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Review Article

Phytochemicals, Traditional Uses, Biological Effects, and Possible Molecular Mechanisms of *Ephedra alata*

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Abstract

Nowadays, *Ephedra* species have been marked as encouraging natural material for research in the field of pharmacology. This work provides an overview of the botanical, traditional uses, phytochemistry, pharmacological properties, molecular mechanisms, and toxicity of *Ephedra alata*. The following databases were utilized to search for primary literature references: PubMed, Web of Science, Scopus, Scientific Information Database, Science Direct, Google, and Google Scholar. The present review demonstrates that various compounds have been extracted from *E. alata*, such as fatty acids, sphingolipids, volatile compounds, reducing sugars, flavonoids, phenolic compounds, and alkaloids. These natural compounds show valuable biological activities, such as antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic, antihypertensive, anti-obesity, nephroprotective, hepatoprotective, antipyretic, analgesic, anti-acetylcholinesterase, antityrosinase, and anti-urease activities. Several mechanisms are proposed to understand the biological effects of *E. alata*. In summary, *E. alata* constitutes good natural material for utilization in food and medicine applications.

Introduction

The plant species existing in Mother Nature have been a huge source of medicinal materials.[1] Modern research has confirmed that the first medications were previously taken from herbs and plants.[2] About 80% of medications used for antimicrobial, cardiovascular, immunosuppressive, and anticancer purposes originate from plants.[3] Consequently, several scientists intend to screen medicinal plants for their phytochemistry and bioactivity.[2]

Recently, many researchers have focused their studies on the species of *Ephedra*, and their isolated phytochemicals form an important basis for natural medications and nutrient complements.[4–16] *Ephedra* species (Ephedraceae family) are rich in alkaloids of the ephedrine type and act as sympathomimetics.[17] In general, *Ephedra* species have been traditionally used to treat bronchial asthma, chills, allergies, colds, coughs, edema, fever, flu, nasal congestion, and headaches.[18] *Ephedra alata* is a small perennial, xerophytic, gymnosperm, and dioecious shrub.[19,20] It is native to many areas throughout Northern Africa, mainly the Sahara, and spans throughout the Middle East.[21]

Over the last several years, *E. alata* and other *Ephedra* species have been screened for their chemical constituents and reported for their various medicinal properties.[5–16] Several mechanisms have been suggested to comprehend the biological effects of *E. alata*, namely cell cycle arrest, mitochondrial repression, apoptosis, and vital enzyme blockage.[12,13,15] To date, no review has covered the several phytochemistry and bioactivity effects of *E. alata* or highlighted its molecular mechanisms of action. Therefore, this work provides an overview of the botanical, traditional uses, phytochemistry, pharmacological properties, molecular mechanisms, and toxicity of *E. alata*. To sum up, our work supplies the reader with information concerning the phytochemicals, traditional uses, biological effects, and possible molecular mechanisms of *E. alata* and orients the direction of health professionals and researchers in scientific fields to discover this plant and to develop new drug formulations to treat several types of diseases.

Traditional medicine utilization

E. alata mostly grows in the deserts distributed in Africa, including Algeria, Egypt, Libya, Morocco, Tunisia, Mauritania, Chad,

Keywords: *Ephedra alata* L.; Medicinal uses; Botanical characterization; Phytochemical; Pharmacology.

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AcE, acetonitrile extract; AChE, Acetylcholinesterase; AE, alkaloid extract; BChE, butyrylcholinesterase; BE, betanol extract; COX, cyclooxygenase; DE, dicloromethane; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EAE, ethyl acetate extract; EE, ethanolic extract; EO, essential oil; FA, fatty acid; FRAP, ferric reducing ability of plasma; HE, hydraulic extract; HEE, hydroethanolic extract; HEPG2, human liver cancer cell line; HME, hydromethanolic extract; IC_{50}, half-maximal inhibitory concentration; MCF-7, human breast cancer cell line; ME, methanol extract; PC3, human prostate cancer cell line; TBARS, thiobarbituric acid reactive substance.

