



# The Genus *Artemisia* (*Asteraceae*): A Review on its Ethnomedicinal Prominence and Taxonomy with Emphasis on Foliar Anatomy, Morphology, and Molecular Phylogeny

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**Abstract:** *Artemisia* is the largest genus in the tribe *Anthemideae* having ecologically, morphologically, and chemically diverse species. These species are found mostly in the Northern hemisphere, with the distribution of fewer taxa in the Southern hemisphere of the world. Species of this genus have great therapeutic potential and constitute a remarkable natural asset and are still utilized as folk remedies against different health-related problems all over the world. The taxonomy of *Artemisia* has long been problematic essentially due to the morphological complexities within its species. The recent classification divides the genus into six major groups like *Absinthium* DC., *Artemisia* L., *Dracunculus* Besser, *Pacifica*, *Seriphidium* Besser, and *Tridantatae* (Rydb.) McArthur. However, its infrageneric classification is still indistinguishable. The current review comprehensively enlightens the ethnomedicinal significance and recent advancements in the taxonomy based on foliar anatomy, pollen features, morphology, and molecular phylogeny of *Artemisia* to understand the classification of this economically significant genus.

## 1. INTRODUCTION

The genus *Artemisia* L. belongs to the *Asteraceae* family having economical and medicinal significance. Species of this genus are commonly found in the temperate sectors of the northern hemisphere with a limited number of species in the southern hemisphere of the world [1]. It contains ~500 species of both herbs and shrubs [2] and is a diverse genus of the *Anthemideae* tribe [3]. The economic importance of many *Artemisia* species is due to their utilization as aesthetics, feedstuff, fodder, therapeutics, and soil binder, while few species are allergic and toxic weeds. Some major crops are being affected because of their susceptibility to the toxic effects of *Artemisia* species [4]. Many *Artemisia* species are perennial while some are annual or biennial [5].

Nearly, 87% of human health-related problems including cancer, bacterial infections, and immunological ailments are treated with naturally

occurring compounds and their allied medicines. It has been shown that almost 25% of approved drugs around the world are obtained from plant origin. Additionally, in many developing countries, more than 80% of the population relies on traditional medicines from plants to meet their health care requirements [6].

Many extracts obtained from *Artemisia* species are used for the treatment of anxiety, epilepsy, depression, insomnia, irritability, psychoneurosis, and stress [7]. These species possess a wide range of biological activities like antimalarial, antiseptic, antibacterial, antitumor, hepatoprotective, antispasmodic, and antirheumatic activities [6, 7, 8].

Malaria is a universal and life-threatening disease, leading to the annual death toll of around one million. The treatment of malaria and other parasitic and pathogenic diseases is possible using Artemisinin containing antimalarial drugs.

Artemisinin is a sesquiterpenoid/lactone compound obtained from the trichomes of annual *Artemisia* species like *Artemisia annua* [9]. The chemical composition of Artemisinin confirms the presence of an endo-peroxide bridge in the 1, 2, 3-trioxane structure. This endoperoxide bridge acts as a pharmacophore and gives antimalarial activity against *Plasmodium falciparum*. The details of other essential compounds with potential biological activities obtained from *Artemisia* species are provided in Table 1. Currently, WHO recommends Artemisinin combination therapy (ACT) [10] to halt the transmission of malarial parasite *P. falciparum* [11]. According to WHO, there has been a record of 219 million cases of malaria in 90 countries of the World. The number of deaths caused by malaria has now reduced to 435,000 in 2017, because of the ACT approach.

The taxonomy of this economically important genus is not clear for many years [4]. This is because of the greater population size and the presence of morphological complexities in its species. In the previous taxonomic investigations based on morphology, *Artemisia* and its allies were separated as sections, but recent studies based on molecular data again pooled the previously separated subgenera i.e., the subgenera *Seriphidium*. So far, the genus has been divided into 5 to 6 different genera. The six representative species from 5 subgenera of the genus *Artemisia* are given in Fig. 1.

This comprehensive review provides important data about recent advancements in the ethnobotany, morphology, foliar anatomy, pollen morphology and molecular phylogeny of the genus *Artemisia* to understand its economic importance with special emphasis on the taxonomy and classification.

## 2. METHODOLOGY

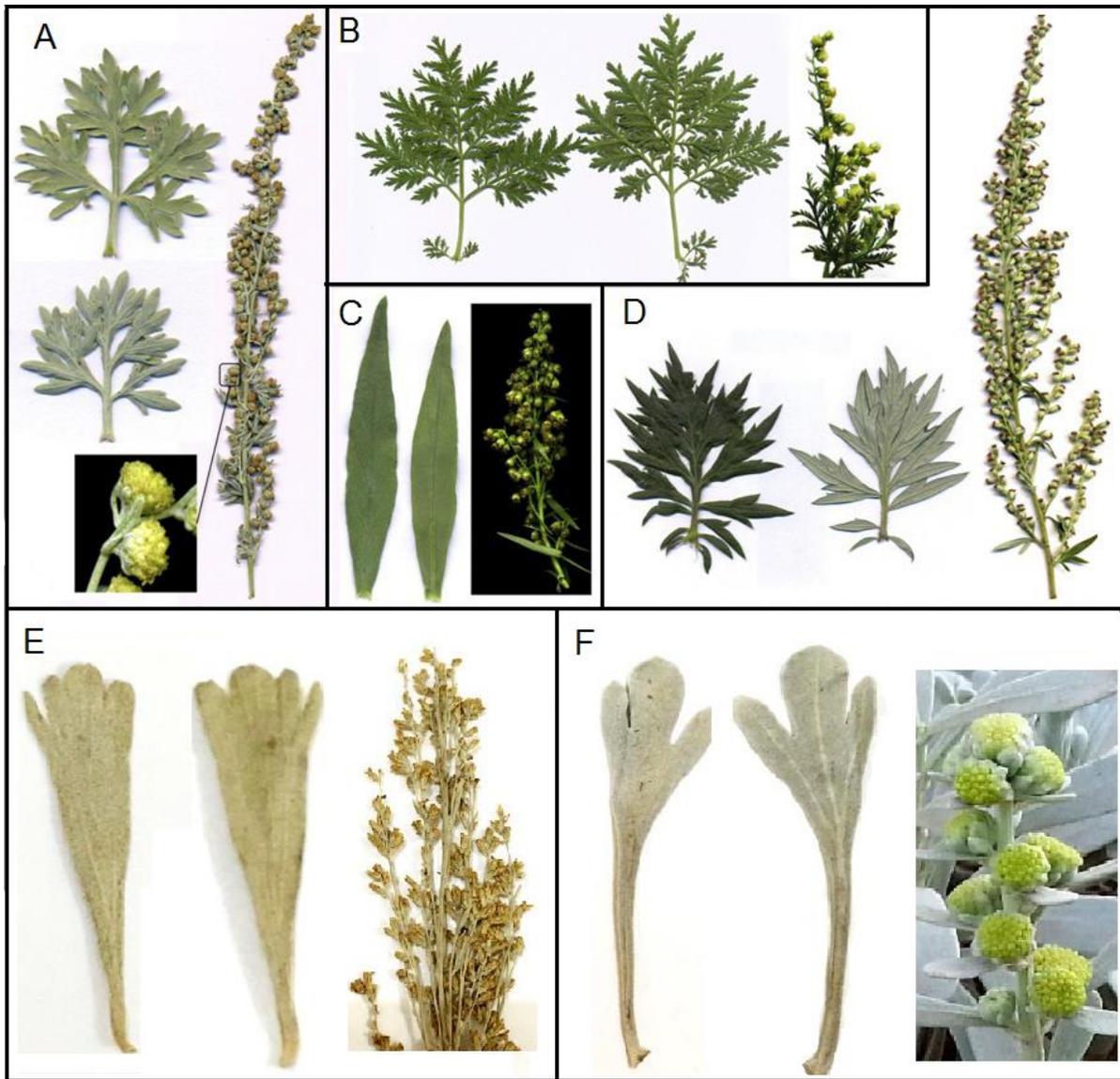
In this review, the data was collected from different search engines like Google Scholar, Science Direct, Scopus, PubMed, Research Gate, Web of Science, and Medline. The species names and taxonomic descriptions with synonyms were checked and confirmed from the database (<http://www.eflora.org/> and <http://www.theplantlist.org/>). More than 200 research articles published on the genus *Artemisia* were studied and information on ethnobotany,

morphology, foliar epidermal anatomy, pollen morphology and molecular phylogeny of the genus *Artemisia* has been provided.

