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REVIEW ARTICLE

Nutraceutical Potential of Molokhia (*Corchorus olitorius* L.): A Versatile Green Leafy Vegetable

Faiyaz Ahmed

Department of Clinical Nutrition, College of Applied Health Sciences in Ar Rass, Qassim University, Al Qassim Region, Saudi Arabia

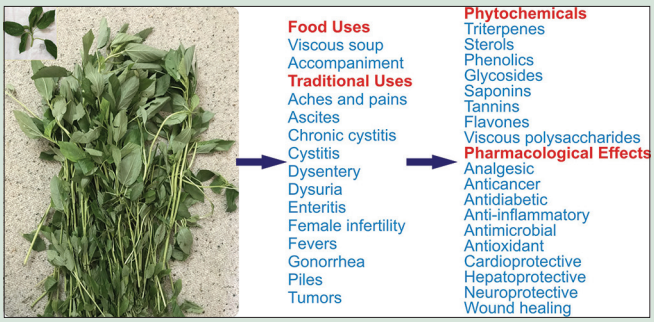
ABSTRACT

Molokhia is a nutritious green leafy vegetable consumed in African and Middle Eastern countries as a viscous soup. Although the leaves are used in the traditional medicine and also reported to exhibit a number of pharmacological effects, its utilization is limited to some cultures and does not find wider utilization in the mainstream dietary habits. This review is aimed to present the nutritional and nutraceutical potential of molokhia to promote consumption of this valuable leafy vegetable. An unbiased literature search was conducted using online resources such as Google Scholar and PubMed to collect published reports on various biological/pharmacological activities of molokhia. Chemical structures of bioactive compounds were downloaded from PubChem. The leaves of molokhia are rich sources of Vitamin A (β-carotene), C, E, B1, B2, folic acid and minerals such as iron and calcium in addition to common macromolecules. Among carbohydrates, acidic polysaccharides are of particular interest because of their notable biological effects including antidiabetic and antioxidant. The vegetable is also a good source of a diverse category of phytochemicals including alkaloids, saponins, tannins, terpenes, flavonoids, and phenolics. Different extracts exhibit potent antidiabetic, antioxidant, antiinflammatory, anticancer, antimicrobial, hepatoprotective, cardioprotective, neuroprotective, analgesic, and wound healing effects. The extracts have shown to safe even at a dose of 3.2 g/kg body weight in experimental animals. Molokhia is a nutritious leafy vegetable loaded with essential micronutrients and phytochemical that could be handy in promoting general health. Further research is warranted to develop novel food product formulation using molokhia.

Key words: *Corchorus olitorius,* green leafy vegetable, molokhia, pharmacology, polysaccharides, toxicity

SUMMARY

- Molokhia is a leafy vegetable widely consumed in Middle-Eastern countries and valued for its nutrient composition. The leaves find its use in folkloric medicine for the treatment of a number ailments and have been ascribed with a diverse pharmacological activities due to presence of polysaccharides and phenolic compounds. This review would be beneficial to popularize the utilization of molokhia as functional food ingredient in food formulations.



Abbreviations Used: ASL: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; MDA: Malonaldehyde; CCl_4: Carbon tetrachloride; LD_{50}: Median lethal dose; IC_{50}: Median inhibitory concentration; OECD: Organization for economic co-operation and development; SOD: Superoxide dismutase; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; FRAP: Ferric reducing antioxidant potential; DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; BHT: Butylated hydroxy toluene; BHA: Butylated hydroxy anisole; TBARS: Thiobarbituric acid reactive substances; GSH: Glutathione.

Correspondence:
Dr. Faiyaz Ahmed,
Department of Clinical Nutrition, College of Applied Health Sciences in Ar Rass, Qassim University, Al Qassim Region 51921, Saudi Arabia.
E-mail: f.masfoor@qu.edu.sa

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INTRODUCTION

Corchorus olitorius L. commonly known as molokhia in Middle-Eastern countries is an annual herbaceous plant with slender stem belonging to Tillaceae family [Table 1]. Although molokhia is believed to be native to Africa and Asia, it is cultivated in a number of countries, including Australia, South America, and some parts of Europe for food and industrial use.[1,2] Molokhia is used as green leafy vegetable in Egypt, Sudan, India, Bangladesh, Philippines and Malaysia, Africa, Japan, South America, the Caribbean, and Cyprus.[3]