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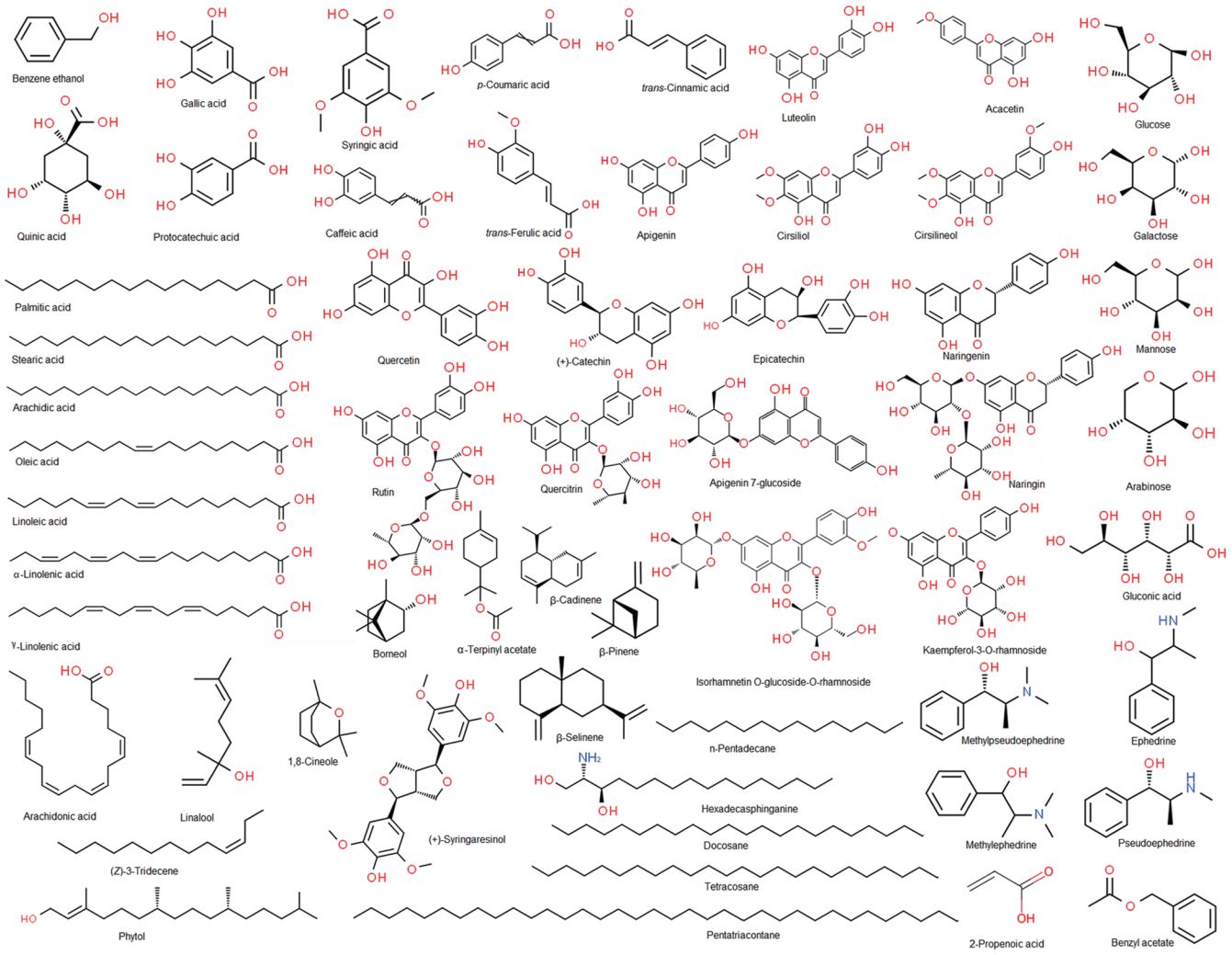

Fig. 1. Structures of the phytochemicals present in *Ephedra alata*.

and Mali, as well as in Asia, including Saudi Arabia, Iraq, Iran, Palestine, Lebanon, Jordan, and Syria.[22–24] In traditional medicine, the plant's dried stems are employed and typically boiled in water for approximately 30 min; it is ingested orally as a hot tea, with dosages ranging from 1.5 to 9 g.[25] In general, the decoction of *E. alata* stems has demonstrated efficacy in addressing issues related to the kidneys, bronchi, circulatory system, and digestive system as well as in alleviating asthma attacks and cancer treatment; moreover, chewing the plant stems has been employed to treat bacterial and fungal infections.[26] In the traditional Chinese Pharmacopoeia, *E. alata* has been taken to treat hay fever, cough, cold, asthma, chills, allergies, and edema. In traditional Russian medicine, it has been utilized for respiratory disorders and rheumatism.[4,27] In Algeria and Tunisia, *E. alata* has been used as a traditional remedy for treating cancers.[28] Furthermore, in Algeria, it has been used to alleviate asthma, allergies, headaches, general wounds, chills, fever, nasal congestion, whooping cough, ulcers, diabetes, abortion, obesity, renal disorders, and hypotension.[29,30] In Palestine, it is currently used for cancer treatment.[19] In Morocco, *E. alata* has been used to fight diabetes.[27] Additionally, Bedouins residing in Egypt's Sinai Peninsula have used *E. alata* as a treatment herb for central nervous disorders and various other healing purposes.[31]

Chemical constituents

Figure 1 and Table 1 illustrate the various phytochemicals belonging to different chemical classes present in *E. alata*.[13,32–39]

Lipids

The total lipid content of the aerial parts of *E. alata* is 9.81 mg/g of fresh weight. The fatty acid composition has been characterized by the presence of linoleic (22.97%), arachidonic (21.31%), arachidic (17.72%), stearic (14.64%), α-linolenic (13.46%), and oleic (9.90%) acids.[40] Different results were obtained by Mighri *et al.*, who reported that the main fatty acids are oleic (12.88%), palmitic (9.948%), behenic (6.17%), and linolelaidic (2.87%) acids.[21] In a recent study, Dbeibia *et al.* found that the main fatty acids are γ-linolenic acid (23.69%), linoleic acid (23.08%), palmitic acid (18.91%), and oleic acid (17.43%).[32] Sphingolipids also have been detected in the methanol extract (ME) of *E. alata* pulp with the predominance of hexadecasphinganine (24.17%).[13]