## 3. ETHNOMEDICINAL PROMINENCE OF *ARTEMISIA*

Ethnobotanical practices have been applied by many communities to cure health-related problems. These communities have their customs regarding the utilization of medicinally important plants [58]. The ethnobotanical data of plants is a way to obtain better information on traditional applications and the information is used to explore plants' therapeutic properties scientifically [59]. Plant-based inquiries also validate the transfer of traditional information and conservation strategies of biological diversity of plants in a better way [60]. It has been shown that about 5,000 different plant species from angiosperms are globally used in traditional medicine direct or indirectly against different health problems [61]. The center of the diversity of *Artemisia* is primarily from Asia, Europe, and North America. Asia is the prominent continent with a large number of *Artemisia* species. Literature has shown that 150 accessions have been acknowledged until now from China, 174 from the ex-USSR, 50 species were reported from Japan, 38 species from Pakistan [4], and 35 species from Iran.

*Artemisia* genus has a therapeutic history from back over two millennia [62] because of its promising therapeutic folk remedies [63] for the treatment of cancer, hepatitis, inflammation malaria, and microbial infections. Several inquiries documented the folk medicinal utilization of *Artemisia* species globally [64-79]. *Artemisia Abrotanum* (southernwood) is a perennial plant used as a traditional medicine to treat many diseases including upper airway infections. It is also used in culinary reasons and cosmetics industry [68]. From ancient time, few *Artemisia* species like *Artemisia annua*, *Artemisia Absinthium*, and *Artemisia vulgaris* are utilized as a traditional medicine in Asia and Europe [80]. Other species of *Artemisia* were also reported for their biological activities with folk medicinal uses against diabetes, high blood pressure, and stomach problems [81]. Hayat *et al.* [4] validated that the leaf powder of *A. absinthium* is used to cure gastric problems and intestinal worm in Pakistan. Seed powder of *A. Absinthium* is taken



**Fig. 1.** Adaxial and abaxial surfaces of leaves and fluorescence of six *Artemisia* species from five subgenera. (A) *A. absinthium* from subgenus *Absinthium* (B) *A. annua* from subgenus *Artemisia* (C) *A. dracunculoides* from subgenus *Dracunculoides* (D) *A. vulgaris* from subgenus *Artemisia* (E) *A. tridentata* from subgenus *Tridentata* (F) *A. chinensis* from subgenus *Pacifica*. The leaves placed in the right side show the abaxial face while, left side show the adaxial face. Photograph adapted and modified from Valles *et al.* [12].

orally to treat rheumatism and applied directly to the tooth to relieve pain. Amiri and Joharchi [76] reported the traditional medicinal uses of *A. absinthium* as an anthelmintic and appetizer.

*Artemisia annua*, (sweet wormwood or qinghao in China) is used as a traditional medicine to treat chills and high fever [82]. This plant is native to Asian, North American, and European countries, but its cultivation has also been done in African countries where it is used to treat malaria.

An important compound present in leaves of *A. annua* is Artemisinin, having potential anti-malarial action. At present, the artemisinin derived compounds are used as an anti-malarial drug with promising therapeutic and inhibitory action against *Plasmodium* parasite [24, 83]. A study [4] from the North region of Pakistan documented the utilization of *A. annua* decoction against malaria. Its leaves decoction is effective against fever, common cold, and cough. Powder of its dried leaves, when taken orally, cures diarrhea [4]. Another crucial member

**Table 1** Reported pharmacologically important compounds in different *Artemisia* species

Species	Compounds	Reference
<i>A. absinthium</i> L.	Linalool and Myrcene	[13]
	Apigenin, Chlorogenic, Luteolin, Myricetin, p-coumaric, p-hydroxyphenylacetic, Quercetin, Rutin, Spinacetin, and Vanillic	[14]
	Artemitin, 5-hydroxy-3,3',4',6,7-pentamethoxyflavone, 5,6,3',5'-tetramethoxy and 7,4'-hydroxyflavone	[15]
	Absinthin, Arabsin, Artabsin, Artemetin, Artemolin Isoabsinthin, and Matricin	[13]
	Chamazulene	[16]
	Artenolide, 24X-ethylcholesta-7, Parishin B, Parishin C, 22-dien-3b-ol	[17]
	$\alpha$ -bisabolol, $\beta$ -curcumin, matricin, spathulenol	[18]
	Ergosterol, Stigmasterol, $\beta$ – Sitosterol, Campesterol	[15]
	Camphor	[19]
	$\beta$ -pinene	[20]
	Trans-sabinyl acetate	[21]
	$\beta$ -thujone	[20]
	$\alpha$ -thujone, Artemisia ketone, $\beta$ -thujone, Carvone, Chamazulene, 1,8-cineole, Curcumene, (E)-sabinyl acetate, Neryl 2-methylbutanoate, Linalool, Monoterpenes, Myrcene, Neryl butyrate, Neryl 3-methylbutanoate, Sabinene, Trans-epoxyocimene, and Trans-verbenol	[22]
<i>A. afra</i> Jacq. ex Willd.	Acacetin, $\alpha$ -amyrin, betulinic acid, 12 $\alpha$ ,4 $\alpha$ -dihydroxybishopsolicepolide, Pentacyclic triterpenoid, Phytol and Scopoletin	[23]
	Camphor	[19]
<i>A. annua</i> L.	Artemisinin	[24, 25]
	Artemisinin, Deoxyartemisinin and Dihydro epideoxyarteannin B	[26]
	Camphor	[19]
	Linalool	[27]
	Artemisia ketone	[28]
<i>A. biennis</i> Willd.	$\alpha$ -pinene, Artemisia ketone, Camphor and 1,8-cineole	[29]
	$\alpha$ -pinene, $\alpha$ -Terpinene, Artemisia ketone, Benzaldehyde, Camphor, (E)-beta-Farnesene, Farnesene, Para-Cymene, (2E)-Hexenal, Santolina triene, Limonene, Sabinene, Terpinen-4-ol, trans-ocimene, (Z)-beta-Ocimene, (Z)-en-yn-Dicycloether	[21]
	Chrysoplenol, Jaceidin and Jaceosidin	[30]
	Camphor and Eucalyptol	[31]
	$\alpha$ -thujone, artemisia ketone, Camphor, Isothujone and 1,8-cineole	[32]
<i>A. campestris</i> L.	Caryophyllene oxide and Germacrene D	[33]
	Limonene, Trans-anethole and Trans-ocimene	[34]
	Trans-ocimene	[21]
	Artemisinin	[25]
	Chavicol, Limonene, Cis- and trans-ocimene, Methyl chavicol and Trans-ocimene	[35]

Table 1 Continued...