Molokhia is valued as a nutritious leafy vegetable due to its high vitamin, mineral, and phenolic content.[4] The leaves also contain high amounts of mucilaginous polysaccharides with gives viscous consistency and widely consumed as soup in Middle Eastern countries. Dried leaves are used in herbal tea, while seeds are used as flavoring agent.[5] Apart from its food uses, it is valued as an herbal remedy in fevers, enteritis, dysentery, chronic cystitis, aches, and pains.[6] Different parts of *C. olitorius* are reported to exhibit a range of biological effects antimicrobial, antidiabetic, antihistaminic, cardioprotective, hepatoprotective, nephroprotective, anticonvulsant, antiestrogenic, and antimalarial effects.[7-16]

The leaves are reported to contain triterpenes, sterols and fatty acid, phenolics, ionones, oxydase, chlorogenic acid, glycosides, saponins, tannins, and flavones in addition to carbohydrates, protein, fat, fiber,

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Table 1: Taxonomy of *Corchorus olitorius* Linn.

Kingdom	Plantae
Phyllum	Angiosperms
Subphyllum	Eudicots
Class	Rosids
Order	Malvales
Family	Malvaceae
Genus	*Corchorus*
Species	*Olitorius*

Table 2: Nutrient composition of molokhia leaves

Nutrient	Raw
Moisture (%)	82-87
Protein (%)	5-6
Carbohydrate (%)	5-7
Fat (%)	0.3-1
Ash (%)	2.4-2.6
Fiber (%)	1-5
Vitamin A (IU)	3000
Thiamine (mg/100 g)	0.1
Riboflavin (mg/100 g)	0.3
Niacin (mg/100 g)	1.5
Vitamin C (mg/100 g)	10-100
Iron (mg/100 g)	4-8
Calcium (mg/100 g)	250-266

ash, acidic polysaccharides, lignin, and other[17-20] mucilaginous polysaccharides. Nutrient composition of molokhia leaves is presented in Table 2.[4,20,21] The leaves are rich sources of Vitamin A (β-carotene), C, E, B1, B2, folic acid, and minerals such as iron and calcium.[22-27] Among soluble polysaccharides abundantly present in molokhia leaves, an acidic polysaccharide rich in uronic acid rhamnose, glucose, galcturonic acid, and glucuronic acid has been isolated.[5] It is noteworthy that, molokhia leaves retain key nutrients even after cooking.[28] The seeds contain alkaloids, flavonoids, tannins, cardiac glycosides, steroids, saponins, and anthraquinones.[29] The roots reportedly contain triterpenes including corosin and sterols including sitosterol and stigmasterol.[19,30,31] It is noteworthy that molokhia is also known to contain antinutritional factors including phytates, hydrocyanic acid, oxalic acid, and tannins.[27,32-34] This review is aimed to provide a comprehensive account of molokhia in terms of its food/traditional uses, bioactive compounds, and biological effects in view of the many recent findings on this leafy vegetable. An unbiased literature search was conducted using online resources such as Google Scholar and PubMed to collect published reports on various biological/pharmacological activities of *C. olitorius* to serve as a collective reference for the researchers to undertake intensive research leading to the development of molokhia as a novel nutraceutical ingredient.

DESCRIPTION AND DISTRIBUTION

Molokhia is an annual herb up to 2–4 m tall with tough and fibrous stems [Figure 1]. It is commonly known as Jew's mallow, bush okra, long-fruited jute, tossa jute, and jute.[35] Leaves alternate, simple; stipules narrowly triangular with long point; petiole 1–7 cm long; blade narrowly ovate, ovate or elliptical, 4–15 cm × 2–5 cm, cuneate or obtuse and with setaceous appendages up to 2.5 cm long at base, acuminate to acute at apex, margin serrate or crenate, almost glabrous, usually shiny dark green, 3–7-veined from the base. Inflorescence a 1–4-flowered axillary fascicle, bracteate. Flowers bisexual, regular, usually 5-merous, shortly stalked; sepals free, narrowly obovate, 5–7 mm long; petals free, obovate, 5–7 mm long, yellow, caducous; stamens numerous; ovary superior, usually 5-celled, style short from a cylindrical capsule up to 7–10 cm long, ribbed, with a short beak, usually dehiscing by 5 valves, many-seeded. Seeds angular, 1–3 mm long, dark grey. Seedling with epigeal germination; hypocotyl 1–2 cm long; cotyledons foliaceous, broadly elliptical to circular, 3–8 mm long.[36]