Table 1. Classification of the constituents *of Ephedra alata*

Class	Constituents of *Ephedra alata*	Reference
Fatty acids	γ-Linolenic acid, linoleic acid, palmitic acid, oleic acid, α-linolenic acid, stearic acid, eicosatrienoic acid, and vaccenic acid	32
Sphingolipids	Hexadecasphinganine	13
Carbohydrates	Glucose, galactose, mannose, arabinose, and gluconic acid	33
Alkaloids	Ephedrine, pseudoephedrine, methylephedrine, and methylpseudoephedrine ephedralone	34
Phenolic acids	Quinic acid, gallic acid, protocatechuic acid, syringic acid, caffeic acid, *p*-coumaric acid, trans-ferulic acid, and trans-cinnamic acid.	9,36
Flavones	Apigenin, luteolin, cirsiliol, cirsilineol, and acacetin	36
Flavanols	Quercetin and kaempferol	36
Flavan-3-ols	(+)-Catechin and epicatechin	9,36
Flavanol glycosides	Rutin and quercitrin	9,36
Flavone glycosides	Apigenin-7-*O*-glucoside and naringin	36
	Kaempferol 3-*O*-rhamnoside and isorhamnetin *O*-glucoside-*O*-rhamnoside	13
Flavanones	Naringenin	36
Terpenes	β-Pinene, α-terpinyl acetate, β-selinene, borneol, β-cadinene, linalool, (*Z*)-3-tridecene, and *n*-pentadecane	37
	1,8-Cineole, pentatriacontane, docosane, tetracosane, phytol, and benzene ethanol	36
Lignans	(±)-Syringaresinol	35
Organic acids	2-Propenoic acid, 3-phenylbenzoic acid, 2-propenoic acid, 3-phenylmethyl benzene-acetic acid, α-hydroxybenzene-dicarboxylic acid, 1,2-hexadecanoic acid, benzoic acid 4-hydroxyacid ethyl ester, and benzene propanoic acid	38
1,2-benzenedicarboxylic dinonyl ester	Alatine	39

Polysaccharides

The yield of water-soluble polysaccharides isolated from *E. alata* stems (4%) has been reported to be approximately five-fold higher than that from *E. sinica* stems (0.85%) and in the same range as that extracted from *E. sinica* stems (4.9%).[41,42] The predominant component of *E. alata* polysaccharides has been discovered to be total carbohydrates (73.74%). However, lipids, proteins, uronic acid, and ash have been demonstrated to be the minor constituents (1.09, 5.68, 6.82, and 10.24%, respectively). The monosaccharide composition has been revealed to consist of glucose (43.1%), galactose (36.4%), mannose (14.9%), arabinose (3.7%), and gluconic acid (1.7%).[33]

Alkaloids

The total amount of alkaloids obtained from the aerial parts of *E. alata* was 1.34%.[34] Four ephedrine alkaloids were detected in the aerial parts of *E. alata*, namely ephedrine (17%), pseudoephedrine (69%), methylephedrine (5%), and methylpseudoephedrine (10%), as reported by Sioud *et al.*[34] Ephedralone, a 7-methoxylated derivative also has been isolated from *E. alata*.[35] The biosynthesis of the ephedrine alkaloids, more strictly aromatic amines,[43] occurs from phenylalanine.[44]

Polyphenols

The contents of total phenols, flavonoids, and condensed tannins of ME from Tunisian *E. alata* seeds are reported to be 2.5 mg of gallic acid equivalents/g, 3.8 mg of quercetin equivalents/g, and 1.03 mg of catechin equivalents/g, respectively.[9] The ME of Palestinian *E. alata* aerial parts had the highest total phenol (47.62 mg of gallic acid equivalents/g) and flavonoid (0.52 mg of rutin equivalents/g) contents.[45] However, Ibragic and Sofić found that the total phenol content in the ME of German *E. alata* aerial parts was 53.30 mg of gallic acid equivalents/g and the total flavonoid contents was 28.00 mg of rutin equivalents/g.[15] A phytochemical characterization of the hydromethanolic extract (HME) from Tunisian *E. alata* aerial parts revealed the presence of 22 phenolic compounds categorized into 8 phenolic acids (quinic acid (85.85%), gallic acid (0.15%), protocatechuic acid (0.11%), syringic acid (0.01%), caffeic acid (0.02%), *p*-coumaric acid (0.47%), trans-ferulic acid (0.34%), and trans-cinnamic acid (0.02%)); 5 flavones (apigenin (0.01%), luteolin (0.08%), cirsiliol (0.52%), cirsilineol (0.04%), and acacetin (0.02%); 2 flavonols (quercetin (0.09%) and kaempferol (0.08%)); 2 flavan-3-ols ((+)-catechin (0.34%) and epicatechin (7.19%)); 2 flavanol glycosides (rutin (1.57%) and quercitrin (0.88%)); 2 flavone glycosides (apigenin-7-*O*-glucoside (0.12%) and naringin (1.83%)); and 1 flavanone (naringenin (0.17%)), as reported by Mighri *et al.*[36] For the ME of Tunisian *E. alata* seeds, quercetin (26.42%), naringin (19.95%), caffeic acid (14.77%), epicatechin (13.46%), quinic acid (7.76%), rutin (7.13%), cirsiliol (4.54%), and quercetrin (1.38%) were the predominant phenolic components.[9] In addition, kaempferol 3-*O*-rhamnoside (0.046%) and isorhamnetin *O*-glucoside-*O*-rhamnoside (0.2%) have been detected in the ME of *E. alata* pulp.[13]

Essential oils (EOs)