<i>A. dracunculus</i> L.	$\alpha$ -copaene, $\alpha$ -pinene, $\alpha$ -thujene, 1,8-cineole, Camphene, (E)- $\beta$ -ocimene, Elemicin, Myrcene, Terpenolene, Trans-Anethole and (Z)- $\beta$ -ocimene	[36]
	Camphor, 1,8-cineole	[21]
<i>A. frigida</i> Willd.	Bicyclogermacrene, Borneol, Camphor, 1,8-cineole, Cis- $\rho$ -menth-2-en-1-ol and Lavandulol	[37]
	$\alpha$ -pinene, Bornyl acetate, Borneol, Camphene, Camphor, 1,8-cineole, Germacrene D and Terpene-4-ol	[38]
	Chrysanthenyl propionate	[39]
<i>A. herba-alba</i> Asso.	Acetic acid, $\alpha$ -Bisabolol oxide, $\alpha$ -bergamotene, $\alpha$ -bulnesene, $\alpha$ -thujone, $\alpha$ -longipinene, Bergamotol, $\beta$ -Guaiene, $\beta$ -thujone, Camphor, 1-Butanol, Cis-sabinol, Chrysanthenone, Ethyl linoleate, Fenchol, Farnesene epoxide E, Linoleic acid, Piperitone, Terpinene-4-ol, Verbenol, Widdrene, 3-methyl-, 1,2-15, trans-(Z)- $\alpha$ bisabolene epoxide, 16-Diepoxyhexadecane butyl ester and Z- $\alpha$ -trans	[40]
<i>A. indica</i> Willd.	$\alpha$ -Pinene, $\beta$ -Pinene, $\beta$ -Myrcene, 1,8-cineole, Chrysanthenyl, and Divanone Linalool	[41]
	Artemone, Aromadendrene, $\alpha$ -Pinene, $\alpha$ -Terpinene, $\alpha$ -Terpinyl acetate, Bornyl acetate, $\beta$ -Pinene, Chrysanthenyl propionate, Camphene, Cis-Davanone, Elixene, 2,3-Dehydro-1,8-cineole, and Santolina alcohol	[39]
	Bornyl acetate, Borneol, Camphor, Eucalyptol and Germacrene-D	[42]
<i>A. maritima</i> L.	Camphor and 1,8-cineole	[43]
	1,8-cineole	[44]
	$\rho$ -cymene and $\beta$ -myrcene	[45]
<i>A. scoparia</i> Waldst. & Kit.	Limonene	[46]
	$\beta$ -pinene	[47]
	$\gamma$ -terpinene	[48]
	$\alpha$ -thujone, $\beta$ -thujone	[49]
<i>A. sieversiana</i> Ehrhl. Ex Willd.	Eucaliptol	[50]
	$\alpha$ -Thujone, $\beta$ -Thujone, 1,8-Cineol, Camphor, Linalool, cis- $\rho$ -Mentha -2-en-1-ol, $\rho$ -Cymene, b-Caryophyllene and Myrcene	[51]
<i>A. stelleriana</i> Besser.	1,8-Cineole, Artedouglasia oxide A, Artedouglasia oxide B, Artedouglasia oxide D, Germacrene D, Vulgarone B, and Sartedouglasia oxide C	[52]
	Acetylenic spiroethers, Caryophyllene, 1,8-cineol, Camphor, Himachalenes, Longifolene, Neryl, and Nerol	[53]
<i>A. tridentata</i> Nutt.	2,2-Dimethyl-6-isopropenyl-2H-pyran, 2,3-dimethyl-6-isopropyl-4H-pyran, 2-isopropenyl-5-methylhexa-trans-3,5-diene-1-ol	[54]
<i>A. verlotiorum</i> Lamotte.	$\alpha$ -thujone and Camphor	[55]

Table 1 Continued...

<i>A. vulgaris</i> L.	Artemisia ketone, Caryophyllene, cis-Thujone, Chrysanthenyl acetate, b-Pinene, Cadinene, 1,8-Cineole, epi- $\alpha$ -Muurolol, Germacrene D, Sabinene, Salvia-4(14)-en-1-one and trans-Thujone	[56]
	1,8-Cineol, 5-Humulene, $\beta$ -Cubebene, Trans-Caryophyllene and Trans-Salvene	[57]

of the *Artemisia* genus is *Artemisia afra* (Wildeals). This plant is commonly found in South African regions and traditionally used against asthma, bronchitis, colds, coughs, diabetes, and heartburn [73]. Its leaves are employed against fever in Kenya [84] and also used to treat malaria, abdominal pain, and headache in Ethiopia [78].

*Artemisia arborescens* (arborescent mugwort or great mugwort) is a member of *Artemisia* with silver to grey/green leaves. It is a shrubby plant with 1-meter size and indigenous to the Mediterranean region. The prevalent folklore authenticates its applications as an anti-inflammatory agent [85]. *Artemisia argyi* (Also known as “Gaiyou” in Japan and “Ai ye” in China) is a perennial herb having a creeping rhizome. It is native to China, Eastern Soviet Union, and Japan. This herb is used traditionally against liver, spleen, and kidney infections [65]. *Artemisia aucheri* is another Iranian native species used as an anti-leishmanial remedy [86]. The leaves powder of *Artemisia biennis* is used in folk medicine as an antiseptic in the North American regions. This plant is effective against sores; wound healing and chest infections [67].

*Artemisia brevifolia*, an indigenous Pakistani herb possess anthelmintic activity [72] is employed as a traditional medicine against stomach related problems. Its economic and medicinal value has been documented [87] in Pakistan. Extracts from this plant are utilized as vermifuge and the powder of leaves and inflorescence are used as a cure for gastric problems [4]. *Artemisia californica* is used in folk traditional medicine in North America. Its stem and leaves decoction is used against asthma, cough, cold, menstrual problems, rheumatism, and respiratory diseases [88].

*Artemisia campestris* (tgouft) is widely found in Southern Tunisia. It is a perennial herb with a very minor aroma. The decoction of its leaves is broadly used as a traditional medicine due to

anti-inflammatory, antimicrobial, anti-rheumatic, and antivenin activities [89]. *Artemisia cana*, another important *Artemisia* species has antiseptic applications in folk traditional medicine [90]. *Artemisia douglasiana* (California mugwort) in Argentina is usually known as “matico” and used as folk medicine. Leaves’ infusions of this plant pose promising results against gastrointestinal problems and peptic ulcers [91]. *Artemisia Dracunculus* (tarragon), is a perennial herb with long folk medicinal history. The French and Russian cultivars of *A. dracunculus* are frequently used as traditional medicine worldwide. The chemical composition of the essential oil of this plant has been acknowledged [92]. *Artemisia dubai* is another remarkable species with great folk medicinal significance. Its leaves decoction is widely used against leprosy and other health-related ailments. It is also used as a fermenting medium [93]. In Nepal, *A. dubai* is used to treat asthma, ulcers, and skin diseases [94]. Likewise in Pakistan, leaf powder of *A. dubai* is a perfect remedy against gastric problems and intestinal worms. Fresh leaves paste of this plant when applied directly on wound and skin provides better effects [4].

*Artemisia frigida* stem and leaves decoction have therapeutic applications against cough and diabetes [68]. *Artemisia fukudo* is distributed in Japan, South Korea, and Taiwan used as a great flavor enhancer in foods and preparations of different cosmetic products. This plant also possesses anti-inflammatory, antibacterial, and antitumor actions [79]. In Iran, a significant *Artemisia* species *Artemisia haussknechtii* is used as a flavor enhancer in foods. It is also used for the preparations of perfume products and drugs [95]. *Artemisia herba-alba* has been reported with its applications as folk medicine from the Northern areas of Pakistan. Powder of *A. herba-alba* whole plant has shown promising results against diabetes. Fumigation of its decoction gives relief against muscular pain [4]. In the Badia region of Jordan,

*A. herba-alba* leaves decoction is traditionally used against fever and menstrual problems. In an inquiry, Alzweiri *et al.* [96] reported better effects of this plant against neurodegenerative disease.

*Artemisia iwayomogi* (dowijigi or hanin-jin) is a native Korean species. It is a perennial herb used as a traditional medicine against liver complications

especially hepatitis [97]. *Artemisia japonica* (Burmar, Kanyarts, Basna, Kharkhaliech, and tashang) in Pakistan is utilized as a remedy against malaria [4]. *Artemisia judaica* (shih in the Middle-East) is a perennial aromatic shrub distributed mostly in the deserts of Middle-East, Egypt, and North African countries and traditionally used as an anthelmintic drug [73]. *Artemisia ludoviciana* is

**Table 2** Reported folk medicinal uses of different *Artemisia* species from different regions of the world

<i>Artemisia</i> species	Part Used	Disease cure/Uses	Country	References
	Whole Plant	Anthelmintic, fever, malaria	Pakistan	[103]
	Whole plant	Health tonic, insecticide	Pakistan	[4]
	Leaves	Intestinal worms, gastric problems		
	Seeds	Dental pain relief, rheumatism		
	Aerial part	Aphrodisiac, intestinal parasites, stomachic	Cuba	[104]
	Floral top	Emetic	Catolina	[66]
	<i>A. absinthium</i> L.	Whole plant	Gastric problem, malaria, intestinal worm, health tonic	Pakistan
Leaves and seeds		Rheumatism	Pakistan	[75]
Shoots		Conceiving pregnancy, typhoid		
Aerial part		Anthelmintic, indigestion, appetizer		
Whole plant		Diabetes, obesity, liver infections	India	[105]
Leaves		Ear diseases	Pakistan	[106]
Aerial part		Asthma, intestine problems	Algeria	[107]
<i>A. afra</i> Jacq. ex Willd.	Leaves	Headache, abdominal pain, malaria	Ethiopia	[78]
	Leaves	Asthma, cold, cough, sore throat, influenza	South Africa	[73]
	Leaves	Constipation, respiratory ailments, malaria, and wound healing	Africa	[108]
	Leaves	Fever	Kenya	[84]
<i>A. annua</i> L.	Whole plant	Fever, malaria, cough, cold, diarrhea, perfumes	Pakistan	[4]
	Aerial parts	Blood pressure, diabetes, dysentery and cough	Pakistan	[109]
	Whole plant	Malaria, fever, indigestion, tuberculosis, washing for a scab, mosquito bite	China	[82]
<i>A. biennis</i> Willd.	Leaves	Wounds, sores, chest infections	North America	[67]

Table 2 Continued...

