et al., 2016 and references therein) on the phylogenetic trees. According to Saslis-Lagoudakis et al. (2012), phylogenies could be very useful in tracing medicinal folk data for the documentation of lineages with favorable medicinal attributes. Additionally, lineages with greater traditional uses are equivalent with the ones overrepresented in species with pharmacological action. The monophyly of the genus *Artemisia* in the present ITS phylogeny is strongly supported (mL BS = 99 %). The subgeneric classification based on ITS phylogeny was resolved. Some exceptions in lineages were observed, which were already reported in earlier studies (Torrell et al., 1999; Vallès et al., 2003; Sanz et al., 2008; Garcia et al., 2011; Pellicer et al., 2010; Riggins and Seigler, 2012; Hobbs and Baldwin, 2013; Malik et al., 2017; Pellicer et al., 2018 and references therein). Similarly, the position of some *Artemisia* species like *Artemisia leucodes* Schrenk., *Artemisia atlantica* Coss. & Durieu., *Artemisia batakensis* Hayata., *Artemisia lavandulifolia* DC., *Artemisia constricta* Sòn.Garcia, Garnatje, McArthur, Pellicer, S.C.S., *Artemisia spinescens* D.C. Eaton., *A. iwayomogi*, *Artemisia flahaultii* Emb. & Maire., and *Artemisia eremophila* Krasch. & Butkov ex Poljakov. M., was not clearly addressed in previous studies concerning evolutionary relationships of the genus *Artemisia*, resulting from the analysis of DNA sequences. This study found that *A. leucodes*, *A. atlantica*, *A. batakensis*, *A. lavandulifolia* and *A. constricta* appeared within the clades corresponding to the species of the subgenus *Artemisia*. Similarly, *A. spinescens* appeared within the clades corresponding to the species of the subgenus *Tridentatae*. Two species, *A. iwayomogi* and *A. flahaultii* appeared within the subgenus *Dracunculus* clade. Moreover, *A. eremophila* was appeared within the subgenus *Seriphidium* clade.

Furthermore, broad morphological, anatomical, karyological and phytochemical investigations coupled with molecular data on these species are required to confirm their phylogenetic relationships and to systematically identify candidate taxa from the genus for additional probing particularly for *Artemisia* due to complicated evolutionary relationships among its species (Pellicer et al., 2018).

Despite having possible antiviral applications and the presence of artemisinin, no drug from *Artemisia* species is currently in clinical trials against SARS-CoV-2 (COVID-19) disease. The antiviral information on *Artemisia* taxa presented herein is therefore essential for advancing the drug development of novel treatments against SARS-CoV-2 disease.

5. Conclusions

This study delivers baseline information on *Artemisia* against viral diseases and proposes some candidate taxa for the possible treatment of SARS-CoV-2. A leading conclusion of this inquiry is that *Artemisia* species from different subgenera with antiviral activities are extensively distributed in the genus. Specifically, the subgenus *Artemisia* had the greatest number of species with antiviral activities. Numerous vital flavonoids, such as polymethoxyflavonols and terpenes, like artemisinin and artesunate, have been detected in different *Artemisia*with potential antiviral activity. A detailed analysis of these antiviral taxa with a focus on taxa from all subgenera, particularly the subgenus *Artemisia*, is therefore proposed to discover more antiviral species and compounds with potential anti SARS-CoV-2 activity.

CRediT authorship contribution statement

A.H. conceived of the presented idea, designed and performed the data compilation, derived the analysis and analysed the data and wrote the manuscript.

Declaration of Competing Interest

The author declare that there is no conflict of interest.

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Appendix A. Supporting information

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