The EO yield of the *E. alata* aerial parts has been reported to be 1.79% by Chouitah.[46] In their study, the Eos of the aerial parts of *E. alata* mainly contained β-pinene (42.57%), α-terpinyl acetate (28.85%), β-selinene (10.88%), borneol (7.56%), and β-cadinene (4.23%).[46] Moreover, Jerbi *et al.* have reported that linalool (19.3%), (*Z*)-3-tridecene (7.8%), *n*-pentadecane (7.6%), and 1,8-cineole (7.1%) were the main compounds of *E. alata* stems.[37] Meanwhile, Mighri *et al.* have reported that hydrocarbons (pentatriacontane (25.80%), docosane (10.50%), and tetracosane (6.57%)) represented the largest group of volatile compounds, followed by alcohols (phytol (6.37%) and benzene ethanol (4.76%)) in the fresh aerial parts of *E. alata*.[24]

Other constituents

E. alata yielded the furanofuran lignan (±)-syringaresinol and the digalloylglucose nilocitin.[44] A total of 14 acids were extracted from the dichloromethane extract of *E. alata* leaves, including 2-propenoic acid, 3-phenylbenzoic acid, 3-phenylmethyl-1,2-hexadecanoic acid, benzene-acetic acid, α-hydroxybenzene-dicarboxylic acid, benzoic acid 4-hydroxyacid ethyl ester, and benzene propanoic acid.[38]

Pharmacological effects and molecular mechanisms of *E. alata*

Table 2 illustrates the different pharmacological activities of *E. alata*.[9–13,24,31,33,34,36–39,47–59]

Antimicrobial activity

The EOs of *E. alata* aerial parts have shown an interesting antibacterial effect against four types of bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, and *Bacillus cereus*). *E. alata* EO is rich in β-pinene (42.52%), α-terpinyl acetate (28.85%), β-selinene (10.88%), and borneol (7.56%), as reported by Chouitah.[46] However, the antibacterial effect of the EO from *E. alata* stem was due to the presence of linalool (19.30%), (*Z*)-3-tridecene (7.80%), *n*-pentadecane (7.60%), and 1,8-cineole (7.10%) as major components.[37] The acetonitrile extract of *E. alata* stem has been investigated for its potent antimicrobial activity against four types of bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*) and four types of fungi (*Penicillium italicum*, *Aspergillus fumigatus*, *Candida albicans*, and *Syncephalastrum racemosum*). Thin-layer chromatography has been used to separate components in the acetonitrile extract. Alatine, a new benzenedicarboxylic dinonyl ester compound, was identified in the acetonitrile extract of *E. alata* stem as having potent antimicrobial activity.[39] Meanwhile, the aqueous extract of *E. alata* was also found to be effective in controlling the growth rate and conidial production by *Aspergillus flavus*.[23,26] Moreover, the antibacterial activity of flavonoid extracts from *E. alata* flowers and leaves using different solvents (dichloromethane, ethyl acetate, and butanol) was carried out on Gram-positive (*Enterococcus faecalis, Bacillus subtilis*, and *Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa, Escherichia coli*, and *Serratia marcescens*) pathogenic bacteria. The results showed that the butanol extract of *E. alata* flowers was active against Gram-positive bacteria, whereas it was ineffective against *Serratia marcescens*. Furthermore, the ethyl acetate extract (EAE) and dichloromethane extract of *E. alata* flowers were effective in all bacteria strains tested. The butanol extract of *E. alata* leaves was effective against all bacterial strains except *S. marcescens* and *E. faecalis*. The EAE and dichloromethane extract of *E. alata* leaves were effective only against *S. aureus*, *B. subtilis*, and *B. cereus*.[60] In a study by Danciu *et al.*,[47] the Gram-positive bacteria *S. aureus* and the fungi *Candida* spp. (*C. albicans* and *C. parapsilosis*) were the most sensitive strains to the ethanolic extract of *E. alata* aerial parts compared to the other Gram-positive (*E. faecalis*) and Gram-negative (*Salmonella enterica*, *Klebsiella pneumonia*, *P. aeruginosa, E. coli*, and *Shigella flexneri*) strains. According to Alshalmani *et al.*,[48] the ME derived from *E. alata* aerial parts had significant antibacterial activity against both Gram-positive strains (*B. subtilis* and *S. aureus*) and Gram-negative strains (*P. aeruginosa* and *E. coli*), while no antifungal effects were observed against *A. flavus* and *C. albicans.* Recently, the main phenolic compounds identified in *E. alata* extract were kaempferol (15.55 µg/mg) and quercetin (2.63 µg/mg). Several studies have determined the potent antimicrobial activity of kaempferol and quercetin.[61–66] These compounds might exert their microbial inhibitory effects by influencing the functionality and structure of the cell membrane. Their ability to cross cell membranes allows them to penetrate the cell and interact with crucial intracellular sites, including enzymes and proteins, ultimately resulting in cell death.[67]