<i>A. campestris</i> L.	Aerial parts	Bronchitis, stomach pain	Algeria	[107]
	Leaves	Intestinal bloating, intestinal parasites	Algeria	[110]
	Aerial parts	Hepatic protector	Catolina	[66]
<i>A. californica</i> Less.	Stem and leaves	Asthma, cough, cold, menstrual problems, respiratory ailments, and rheumatism	America	[88]
<i>A. dracunculus</i> L.	Leaves	Gastro problems, fodder for sheep	Pakistan	[4]
	Whole plant	Toothache, gastrointestinal problems, and fever	India	[111]
	Leaves	Appetizer, anthelmintic, dyspepsia, antacid and carminative	Iran	[76]
<i>A. frigida</i> Willd.	Leaves and roots	Cough, anti-convulsive, wound healing, tonic, lung problems	America	[64]
<i>A. herba-alba</i> Asso.	Whole plant	Diabetes, muscular pain	Pakistan	[4]
	Whole plant	Gastrointestinal disorders, jaundice	Jordan	[112]
	Leaves	Stomach pain, genital diseases	Algeria	[110]
<i>A. indica</i> Willd.	Aerial parts	Stomach-ache, ulcer	Algeria	[107]
	Whole plant	Ear diseases, insecticide	Pakistan	[106]
	Whole plant	Anti-leech, indigestion	Nepal	[113]
	Whole plant	Folk medicine	Nepal	[114]
	Leaves, buds and flowers	Anthelmintic, gastrointestinal ailments and boils	Pakistan	[103]
<i>Artemisia maritima</i> L.	Leaves and stem	Anti-inflammatory, antiseptic, malaria, intestinal worms	Pakistan	[4]
	Leaves	Stomach worm	India	[111]
	Leaves and stem	Skin infections, inflammation, and intestinal parasites	Pakistan	[74]
	Leaves	Skin infections	Pakistan	[99]
	Aerial part	Abdominal parasite, antiseptic, vermifuge, blood purifier,	India	[115]
<i>A. scoparia</i> Waldst & Kit.	Leaves and stem	Fever, stomach pain	Pakistan	[109]
	Whole plant	Pain killer, burns, depurative	Pakistan	[4]
	Shoots	Making brooms, Fuel purpose	Pakistan	[75]
	Leaves	Stomach problems (indigestion, intestinal worms) and joint pain	India	[116]
	Whole plant	Antidote, burns, gastric problems, ENT problems, fever, malaria	Pakistan	[77]
<i>A. stelleriana</i> Bess.	Shoots and Roots	Diabetes, rheumatism and blood pressure,	Pakistan	[109]
	Whole plant	Stomachic, cold, skin diseases hair tonic and carminative	China	[117]
	Leaves	Carminative, peptic ulcer		[118]
<i>A. tridentata</i> Nutt.	Whole plant	Cold, fever, pneumonia, laryngitis, stomach ache, tuberculosis, gum, mouth diseases, pulmonary problems	America	[88]

Table 2 Continued...

<i>A. vulgaris</i> L.	Leaves	Anthelmintic, skin diseases	Pakistan	[119]
	Leaves and tomentum	Fever, moxa	Pakistan	[4]
	Whole plant	Cardiac problems	Pakistan	[120]
	Leaves	To stop nosebleeding, mouth ulcers	Nepal	[121]
	Flowers	Sexual impotency, Nerve tonic, menstrual regulator	Iran	[76]
	Leaves	Fever, malaria	Pakistan	[74]
	Leaves	Fever, vermifuge, gastric problems, antimalarial	Pakistan	[77]

used against diarrhea in Northeastern Mexico [98]. *Artemisia laciniata* is employed for the treatment of high fever, gall bladder, and jaundice problems [99]. *Artemisia maritima* (Tarkh, Rooner, or Zoon) in the North region of Pakistan is utilized as an anti-inflammatory and antiseptic agent. Its decoction possesses promising antimalarial activity. The leaves powder of *A. maritima* is used against intestinal worm [4]. Additionally, the leaf paste of *A. maritima* has a therapeutic effect on skin infections [99]. *Artemisia nilagirica* (Indian wormwood) is native to the mountain regions of India where it is used as an insecticide [100].

A Japanese native herb *Artemisia princeps* (Japanese mugwort or yomogi) is used to prepare popular Japanese confection called kusa-mochi. It is used as a traditional Asian remedy against diarrhea, inflammation, and circulatory disorders [97]. *Artemisia roxburghiana* (locally called garrotra in Pakistan) is traditionally employed against fever in the form of decoction. Its powder has better effects against intestinal worms [4].

*Artemisia rubripes* is used in traditional medicine as a hemostatic agent in Korea. It is also used against stomach problems like diarrhea and vomiting [79]. *Artemisia rutifolia* from the Chitral region of Pakistan has been reported with its traditional uses against gastrointestinal problems. This plant is also used against fever and stomach worms [101]. *Artemisia santolinifolia* (locally called churi-saroj, dona, dron, jhau, lasaj, marua, jaanh and jaukay" in Northern Pakistan) has folk medicine uses against intestinal worms and other stomach related problems [4]. *Artemisia scoparia*, (Redstem wormwood) is an annual herb,

slightly aromatic and native to Southwest Asia and Central Europe. This plant possesses antibacterial, anticholesterolemic, diuretic, antipyretic, insecticidal, vasodilatory, and purgative activities. Infections like jaundice, hepatitis, inflammation of gall bladder are treated with *A. scoparia* [68]. This plant is used as a depurative and utilized against burns [4].

*Artemisia tridentata* (Big sagebrush) is a shrub native to North American. This plant is a major source of food for many invertebrates [90] with folk traditional medicinal uses. A perennial weed *Artemisia vulgaris* (mugwort) is native to Asian, European, and North American regions. It is locally called herbaka in the Philippines and is widely used as folk medicine because of anthelmintic, anti-inflammatory, antispasmodic and carminative activities. *A. vulgaris* has long been used to treat dysmenorrhoea and in the induction of labor/miscarriage. This plant is also used as an antihypertensive remedy [102]. *A. vulgaris* (locally called nagdowna, tatwan, and tarkha in Pakistan) is traditionally used to cure fever and its tomentum is utilized as moxa in Pakistan [4]. Some reported traditional uses of different *Artemisia* species from different parts of the world are given in Table 2.

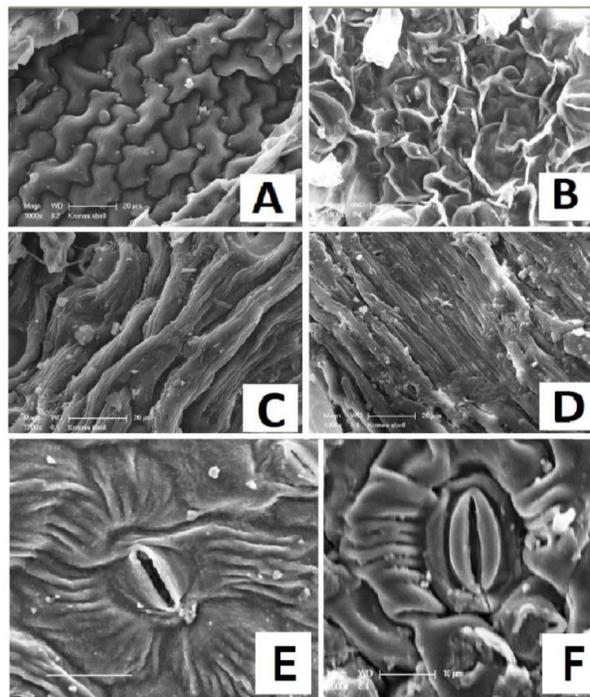
#### 4. FOLIAR EPIDERMAL ANATOMY OF *ARTEMISIA*

The epidermis of the leaves possess significant characters for the identification of species. These characters include the size and shape of epidermal cells, distribution and orientation of stomata, size of guard cells, number of subsidiary cells, and types of specialized glandular and non-glandular

trichomes [122]. The foliar epidermis of plants is an important marker for the taxonomic and biosystematics studies and taxonomic questions of plant families are addressed based on leaf epidermis characteristics [123, 124].