There is no consensus on the origins of *C. olitorius* because it has been cultivated and used in Africa and Asia for centuries and also occurs in the wild in both continents.[1,2] Currently, since it is used as a leafy vegetable, it occurs in all of tropical Africa including Benin, Nigeria, Ivory Coast, Cameroon, Sudan, Kenya, Uganda, and Zimbabwe. It is a popular soup vegetable in Caribbean, Cyprus, Brazil, India, Bangladesh, Sri Lanka, China, Japan, Philippines, Malaysia, Egypt, and the Middle Eastern countries. Apart from food use, it is grown as a commercial crop for jute production in India, Bangladesh and China.[37]

FOOD USES

Molokhia leaves contains high amounts of mucilaginous polysaccharides which yield viscous soup when cooked and usually used as an accompaniment for main dishes.[38] In Middle Eastern countries, the leaves are cut into small pieces and boiled in water with salt and pepper to make soup. Molokhia soup is very popular in the Middle east. In Mediterranean regions, young green leaves and shoots are used to add flavor and viscous texture to soups and stews. Seeds are used for flavoring. Tender leaves and shoots are also eaten raw as salad vegetable in Egypt and India.[39] Dried leaves are used in the preparation of herbal tea. The leaves are used to prepare a stew called "ewedu" in Nigeria, while in Philippines the leaves along with bamboo shoots are consumed as a leafy vegetable. In Nigeria, sticky sauce comparable to okra is prepared and eaten as an accompaniment for starchy dumplings made from cassava, yam or millet. Since, Molokhia is an annual herb dried leaf powder is used to make this sauce during off season. Sauce is also prepared from powdered and dried immature fruits (bush okra). In East Africa, it is cooked with cowpeas, pumpkin, cocoyam leaves, sweet potato, milk, butter, and meat flavored with pepper and lemon.[40,41] Recently, molokhia leaves are also used for the development of Sushi wrap as a promising viable substitute for Nori.[42]

TRADITIONAL AND FOLKLORIC USES

The leaf extract of the plant is also employed in folklore medicine in the treatment of gonorrhea, pain, fever, and tumors. Its leaves and roots are eaten as herbal medicine in South east Asia.[33] In some part of Nigeria, its leaves decoctions are used for treating iron deficiency, folic acid deficiency, as well as treatment of anemia. Leaves are used in ascites, pains, cystitis, piles, dysentery, dysuria, pectoral pain, tumors, gonorrhea, and female infertility.[27,43] The leaves are particularly used as an herbal medicine in typhoid and malarial fevers.[38] Leaves are also used as blood purifier and leaf twigs are used cardiac problems while, leaf infusion is taken as a tonic and appetite enhancer. The leaves are also used in the treatment of constipation in Tanzania.[43-45] In Benin leaves are used as tonic, diuretic, emollient, as blood purifier, in heart disease and infantile malnutrition.[46] Root scrapings are used to treat toothache, while decoction of the roots is used as a tonic to increase strength. In Nigeria, seeds are used as purgative and febrifuge.[47]

PHARMACOLOGICAL PROPERTIES

Different parts of *C. olitorius* have been reported to exhibit a wide range of biological activities *in vitro* and *in vivo* which are attributed to its phytochemical composition. Some of the important phytochemicals found in molokhia leaves and seeds are presented in Table 3. Chemical structures of some important bioactive compounds present in molokhia leaves are presented in Figures 2 and 3.