Antioxidant activity

The antioxidant activity of *E. alata* was evaluated by 2,2-diphenyl-1-picrylhydrazyl,[9–13,24,31,33,36,48,49–57] 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid,[9,49,50,54,55] chelating power,[13,33] cupric reducing power,[50,52,55] ferric reducing ability of plasma,[11,24,50] reducing power,[9,12,33,50,52,53,55] thiobarbituric acid reactive substances,[52] ammonium molybdate,[51] phosphomolybdenum,[9,12,25,33] β-carotene bleaching,[33,52–57] dimethylsulfoxide alkaline,[57] reducing silver nanoparticle,[57] *O*-phenonthroline,[57] galvinoxyl radical,[57] hydroxyl radical,[57] and superoxide anion assays.[12,68] In these studies, the main contributors to the antioxidant potential were phenolic compounds. Numerous phenolic compounds have been discovered in the extract of *E. alata*,[9,12,13,26,50,51,55–57,67–69] representing different classes but mainly phenolic acids (quinic acid, chlorogenic acid, coumaric acid, *trans*-cinnamic acid, and gallic acid) and flavonoids (hydroxypuerarin isomer 1, herbacetin derivatives, isovitexin derivatives, quercetin derivatives, myricetin derivatives, kaempferol derivatives, and luteolin derivatives). In addition to phenolic compounds, the alkaloid extract (AE) of *E. alata* aerial parts was found to possess a significant reducing power. The main alkaloid of *E. alata* aerial parts was determined to be pseudoephedrine (51.85%), which forms ephedrine as the most active constituent of this plant.[23] Soua *et al.* also investigated the antioxidant activity of polysaccharides extracted from *E. alata* stem.[33] The EO of *E. alata* stem has been studied for its antioxidant activity using a 2,2-diphenyl-1-picrylhydrazyl assay. This antioxidant activity might arise from the presence of a significant percentage of the main components or synergy among different EO constituents, particularly linalool (19.30%), (*Z*)-3-tridecene (7.8%), *n*-pentadecane (7.6%), and 1,8-cineole (7.1%), as major components.[37] The mechanisms involved in the antioxidant assays varied, depending on whether these different bioactive compounds participated as antioxidants by suppressing reactive oxygen species and forming stable products.[12,13] Generally, they were antioxidants in one or more of the following pathways: direct reaction with reactive oxygen species/reactive neutral species, inhibition of oxidant enzymes, interaction with redox signaling pathways, and chelation with transitional metals.[70]

Anti-inflammatory activity

The utilization of *E. alata* extract as an anti-inflammatory remedy may be attributed to its substantial antioxidant potential as well as

Table 2. Pharmacological activities of *Ephedra alata*

Bioactivity	Extract	Part used	Bioactive compound	Potency	Reference
Antimicrobial activity	Essential oil	Aerial parts	Terpenes (β-pinene, α-terpinyl acetate, β-selinene, and borneol)	An interesting antibacterial effect for four bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*)	37
	Acetonitrile extract	Stems	Alatine	Potent antimicrobial activity against four bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli*) and four fungi (*Aspergillus fumigatus*, *Penicillium italicum*, *Syncephalastrum racemosum*, and *Candida albicans*)	39
	Butanol extract	Flowers	Flavonoids	Active against *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*	38
	Ethyl acetate and dichloromethane extracts		Flavonoids	Effective against Gram-positive (*B. subtilis*, *E. faecalis*, and S. *aureus*) and Gram-negative (*E. coli*, *P. aeruginosa*, and *S. marcescens*) strains	42
	Ethanolic extract	Aerial parts	Flavonoids	Sensitive against the Gram-positive bacteria *S. aureus* and the fungi *Candida* spp. (*C. albicans* and *C. parapsilosis*) as compared to the other Gram-positive (*E. faecalis*) and Gram-negative (*K. pneumonia*, *S. flexneri*, *Salmonella enterica*, *E. coli*, and *P. aeruginosa*) strains	47
	Methanolic extract	Aerial parts	Phenols (kaempferol and quercetin)	Significant antibacterial activity against Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *P. aeruginosa*) strains, while there was no antifungal effect against *A. flavus* or *C. albicans*	48
Antioxidant activity	Methanolic extract; Ethanolic extract; Aqueous extract; Ethyle acetate extract	Aerial parts, leaves, flowers, and stems	Phenolic acids (chlorogenic acid, coumaric acid, trans-cinnamic acid, and gallic acid) and flavonoids (hydroxypuerarin isomer 1, herbacetin derivatives, isovitexin derivatives, quercetin derivatives, myricetin derivatives, kaempferol derivatives, and luteolin derivatives)	Antioxidant activity using DPPH, ABTS, chelating power, cupric reducing power, FRAP, TBARS, ammonium molybdate, phosphomolybdenum, and β-carotene bleaching, dimethyl sulfoxide, alkaline, reducing silver nanoparticle, *O*-phenonthroline, galvinoxyl radical, and hydroxyl radical assays	9–13,24, 31,33,36, 48,49–57
	Alkaloid fraction	Aerial parts	Pseudoephedrine	Reducing power activity	36
	Polysaccharide fraction	Stems	Polysaccharides	Good antioxidant activity	33
	Essential oil	Stems	Linalool, (*Z*)-3-tridecene, *n*-pentadecane, and 1,8-cineole	Good antioxidant activity using a DPPH assay	37
Anti-inflammatory activity	Ethyl acetate extract	Aerial parts	Isoquercetin and rutin	Important anti-inflammatory effect inhibiting nitric oxide (62% at 50 mg/mL)	55

(continued)