The foliar epidermal anatomy of *Artemisia* species has been reported by many researchers around the world [125-131]. Some of the major foliar epidermal features of *Artemisia* species are given in Fig. 2. Nautiyal and Purohit [125] validated anatomical variations with the frequency of stomata in *Artemisia* species leaves. In the Eastern province of Iran, Naseri [126] anticipated an ecological investigation of *Artemisia* with documentation of anatomical attributes of seven *Artemisia* species. Marchese *et al.* [132] showed foliar epidermal anatomy and carbon isotope composition of the economically significant herb *A. annua*. Rabie *et al.* [127] scrutinized five species of *Artemisia* for their foliar anatomical characters from Northern Iran. Zarinkamar [128] unveiled different stomata types in *Artemisia* and recognized anisocytic and anomocytic stomata types. The foliar anatomical study of twenty-eight *Artemisia* species with taxonomic implication was performed by Noorbakhsh *et al.* [129]. Based on foliar anatomy, they corroborated 3 different groups within *Artemisia*. They additionally, suggested that anatomical features are very important for the identification of species.

In a study, Hayat *et al.* [130] acknowledged the taxonomic importance of foliar epidermal cells with stomatal diversity in 24 *Artemisia* species from Pakistan. They noticed some novel stomata types with variation in epidermal cells. They observed long regular or smooth epidermal cells in the majority of species from the subgenus *Seriphidium* and curvy margined irregular shape of epidermal cells with slight exceptions in other *Artemisia* species. They endorsed foliar epidermal features as favorable traits to resolve taxonomic issues in the genus. Rani *et al.* [133] analyzed foliar anatomy of *A. nilagirica* from the Indian district Tamil Nadu; and observed wide, thick, and wavy anticlinal epidermal cell walls. Their investigation also noticed anomocytic type of stomata without having subsidiary cells in both abaxial and adaxial surfaces of the leaves. Konowalik and Kreitschitz [134] studied anatomy and comparative morphology of two varieties of



**Fig. 2.** SEM micrographs of foliar epidermal cells and stomata of *Artemisia*. (A) Irregular and wavy margined epidermal cells of *A. annua* (B) Polygonal epidermal cells of *A. argyi* (C-D) Smooth epidermal cells of *A. maritima* (E) Anomocytic stomata of *A. scoparia* (F) Anomotetracytic stomata of *A. chinensis*. Micrographs adapted with permission from Hussain *et al.* [131] and Friends Science Publishers (<http://www.fspublishers.org>).

*Artemisia*, i.e. *A. absinthium* var and calcigena in Western Carpathians Poland. A cuticle with a thin layer was observed in the foliar epidermis of investigated *Artemisia* species. They corroborated wavy shaped margins in the anticlinal walls of the epidermal cells. Bakr [135] studied an important perennial aromatic shrub; *A. judaica* for its foliar microscopical attributes from Egypt. A squarish epidermal cell with thick cuticle and one layer cells were acknowledged. Srilakshmi and Naidu [136] investigated the foliar epidermal attributes of *A. vulgaris* and reported irregular sinuous with anticlinal walls of the epidermis. They also noticed anomocytic type of stomata in *A. vulgaris*.

In one study, polygonal/irregular epidermal cells with undulate or straight walls of *A. persica* have been validated [137] with anisocytic type of stomata in different *Artemisia* species. The abaxial and adaxial epidermis surfaces of *A. Abrotanum*

leaves were investigated by Ivashchenko and Ivanenko [138] where they found cuticle in the epidermal surface. The observed amphistomatic leaf blades having an oval anomocytic type of stomata in the foliar epidermal surface. Hussain *et al.* [131] reported three different types of epidermal cells in *Artemisia* species from the Gilgit-Baltistan region of Pakistan. Their results validated irregular, smooth, and polygonal type of epidermal cells in *Artemisia* species. Four different types of stomata in *Artemisia* species including, anomocytic, anomotetracytic, diacytic, and anisocytic types were also documented. It could be confirmed that the foliar anatomical features are useful taxonomic traits for the taxonomy and species delimitation within the genus *Artemisia*.

## 5. FOLIAR TRICHOMES OF ARTEMISIA

The micromorphological characters of trichomes in the leaf surface of plants play a fundamental role in the taxonomy, particularly for specific groups at both generic and species levels. Numerous inquiries on the foliar trichomes have attracted plant systematists and morphologists to resolve taxonomic issues with different plant genera [139]. Trichomes are present in plants upper leaves surface that protects the plants from pathogens and herbivores attack [140, 142] by acting as a defender and physical barrier [142]. Glandular trichomes in many species of *Artemisia* are the major sources where the production of secondary metabolites takes place [143]. It has been substantiated that *Artemisia* species are a rich source of sesquiterpenoid lactones [144] which are found in the upper cells of glandular trichomes.

The foliar trichomes attributes are also very crucial investigations concerning the taxonomic relationship in genus *Artemisia* [145]. Different types of trichomes (glandular and non-glandular) have been reported in different *Artemisia* species [146]. The most common types in *Artemisia* are capitate type trichomes reported by Kelsey [147], Slone and Kelsey [143], Ferreira and Janick [146], Ascensao and Pais [148], Lodari *et al.* [149], Hayat *et al.* [150], Hussain *et al.* [131] and references therein. Ascensao and Pais [148] observed capitate trichomes in *A. campestris*, Smith and Kreitner [151] in *A. ludoviciana*, Ferreira and Janick [146] in *A. annua*, Kelsey [147] in *A. nova*, Slone and Kelsey [143] in *A. tridentatae* and Lodari *et al.* [149]

in *A. princeps*. Kelsey [147] unveiled the taxonomic status of *A. arbuscula*, *A. nova*, and *A. tridentata* based on trichomes (glandular and non-glandular). Slone and Kelsey [143] reported taxonomic importance by extracting and purifying glandular excretory cells from *A. tridentate*. Ascensao and Pais [148] studied glandular trichomes of *A. campestris* and elucidated the histochemistry and ontogeny of the secretory products.

In an inquiry, Lodari *et al.* [149] scrutinized the foliar surface of twelve *Artemisia* species with the SEM technique. They validated the species delimitation and their close relationships based on foliar epidermis anatomical attributes within *Artemisia*. In a study, foliar epidermal characters of *A. annua* confirmed the presence of both glandular and non-glandular types of trichomes [152].

The particulars of floral features of *A. annua* with a special focus on trichomes have also been reported [146]. Another attempt validated the foliar epidermal structure of *A. annua* and its biochemical profile [132]. Based on foliar trichomes micromorphological data, Hayat *et al.* [150] described relationships and evolutionary patterns within *Artemisia*. They analyzed 24 species and reported eight trichomes types in *Artemisia* species. Their examination suggested foliar trichomes of *Artemisia* as a significant taxonomic marker and could be employed to resolve taxonomic issues of the genus.

Bercu and Broască [153] examined the histoanatomical features of the stem, leaf, and root structure of *A. alba* subsp. *saxatilis* and validated the presence of both glandular and non-glandular trichomes. Kjær *et al.* [154] proposed the external stress magnitudes on glandular trichomes density and size of *A. annua*. Bryant *et al.* [155] and Wu *et al.* [156] employed comparative genomics to estimate proteins from glandular trichomes of *A. annua*. Tan *et al.* [157] scrutinized the molecular mechanisms of biosynthesis and triggering the development of artemisinin in *A. annua* trichomes. Rostkowska *et al.* [158] showed silicon accumulation in *A. annua* glandular trichomes for the production of antimalarial compound Artemisinin. In a recent anatomical investigation from the Northeast region of Pakistan, Hussain *et al.* [131] documented 10 types of glandular and non-glandular trichomes

in *Artemisia* species. Their results validated four types of glandular trichomes including capitate, peltate, pluricellular, and thin necked ones, whereas, the six types of non-glandular trichomes observed were aduncate, conical type, stinging hair type, unicellular calavate, unicellular filiform and unicellular tector in *Artemisia* species.