Table 2. *(continued)*

Bioactivity	Extract	Part used	Bioactive compound	Potency	Reference
Anticancer activity	Hydroethanolic extract	Aerial parts	Kaempferol and quercetin	Potential cytotoxic effect against the human breast cancer cell line MCF-7	47
Antidiabetic activity	Ethyl acetate extract	Leaves	Flavonoids	High activity toward key enzymes related to hyperglycemia such as α-amylase (IC_{50} = 0.28 mg/mL)	58
Antihypertensive activity	Water-soluble polysaccharide extract	Stems	Glucose, galactose, mannose, arabinose, and gluconic acid	Effective against angiotensin I-converting enzyme inhibitors for the treatment and prevention of hypertension	33
Anti-obesity activity	Methanol extract	Leaves	Phenols (quinic acid, apigenin-derivatives, erydictiol-*O*-hexoside, quercetin derivatives, and rosmarinic acid hexoside)	Stronger inhibitory activity against key enzymes related to obesity such as lipase (IC_{50} = 1.296 mg/mL)	58
Nephroprotective activity	Alkaloid extract	Aerial parts	Alkaloids (ephedrine, pseudoephedrine, methylephedrine, and methylpseudoephedrine)	Reduce kidney damage caused by cisplatin by reducing the level of oxidative stress and improving the antioxidant capacity of the body	34
Hepatoprotective activity	Alkaloid extract	Aerial parts	Alkaloids (ephedrine, pseudoephedrine, methylephedrine, and methylpseudoephedrine)	Decreased the liver damage caused by cisplatin by reducing the oxidative stress and improving the antioxidant activity of the body	34
Antipyretic activity	Methanol extract	Leaves	Flavonoids (diazein, epicatechin, rutin, quercitin, and myricetin derivatives) and alkaloids (ephedrine, pseudoephedrine, and ephedroxane)	After administration of the extract, the antipyretic effect started from the second hour, and the effect was maintained for 4 h.	58
Analgesic activity	Methanol extract	Leaves	Flavonoids, namely diazein, epicatechin, rutin, quercitin, and myricetin derivatives	A significant diminution effect of cramping in a dose-dependent manner (49.60 and 55.86%, respectively) as compared to the control	58
	Methanol extract	Leaves	Alkaloids (ephedrine, pseudoephedrine, and ephedroxane)	Analgesic effect on acetic acid-induced pain	59
Anti-acetylcholinesterase and anti-butyrylchlonesterase activity	Hydromethanol extract	Aerial plants	Phenolic acids and flavonoids (caffeic acid, gallic acid, apigenin, quercetin, luteolin, and kaempferol)	An impressive inhibitory potential of both AChE (IC_{50} = 22.46 µg/mL) and BChE (IC_{50} = 28.91 µg/mL) activities compared to the positive control galantamine ($IC_{50\ AchE}$ = 6.26 µg/mL and $IC_{50\ AbhE}$ = 28.91 µg/mL)	57
Antityrosinase activity	Hydromethanol extract	Aerial plants	Flavonoids (apigenin, quercetin, luteolin and kaempferol)	A remarkable tyrosinase blocking activity (IC_{50} = 38.04 µg/mL) compared to the positive control kojic acid (IC_{50} = 25.23 µg/mL).	57
Anti-urease activity	Hydromethanol extract	Aerial plants	Flavonoids (apigenin, quercetin, luteolin, and kaempferol)	A blocking property of the urease catalytic site (IC_{50} = 25.23 µg/mL) compared to the positive control (IC_{50} = 11.57 µg/mL)	57

ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); AChE, acetylcholinesterase; BChE, butyrylcholinesterase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing ability of plasma; IC_{50}, half-maximal inhibitory concentration; MCF-7, human breast cancer cell line; TBARS, thiobarbituric acid reactive substance.

its ability to suppress the secretion of pro-inflammatory cytokines while promoting the secretion of anti-inflammatory cytokines.[51] Regarding the anti-inflammatory activities of *E. alata* aerial parts, according to Benarba *et al.*,[55] the ME demonstrated the highest stabilization of human red blood cell membranes at 34.72%, whereas the hydraulic extract displayed the highest inhibition percentage for bovine serum at 99.22% and egg albumin denaturation at 73.31%. In a study by Bourgou *et al.*,[53] the EAE of *E. alata* aerial parts showed an important inhibition of the action of nitric oxide (62% at 50 mg/mL), which could be due to the presence of isoquercitrin (7.60 µg/g) and rutin (3.37 µg/g). Isoquercitrin has been identified as an effective suppressor of eosinophilic inflammation, implying its potential in the treatment of allergies.[71] Additionally, Selloum *et al.* have proved the anti-inflammatory effect of rutin on rat paw edema.[72] On the other hand, previous research studies have shown that *Ephedra* alkaloids, such as ephedrine, pseudoephedrine, and ephedroxane, possess a strong anti-inflammatory activity due to their ability to inhibit prostaglandin E2 biosynthesis.[5,73] The administration of these compounds may cause an anti-inflammatory effect by increasing the expression of interleukin-10 and blocking the production of tumor necrosis factor alpha via the phosphatidylinositol 3-kinase/protein kinase B and peptidoglycan pathways. Additionally, these compounds may act on the phosphatidylinositol 3-kinase, protein kinase B, glycogen synthase kinase-3 beta, and p38 pathways.[74–77] Furthermore, cyclooxygenase is the primary enzyme responsible for converting arachidonic acid (generated as a result of cell membrane damage) into prostaglandins. Mufti *et al.* have determined the *in-vitro* inhibition capacity of *E. alata* pulp extract against cyclooxygenase-1 and cyclooxygenase-2, supporting the use of *E. alata* as a potential anti-inflammatory agent.[13]

Anticancer activity

The hydroethanolic extract of *E. alata* aerial parts has potential antiproliferative, pro-apoptotic, and cytotoxic effects against the human breast cancer cell line MCF-7. The hydroethanolic extract of *E. alata* aerial parts was found to be mainly enriched in kaempferol (15.55 µg/mg) and quercitin (2.63 µg/mg), as mentioned by Danciu *et al.,*[47] Kaempferol has been shown to prevent breast tumors.[78] Additionally, quercitin has been demonstrated to target and destroy breast cancer stem cells.[78] In a recent study, the EAE of Tunisian *E. alata* aerial parts possessed anticancer potential against MCF-7 cells using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (half-maximal inhibitory concentration (IC_{50}) = 26 µg/mL) and a resazurin assay (IC_{50} = 16 µg/mL).[53] Alshalmani *et al.* also noted that the ME of Libyan *E. alata* aerial parts had a significant anticancer effect on MCF-7 cells (IC_{50} = 38.7 µg/mL) compared with the positive control doxorubicin (IC_{50} = 28.3 µg/mL).[48] Additionally, Elshibani *et al.* have mentioned that the ME of Libyan *E. alata* had a significant cytotoxic effect against two human cancer cell lines, namely the human liver cancer cell line HEPG2 (IC_{50} = 32.9 µg/mL) and the human prostate cancer cell line PC3 (IC_{50} = 30.4 µg/mL), compared with the positive control doxorubicin ($IC_{50\ HEPG2}$ = 21.6 µg/mL and $IC_{50\ PC3}$ = 23.8 µg/mL).[58] Bensam *et al.* also found that the ethanolic extract of Algerian *E. alata* aerial parts exhibited a good anticancer potential against MCF-7 cells (IC_{50} = 153 µg/mL), HEPG2 cells (IC_{50} = 359.43 µg/mL), and a human colon cancer cell line (IC_{50} = 407.26 µg/mL).[12] The molecular analysis showed that four genes, Bax, p21, retinoblastoma protein, and tumor protein P53, were upregulated in MCF-7 cells treated either with the ethanolic extract of *E. alata* or the drug 5-fluorouracil.[12]

Antidiabetic activity

The EAE of *E. alata* leaves is reported to have a high α-amylase activity (IC_{50} = 0.28 mg/mL). The administration of the EAE from *E. alata* leaves to high-fat-high-fructose diet rats exerted a blocked action on α-amylase activity in the intestine (43%), pancreas (26%), and serum (46%).[72] Meanwhile, Jaradat *et al.* have demonstrated that the ME of *E. alata* fruits had significant inhibitory action against α-amylase (IC_{50} = 43 µg/mL), α-glucosidase (IC_{50} = 9.43 µg/mL), and lipase (IC_{50} = 46.16 µg/mL).[31] Moreover, a study by Lamine *et al.* has revealed that the hydraulic extract of *E. alata* aerial parts had an antidiabetic effect both *in vivo* and *in vitro*.[79] Polyphenols, particularly flavonoids, are bioactive compounds that have a protective effect on diabetes induced by streptozotocin or alloxan.[80] Two targets were characterized as having antidiabetic potential by the examination of protein–ligand interactions via molecular docking,[81] including α-amylase, the pivot enzyme,[80] and lysosomal acid-α-glucosidase.[78]

Antihypertensive activity

E. alata stem polysaccharides were found to be effective angiotensin I-converting enzyme inhibitors for hypertension. The angiotensin I-converting enzyme inhibitory effect of *E. alata* polysaccharides had an IC_{50} = 0.21 mg/mL.[33]

Anti-obesity activity

The inhibition of lipase is among the extensively studied mechanisms employed to limit triacylglycerol absorption, resulting in reduced caloric yield and weight loss.[82,83] Studying the ME of *E. alata* leaves has demonstrated that it had a high inhibition activity on lipase (IC_{50} = 1.296 mg/mL), followed by the water (IC_{50} = 1.639 mg/mL) and ethyl acetate (IC_{50} = 1.897 mg/mL) extracts.[70] In addition, Jaradat *et al.* found that the methanol fraction of *E. alata* fruits had significant inhibitory activity against lipase, with an IC_{50} = 66.48 µg/mL.[31] Moreover, Ziani *et al.* determined that the HME of *E. alata* was mainly enriched in quinic acid, apigenin derivatives, erydictiol-*O*-hexoside, quercetin derivatives, and rosmarinic acid hexoside.[52] According to Duangjai *et al.*, quinic acid has anti-adipogenic and lipolytic properties, suggesting its potential role in anti-obesity effects.[84] Meanwhile, Su *et al.* have elucidated the mechanism of action that underlies the antivisceral obesity effect of apigenin.[85] Furthermore, Nabavi *et al.* have emphasized the role of quercetin in the treatment of obesity.[86] It was also discovered that rosmarinic acid can mitigate obesity and inflammation related to obesity in human adipocytes.[87]

Nephroprotective activity

The nephroprotective effect of the AE from *E. alata* aerial parts on kidney injuries induced by cisplatin has been studied. The kidney damage was restored after treatment with the AE of *E. alata* aerial parts, which were rich in ephedrine, pseudoephedrine, methylephedrine, and methylpseudoephedrine.[35] In fact, Westman *et al.* have documented that the continuous infusion of ephedrine appeared to have favorable impacts on the renal function of patients after elective major vascular surgery.[88] Utilizing molecular docking to investigate the molecular mechanism of the herb *Ephedra* in treating nephrotic syndrome has led to the deduction that active compounds like luteolin, kaempferol, naringenin, and quercetin exhibit good binding with the target protein tumor necrosis factor or protein kinase B. Among them, luteolin and naringenin have

demonstrated binding affinity with protein kinase B.[15]