## 6. POLLEN MORPHOLOGY OF *ARTEMISIA*

The *Asteraceae* family is eurypalynous [159] and species of its genera have zonocolporate type of pollen [160]. With the innovations of scanning electron microscopes, inquiries on pollen have also expanded and gained attractiveness in addressing the taxonomy of plants [161] as the pollen characteristics are useful markers in the evolution and infrageneric classification of the *Asteraceae* family [162]. For example, based on the ornamentation of exine in the subtribe *Artemisiinae*, two types of pollen have been recognized [163]. These include the *Artemisia* type with spinules (microechinate) and the *Anthemis* type with spines (echinate). The presence or absence of spinules in the exine and the exine itself could be considered as a useful diagnostic trait in the *Asteraceae* family [164, 165]. There are plasticity and variation in the pollen of species from *Asteraceae* family [166]. Jiang *et al.* [167] examined the pollen characters of *Artemisia* and proposed two groups (mongolica-type and myriantha-type). These two pollen groups are additionally subdivided into four types, *Anomala*, *Lavandulaefolia*, *Oligocarpa*, and *Sacrorum*. It has been estimated that *Artemisia* species yields ca. 689 pollens per anther [168]. Pollens of *Artemisia* are very distinctive and easily discernable, because of the presence of spines on their surface [169]. These spinules are present in large numbers and are very minute in size [167]. The micromorphology of *Artemisia* pollens with their systematic prominence has been reported worldwide [130, 167, 170-173].

Sing and Joshi [170] reported the pollen morphology of Eurasian *Artemisia* species. Rowley *et al.* [171] studied the sub-structure of exines in the pollen of *A. vulgaris* by partial oxidation process with 2-aminoethanol followed by the growth of exine and with potassium permanganate. Similarly, a palynological inquiry on *Artemisia* and its allies showed the ornamentation of short spinules which are considered good taxonomic markers for the

taxonomy of *Artemisia* and its allies [3].

It has been said that evolution in the morphology of pollens leads to the development of many degenerative structures [130]. Some pollen features are plesiomorphic in *Artemisia* like the dense arrangement of spinules, globular pollen shape, granular exine sculpture, large pollen size, broad spinule base, broad width of colpus, and thick exine. Conversely, in an apomorphic condition, these characteristics transform in to lose arrangement of spinules, oblate pollen shape, sinuolate exine sculpture, without spinule base, small pollen size/volume, thin colpus and compact thickness of exine. This evolution is due to the difference in pollination patterns (entomophily to anemophily). However, variations in a climate with high latitude to low latitude during the relocation from North temperate zone and low evaluation moist areas throughout the glacial epoch are other causes of this evolution in pollen of *Artemisia* [167, 174].

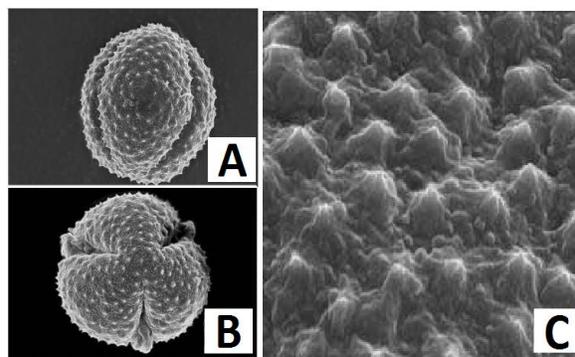
Based on pollen morphology, the monophyletic nature of *Artemisia* was long-established in the earlier reviews [130, 175, 176]. Jiang *et al.* [167] and Hayat *et al.* [130] reported approximate symmetry or globular 3 lobed spheres from the equatorial side while ellipsoid from the polar side with tricolporate arrangement in *Artemisia* species pollen as shown in Figure 3. In a taxonomic inquiry from Iran, twenty-six species of *Artemisia* were scrutinized with microscopic techniques with an emphasis on micromorphological attributes of pollen [172]. Pellicer *et al.* [173] performed a comparative study on *Artemisia* and *Ajania* pollen and substantiated oblate or prolate spherical pollen in *Artemisia*. In a study from Pakistan, Hayat *et al.* [130] quantitatively clarified the pollen of different *Artemisia* species with scanning electron and light microscopy. They constructed a phenogram based on pollen data and proposed the unification of *Artemisia* and *Seriphidium* subgenera as monophyletic which were previously reported as separate genera.

Hussain *et al.* [229] analyzed 15 *Artemisia* species from the Gilgit-Baltistan region of Pakistan for their pollen morphology and revealed tricolpate pollen the shape which was characterized by globular symmetry (Three-lobed rounds from polar view and ellipsoid ball-shaped from the equatorial

side). Hayat *et al.* [130] and Jiang *et al.* [167] endorsed that the classification of *Artemisia* based on pollen micro-morphological characters is a very challenging task; Yet, Martin *et al.* [3] expected that the pollen characteristics of *Artemisia* are better diagnostic taxonomic markers. Hussain *et al.* [229] noticed the presence of reduced spinules and their patterns of distribution on the surfaces of *Artemisia* species and also authenticated these spinules as diagnostic characters for the genus *Artemisia*

## 7. TAXONOMY OF *ARTEMISIA* BASED ON MORPHOLOGY

The morphology of plants is very important to recognize their taxonomic position with cladistics approach [161]. Previous inquiries on *Artemisia* have recommended that the morphology of *Artemisia* is very challenging to address due to similarities in species which causes problems in their identification and delimitation [150]. This complexity is manifested in *A. vulgaris* complex [177]. The subgeneric classification of genus *Artemisia* was primarily done based on its capitular morphology [176] and the genus was divided into three different genera like *Abrotanum*, *Absinthium*, and *Artemisia*. Linnaeus assigned these three separate genera as a single genus called *Artemisia* (s.l). Moreover, Valles and McArthur [2] divided the genus into four sections based on fertility/sterility of disc florets or the presence/absence of ray florets. Those sections include *Absinthium* with pistillate fertile ray florets and perfect fertile disc florets on a hairy receptacle, *Artemisia* with pistillate ray fertile florets; disc perfect fertile florets on a glabrous receptacle *Serphidium* without ray florets, but with perfect fertile disc florets on a glabrous receptacle and *Dracunculus* with pistillate fertile ray florets and staminate disc florets on a glabrous receptacle. Classically, the *Artemisia* genus was categorized into four major groups using the floral characters as taxonomic markers. These groups include *Artemisia* Tournefort., *Absinthium* (Tournefort) de Cand., *Serphidium* Besser, and *Dracunculus* Besser [3]. Another new group in the genus named *Tridantate* (Rydb.) was later documented by McArthur *et al.* [178] that is only native to North America. Cronquist [129] conducted a study and reported the taxonomy of *Asteraceae* based on floral morphology. His inquiry revealed the phylogenetic relationships of the *Asteraceae*



**Fig. 3.** SEM micrographs of pollen of *Artemisia*. (A) Equatorial view (B) Polar view (C) Exine sculpture. Micrographs adapted with permission from Hayat *et al.* [130] and Friends Science Publishers (<http://www.fsublishers.org>).

with other allied families. He further stretched the taxonomic relationship on a classical basis like the *Anthemideae*, from where the *Artemisia* genus belongs. A cladistics inquiry of *Artemisia* and its allies was carried out by Jiang and Lin [180]. They focused on the morphology of *Artemisia* and the outcomes were following the postulate of Ling [181] interpreting the classification of *Artemisia*. After the efforts of Ling [181, 182], Bremer and Humphries [169] the generic monograph of the *Asteraceae* family was distributed, where a more clearly morphological phylogeny of *Artemisia* was presented. Bremer [183] again revised the classification of the *Asteraceae* family and described the classification of *Artemisia* based on morphology. Gustafsson and Bremer [184] started a morphological investigation and acknowledged the phylogeny of the *Asteraceae* family and its allies from the Asterales. One of the vital annual *Artemisia* species is *A. annua*, whose floral morphology was examined by Ferreira and Janick [146] to validate its taxonomic position. Another significant species *A. biennis* was scrutinized for the identification of its morphological characters by Kegode and Christoffers [185]. It has been said that the morphology of achene together with other morphological features contributes a central role in systematics investigations [186]. Kreitschitz and Valles [187] inspected the morphology of achene and measured the distribution pattern of achene slime cells in *Artemisia* species.