Hepatoprotective activity

The hepatoprotective effect of the AE from *E. alata* aerial parts (150 mg/kg) on liver injuries induced by cisplatin (20 mg/kg) has been investigated. Mice treated with the AE of *E. alata* aerial parts exhibited reduced aspartate aminotransferase and alanine aminotransferase activities. The AE of *E. alata* was also found to be rich in ephedrine, pseudoephedrine, methylephedrine, and methylpseudoephedrine.[34] Additionally, Wu *et al.* found that pseudoephedrine/ephedrine showed strong anti-inflammatory activity against tumor necrosis factor alpha-mediated acute liver failure induced by lipopolysaccharide/D-galactosamine.[89,90]

Antipyretic activity

The antipyretic activity of the ME of *E. alata* leaves was studied by Tiss *et al.* in mice having a fever of 39°C.[58] The antipyretic effect started in the second hour and persisted for a duration of 4 h after the administration of the extract. The obtained antipyretic effect of the *E. alata* extract was likely due to the presence of flavonoids (diazein, epicatechin, rutin, quercitin, and myricetin derivatives) and alkaloids (ephedrine, pseudoephedrine, and ephedroxane). These flavonoids and alkaloids might function by blocking the synthesis of prostaglandin E2 (a peripheral fever mediator) through the inhibition of prostaglandin synthesis.[16]

Analgesic activity

The analgesic potential of the ME of *E. alata* leaves (100, 200, and 400 mg/kg) has been studied by Tiss *et al.* in mice that received acetic acid (10 mL/kg) to induce the writhing reflex.[58] The highest doses of 200 mg/kg and 400 mg/kg of the ME of *E. alata* leaves provoked a cramp decrease (49.60% and 55.86%, respectively) compared to the control. Any substance capable of inhibiting acetic acid-induced writhing may potentially possess anti-inflammatory and analgesic effects.[91] As reported by Hyuga *et al.*,[59] the analgesic effects of bioactive drug components in the ME of *Ephedra*, such as ephedrine, pseudoephedrine, and ephedroxane, have been demonstrated on acetic acid-induced pain. Another study by Hyuga *et al.* has demonstrated that herbacetin, a component of the herb *Ephedra*, can alleviate formalin-induced pain.[92] Moreover, the *E. alata* extract was characterized by the presence of flavonoids, namely diazein, epicatechin, rutin, quercitin, and myricetin derivatives.[65] The majority of these compounds have been reported to have antipyretic, anti-inflammatory, and analgesic effects.[92–94]

Anti-acetylcholinesterase activity

Noui *et al.* have studied the acetylcholinesterase (AChE) inhibitory activity of the ME of *E. alata* aerial parts *in vitro* and showed that it inhibits AChE activity (IC_{50} = 11.25 µg/mL).[54] Khattabi *et al.* also noted that the HME of *E. alata* aerial parts had an impressive inhibitory AChE action (IC_{50} = 22.46 µg/mL) compared to the positive control galantamine (IC_{50} = 6.26 µg/mL).[57] The HME of *E. alata* aerial parts was found to be mainly rich in phenolic acids (caffeic acid and gallic acid) and flavonoids (quercetin, apigenin, kaempferol, and luteolin), which exhibit an important inhibitory AChE action.[57] AChE inhibition decreased the level of acetylcholine degradation and increased its concentration in the brain. AChE serves as an enzyme that hydrolyzes acetylcholine by terminating cholinergic neurotransmission.[14]

Antityrosinase activity

Tyrosinase is a key enzyme in melanin biosynthesis, and its inhibitors are frequently used as hypopigmenting agents.[95] Söhretoglu *et al.* have noted that flavonoids can be considered as important constituents for tyrosinase inhibitor drugs.[96] In addition, the HME of *E. alata* aerial parts had high amounts of flavonoids and showed high tyrosinase inhibition (IC_{50} = 38.04 µg/mL). The positive control kojic acid had an IC_{50} = 25.23 µg/mL.[57]

Anti-urease activity

Urease is a necessary enzyme for *Helicobacter pylori* colonization in the acidic milieu of the stomach, which causes gastrointestinal diseases. This enzyme catalyzes the hydrolysis of urea into carbon dioxide and ammonia. It is a key enzyme benefiting bacteria by making its persistence possible; thus, it causes gastritis as well as duodenal cancer, peptic ulcers, and gastric cancer.[97,98] Flavonoids are considered excellent inhibitors of urease activity.[96,99] As the HME of *E. alata* aerial parts are rich in flavonoids, it was found to inhibit the urease catalytic site with an IC_{50} = 25.23 µg/mL, compared to the positive control with IC_{50} = 11.57 µg/mL.[57]

Toxicity of *E. alata*

The toxicity of *Ephedra* species can be attributed to the presence of ephedrine alkaloids.[34] For example, Boubekri *et al.* have noted the intoxication of a 70-year-old female patient who, after ingestion of a broth of this plant at indeterminate doses due to influenza, had disorders of consciousness.[100] These authors highlighted the importance of evoking this diagnosis, raising awareness, and combating the trivialization of its consumption. Moreover, Sioud *et al.* have reported the acute toxicity of *E. alata* to mice by determining the seven-day median lethal dose to be 500 mg/kg; therefore, it can be toxic if incorrectly dosed.[34]

Conclusion

In summary, *E. alata* has historically occupied an important role in the treatment of several illnesses. Additionally, the number of studies on the phytochemical composition and pharmacological effects of *E. alata* continues to grow annually, offering fresh perspectives on understanding its composition and clinical applications.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

WAW and MST conceived, designed the study, and wrote the manuscript. WAW reviewed and edited the manuscript. All research was performed by the authors.

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