In Pakistan, the morphology of the genus *Artemisia* has been examined by Ghafoor [69],

**Table 3.** Historical advancements in the infrageneric classification of *Artemisia* genus based on morphological characteristics

Rank	Infrageneric taxa				Reference	
Genera	<i>Absinthium</i>	<i>Abortanum</i>	<i>Artemisia</i>		[202]	
Genus	<i>Artemisia</i>				[203]	
Genera	<i>Artemisia</i>			<i>Oligosporus</i>	[204, 205]	
Section	<i>Absinthium</i>	<i>Abortanum</i>	<i>Seriphidium</i> *	<i>Dracunculus</i>	[206]	
Section	<i>Absinthium</i>	<i>Abortanum</i>	<i>Seriphidium</i>	<i>Dracunculus</i>	[207]	
Subgenera	<i>Euartemisia</i>		<i>Seriphidium</i>	<i>Euartemisia</i>	[208]	
Subgenera	<i>Absinthium</i>	<i>Abortanum</i>	<i>Seriphidium</i>	<i>Dracunculus</i>	[209]	
Section			<i>Seriphidium</i>	<i>Tridentatae</i>		
Subgenera	<i>Artemisia</i>		<i>Seriphidium</i>	<i>Dracunculus</i>	[210]	
Subgenera	<i>Absinthium</i>	<i>Artemisia</i>	<i>Seriphidium</i>	<i>Dracunculus</i>	[211]	
Section	<i>Artemisia</i>			<i>Dracunculus</i>	[212]	
Subgenera	<i>Artemisia</i>		<i>Seriphidium</i>	<i>Tridentatae</i>	<i>Dracunculus</i>	[178]
Subgenera	<i>Artemisia</i>		<i>Seriphidium</i>	<i>Dracunculus</i>	[213]	
Genera	<i>Artemisia</i>		<i>Seriphidium</i>			
Subgenera	<i>Absinthium</i>	<i>Dracunculus</i>			[214]	
Genera	<i>Artemisia</i>		<i>Seriphidium</i>		[215]	
Genera	<i>Artemisia</i>		<i>Seriphidium</i>		[69]	

Sources: Bremer [183], Haghghi *et al.* [217], Kornkven *et al.* [216], and Hussain *et al.* [194]. \* as “*Seriphidium*” formerly in Besser [206], and subsequently as “*Seriphidium*” described by Besser [218].

Abid, and Qaiser [188] and Hayat [189]. The first morphology of *Artemisia* from the Kashmir region was reported by Kaul and Bakshi [190]. After that, inquiry-based on *Artemisia* morphology was performed by Mumtaz *et al.* [191]. A broad morphological investigation on *Artemisia* was acknowledged by Ghafoor [69] who separated *Seriphidium* from *Artemisia* and provided identification key to appropriately identify *Artemisia* plants on a morphological basis.

Abid and Qaiser [188] studied twenty-four *Artemisia* species and recommended a few morphological features which are significant for taxonomic studies. They examined the cypselas of *Artemisia* and conveyed their taxonomic outcomes. Hayat [189] performed the detailed cladistics analysis and revealed the taxonomy of *Artemisia* based on morphological attributes. He united subgenus *Seriphidium* with *Artemisia* and recognized four groups within the genus *Artemisia* i.e., *Absinthium*, *Artemisia*, *Dracunculus*, and *Seriphidium*. After these morphological readjustments, the *Artemisia* genus was divided into five large groups considered at the subgeneric level. These include *Absinthium* DC., *Artemisia*

(*Abrotanum* Besser), *Dracunculus*, *Seriphidium* Besser, *Tridentatae* (Rydb.) McArthur [175]. There are many agreements concerning the idea that this morphological infrageneric classification of the *Artemisia* genus is not sufficient and it does not clarify the natural classification [2, 178, 192, 193]. Correspondingly, the very up-to-date molecular inquiries of Hussain *et al.* [194], Malik *et al.* [195], Hobbs and Baldwin [196], Riggins and Seigler [197], Garcia *et al.* [198], Pellicer *et al.* [199], Sanz *et al.* [200], Tkach *et al.* [201], Hayat [189], Valles *et al.* [5] and Watson *et al.* [176] concluded that the classification of *Artemisia*, either on morphological or molecular data needs more detailed investigations to validate its natural classification. The historical advancements in the infrageneric classification of the *Artemisia* genus based on morphology are given in Table 3.

## 8. TAXONOMY OF ARTEMISIA BASED ON MOLECULAR PHYLOGENY

For many years, phylogenetic inquiries based on nucleotide sequences from the transcript coding and/or non-coding regions are favorable gears to assess the genetic diversity among organisms.

The phylogenetic relationships of closely related species can also be done by investigating these regions as markers [219]. Linder *et al.* [220] suggested that the evolution rate of some DNA regions like the internal transcribed spacer (ITS) is faster than 18S-26S repeats of nrDNA. A method for the amplification and sequencing of the ETS region for *Asteraceae* and its closed families has also been given by Linder *et al.* [220]. These approaches employed for studies concerning the taxonomy and classification of *Artemisia* provide crucial insights for its classification and also determines changes at the genomic level [221]. The genomic regions in *Artemisia* like internal transcribed spacer (ITS), external transcribed spacer (ETS) of nuclear ribosomal DNA (nrDNA) and the intergenic spacer regions of chloroplast DNA (cpDNA) have been most commonly used to define phylogenetic positions of organisms [175, 200, 201, 216]. Many researchers around the world performed phylogenetic investigations of *Artemisia* and its allies based on molecular data to address its taxonomy [169, 175, 176, 181, 183, 189, 194-199, 222-224]. The detailed historical development in the infrageneric classification of *Artemisia* and the status of subgenus *Seriphidium* based on phylogenetic analysis of molecular data is given in Table 4. The phylogenetic investigations on *Artemisia* have been initiated with the pioneering study of Kornkven *et al.* [223], using ITS markers. After that, the phylogenetic revision based on molecular data for the subtribe *Artemisiinae* of *Anthemideae* tribe was performed with ITS regions by Watson *et al.* [176]. In their results, three major clades representing the radiate genera (*Arctanthemum* and *Dendranthema*), and two major *Artemisia* genus clades have been observed.

Valles *et al.* [5] examined phylogenetic relationships of forty-four *Artemisia* species from five classical subgenera of *Artemisia* with their ITS and ETS nrDNA sequences. Their results confirmed the monophyly of the *Artemisia* genus and the inclusion of 11 species other than ten genera to be closely related to *Artemisia*.

Sanz *et al.* [200] generated sixty-three ETS and ten ITS new sequences to understand the evolutionary history of *Artemisia* and its affinity with other genera of subtribes *Artemisiinae*, *Leucantheminae*, and *Tanacetinae*. The combined

data of ITS and ETS sequences substantiated the monophyly of all *Artemisiinae* genera including *Nipponanthemum* and *Hippolytia*. They suggested that one lineage *Artemisia/Kaschgaria* was perhaps produced from an ancestor with *Artemisia* pollen type, disciform capitula with central hermaphrodite florets. In another investigation, the sequences from nrDNA assessed the interspecific relations between the South American endemic *Artemisia* and its allies with other species of the genus [199]. In a resultant phylogenetic tree, a monophyletic clade with the majority of species *Artemisia* grouped in the American endemic clade, with the elimination of *A. magellanica*. This species seemed separated from American species and forming a clade with *A. biennis* lineage.

In Pakistan, Hayat [189] conducted a phylogenetic study of Pakistani *Artemisia* based on ITS and ETS sequences of nrDNA and also reported the reunion of subgenera *Seriphidium* and *Artemisa*. Mahmood *et al.* [225] assessed the relationship of *Artemisia* species collected from different localities of Pakistan using the rps11 regions of chloroplast DNA. It has been suggested that the subgenus *Artemisia* was defined taxonomically based on plesiomorphies which are still unclear. In the previous investigations, two subgenera (*Artemisia*, *Absinthium*) were pooled as one subgenus (*Artemisia*) [176, 226, 227]. Riggins and Seigler [197] studied the phylogeny of *Artemisia* from Beringian Region and offered new hypotheses concerning migration history, species relationships, and role of reticulate evolution in the genus *Artemisia*. Their study based on ITS regions contained 173 accessions showed the paraphyletic appearance of *Artemisia* with the segregation of some Asian genera and the North American genus *Sphaeromeria*. Phylogenetic study on rpl32-trnL and psbA-trnH regions were constructed that showed consistency with ITS topology with the possibility of introgression among Beringian and new World *Artemisia* species. Their analysis concluded that *Artemisia* species from North America have multiple origins, and western North America has aided as colonizing elements in South America and eastern Asia.

Phylogenetic analysis based on molecular data (nrDNA and cpDNA) for the Hawaiian *Artemisia* in the context of world-wide diversity was performed

**Table 4.** Historical developments in the infrageneric classification of *Artemisia* genus based on molecular data

Rank	Infrageneric taxa					DNA regions (nrDNA/cpDNA)	Reference
Subgenera	<i>Artemisia</i>		<i>Seriphidium</i>		<i>Dracunculus</i>	ITS	[223]
Section			<i>Tridentatae</i>				
Subgenera	<i>Artemisia</i>		<i>Seriphidium</i>		<i>Dracunculus</i>	cpDNA	[216]
Section			<i>Tridentatae</i>				
Groups	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>	<i>Tridentatae</i>	<i>Dracunculus</i>	ITS	[175]
Subgenera	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>		<i>Dracunculus</i>	ITS1 and ITS2	[176]
Section				<i>Tridentatae</i>			
Subgenera	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>	<i>Tridentatae</i>	<i>Dracunculus</i>	ITS1 and <i>trnL-F</i>	[5]
Subgenera	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>		<i>Dracunculus</i>	ITS and ETS	[200]
Subgenera	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>	<i>Tridentatae</i>	<i>Dracunculus</i>	ITS and ETS	[199]
Subgenera	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>		<i>Dracunculus</i>	ETS, ITS, <i>trnM</i> <sup>CAU</sup> , <i>trnS</i> <sup>UGA</sup> , <i>trnC</i> <sup>GCA</sup> and <i>trnS</i> <sup>GCU</sup>	[198]
Section			<i>Tridentatae</i>	<i>Nebulosae</i>	<i>Filifoliae</i>		
Subgenera	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>	<i>Tridentatae</i>	<i>Dracunculus</i>	ITS1, ITS2, <i>rpl32-trnL</i> , <i>psbA-trnH</i> , <i>rbcL-accD</i> , <i>ndhF-rpL32</i> , <i>trnT-trnL</i> , <i>ndhI-ndhG</i> , <i>trnV-ndhC</i> , <i>trnS-trnC</i> , <i>trnM</i> , <i>rpoB-trnE</i> , <i>rps16-trnK</i> , <i>rpL16-trnS</i> and <i>trnC-ycf6</i>	[197]
Subgenera	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>	<i>Tridentatae</i>	<i>Dracunculus</i>	ITS1, ITS2 ETS, <i>psbA-trnH</i> and <i>trnL-trnF</i>	[196]
Section				<i>Nebulosae</i>	<i>Filifoliae</i>		
Section	<i>Artemisia</i>		<i>Seriphidium</i>		<i>Dracunculus</i>	ITS and <i>psbA-trnH</i>	[217]
Subgenera	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>	<i>Tridentatae</i>	<i>Dracunculus</i>	ETS, ITS <i>ndhC-trnV</i> and <i>rpl32-trnL</i>	[195]
Subgenera	<i>Artemisia</i>	<i>Absinthium</i>	<i>Seriphidium</i>	<i>Tridentatae</i>	<i>Dracunculus</i>	ITS, ETS and <i>psbA-trnH</i>	[194]

Source: Hussain *et al.* [194]

[196]. It has been designated that the Hawaiian endemic species (*A. australis*, *A. mauianensis*, and *A. kauaiensis*) established a sister clade with Southeast Asian *A. chinensis*, which has ribbed fruit walls. The clade containing Hawaiian *Artemisia* and *A. chinensis* resolved are from the Asian origin. The natural distribution of *A. chinensis* in littoral habitats of Okinawa, Taiwan, Ryukyu, Bonin islands, and of *A. australis* in alike sceneries in the Hawaiian Islands showed ancestral ecology of the Hawaiian clade, with succeeding colonization of inland, higher-elevation habitats [196]. For the reinvestigation of phylogenetic relationships in *Artemisia*, ITS, and *psbA-trnH* sequences within 3 subgenera of *Artemisia* (subgenus *Dracunculus*, subgenus *Artemisia*, and subgenus *Seriphidium*)

were studied [217]. It has been shown that the three subgenera were not alike and separated from each other, where the heterogamous subgenus *Artemisia* and subgenus *Dracunculus* are closely associated with each other than to the homogamous subgenus *Seriphidium*.

The origin of subgenus *Seriphidium* was not resolved and this subgenus was once considered as a separate genus and omitted from *Artemisia* [181, 183, 169, 222]. Investigations based on molecular data performed by Torell *et al.* [175], Watson *et al.* [176], Hayat [189], Hussain *et al.* [194], Hobbs and Baldwin [196], Riggins and Seigler [197], Garcia *et al.* [198], Pellicer *et al.* [199], Malik *et al.* [195] and D'Andrea *et al.* [224]

confirmed *Seriphidium* as a subgenus of *Artemisia*. Malik *et al.* [195] showed species of *Seriphidium* forming two clades. They authenticated that one large monophyletic group resembled the formerly famous subgenus *Seriphidium* and the second clade was phylogenetically dissimilar from the first one. *Seriphidium* subgenus is characterized by homogamous discoid capitula with the absence of ray florets and the presence of bisexual disc florets.

The subgenus *Dracunculus* has been reserved as a subgenus of the genus *Artemisia* by Watson *et al.* [176] and Hussain *et al.* [194]. This subgenus species are characterized by heterogamous flower heads with sterile, pistillate inner and outer florets. The monophyletic nature of one more subgenus (*Tridentatae*) was long-established in previous inquiries [5, 175, 216, 223]. Subgenera *Pacifica* was also reported to be monophyletic in the molecular phylogenetic investigations of Hussain *et al.* [194], Malik *et al.* [195], and Hobbs and Baldwin [196] and reserved as a subgenus of *Artemisia*.

Haghighi *et al.* [217] reinvestigated the phylogenetic relationships using ITS (nrDNA) and psbA-trnH (cpDNA) sequences within 3 subgenera of *Artemisia* like *Artemisia*, *Dracunculus*, and *Seriphidium*. Their results detached the three subgenera from each other, where the heterogamous *Artemisia* and *Dracunculus* were found to be closely related to each other than to the homogamous subgenus *Seriphidium*. Besides the infrageneric studies using genomic regions on *Artemisia*, some studies also provided evidence of the existence of new and undescribed *Artemisia* species from different regions of the world. For example, Koloren *et al.* [228] analyzed the ITS region of 18S-26S rDNA for nineteen species of *Artemisia* from Turkey and exposed two distinctive haplotypes (Haplotype I and II) within the studied samples. The haplotypes appeared with *A. verlotiorum*, *A. argyi*, and *A. sylvatica* lineages. Hussain *et al.* [194] recently divided the genus *Artemisia* into six subgenera and reported 10 undescribed taxa of *Artemisia* based on ITS, ETS of nrDNA and psbA-trnH sequences of cpDNA from the Northeast (Gilgit-Baltistan) region of Pakistan. Their results showed close relatedness of these undescribed species with *A. japonica*, *A. rutifolia*, and *A. sieversiana* lineages.

## 9. CONCLUSION

The practices of using *Artemisia* species as folk traditional medicine are still popular and continued among different communities around the world. It could be established that *Artemisia* species are important medicinally and represent a precious natural asset. Based on contemporary facts and figures and all available in the literature, it is desirable to conclude that the combination of morphological, foliar anatomical, palynological, and molecular data may deliver a clear infrageneric classification of the *Artemisia* genus. It would be also productive to examine new genomic regions to conduct phylogenetic analysis for clear classification. In the same way, the classification based on morphology must be revised and the samples to be investigated must include a greater number of species representing all subgenera with maximum morphological features to resolve taxonomic conflicts in *Artemisia*.

## 10. ACKNOWLEDGEMENT

The author acknowledges the dedication of the respondents who shared their knowledge in this review. The Higher Education Commission of Pakistan (HEC) is greatly acknowledged for making resources available during the period of this work.

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