Table 3: Important phytochemicals found in different parts of molokhia

Compound	Plant part	Reference
(6S,9R)-roseoside	Leaves	[19]
3,4-di-O-caffeoylquinic acid	Leaves	[48,49]
3,5-dicaffeoylquinic acid	Leaves	[18,19]
4-O-caffeoylquinic acid	Leaves	[48,49]
Alkaloids	Leaves	[21]
Apigenin-7-O-glucoside	Leaves	[48,49]
Apigenin	Leaves	[48,49]
Astragalin	Leaves	[19]
Betulabuside A	Leaves	[19]
Caffeic acid	Leaves	[48,49]
Campesterol	Leaves	[50]
Carvacrol methyl ether	Leaves	[50]
Cedran-5-one	Leaves	[50]
Chlorogenic acid	Leaves	[18,19]
Cholesterol	Leaves	[50]
Cichoriine	Leaves	[19]
Cirsilineol	Leaves	[48,49]
Cirsiliol	Leaves	[48,49]
Cis-β-dihydroterpineol	Leaves	[50]
Corchoiononside A	Leaves	[19]
Corchoiononside B	Leaves	[19]
Corchoiononside C	Leaves	[19]
Corosolic acid	Leaves	[51]
Eicosane	Leaves	[50]
Ethyl salicylate	Leaves	[50]
Ferulic acid	Leaves	[48,49]
Gingerol	Leaves	[48,49]
Heptadecane	Leaves	[50]
Heptadecanoic acid	Leaves	[50]
Hexadecane	Leaves	[50]
Isobutyl salicylate	Leaves	[50]
Isochlorogenic acid	Leaves	[18,19,52]
Isoquercetin	Leaves	[19]
Jugalanin (kaempferol 3-O-α-L-arabinopyranoside)	Leaves	[17]
Kaempferol	Leaves	[48,49]
Linoleic acid	Leaves	[50]
l-menthone	Leaves	[50]
Luteolin	Leaves	[48,49]
Methyl tiglate	Leaves	[50]
Myricetin	Leaves	[48,49]
Naringenin	Leaves	[48,49]
Naringin	Leaves	[48,49]
Nonadecane	Leaves	[50]
Octadecane	Leaves	[50]
Oleanolic acid	Leaves	[17]
Oxocorocin	Leaves	[51]
Palmitic acid	Leaves	[50]
P-coumaric acid	Leaves	[48,49]
Piperonal	Leaves	[50]
Protocatchuic acid	Leaves	[48,49]
Quercetin	Leaves	[48,49]
Quercetin-3-(6-malonylgalactoside)	Leaves	[17,18]
Quercetin-3-galactoside	Leaves	[17,18]
Quinic acid	Leaves	[48,49]
Rosmarinic acid	Leaves	[48,49]
Rutin	Leaves	[48,49]
Saponins	Leaves	[21,53,54]
Scopolin	Leaves	[19]
Stearic acid	Leaves	[50]
Stigmasterol	Leaves	[50,55]
Tannins	Leaves	[49,53,54]
Tetradecane	Leaves	[50]
Tolifolin (kaempferol 3-O-β-D-galactopyranoside)	Leaves	[17]
Trans-cis-Farnesol	Leaves	[50]
Trans-Phytol	Leaves	[50]

Contd...

Table 3: Contd...

Compound	Plant part	Reference
Trans-β-dihydroterpineol	Leaves	[50]
Vanillic acid	Leaves	[48,49]
β-amyrin	Leaves	[50]
β-sitosterol	Leaves	[50,55]
1,2-benzenedicarboxylic acid	Leaves and flowers	[56]
1,2-benzenedicarboxylic acid, dibutyl ester	Leaves and flowers	[56]
Aldehyde	Leaves and flowers	[56]
Benzaldehyde	Leaves and flowers	[56]
Camphene	Leaves and flowers	[56]
Cyclohexane	Leaves and flowers	[56]
Fraxinellone	Leaves and flowers	[56]
Geranyl isobutyrate	Leaves and flowers	[56]
Geranyl propionate	Leaves and flowers	[56]
Germacrene D	Leaves and flowers	[56]
Heneicosane	Leaves and flowers	[56]
Hexadecanoic acid	Leaves and flowers	[56]
Hexanoic acid, methyl ester	Leaves and flowers	[56]
Hexanone	Leaves and flowers	[56]
Hexenyl benzoic acid	Leaves and flowers	[56]
Hydrocarbons	Leaves and flowers	[56]
Isoamyl butyrate	Leaves and flowers	[56]
Limonene	Leaves and flowers	[56]
Methyl tiglate	Leaves and flowers	[56]
Nerolidol	Leaves and flowers	[56]
Nonadecane	Leaves and flowers	[56]
Octadecanoic acid	Leaves and flowers	[56]
Phenyl ethyl tiglate	Leaves and flowers	[56]
Sabinene	Leaves and flowers	[56]
Terpenes	Leaves and flowers	[56]
α-phellandrene	Leaves and flowers	[56]
α-pinene	Leaves and flowers	[56]
α-terpinene	Leaves and flowers	[56]
β-cedrene	Leaves and flowers	[56]
β-myrcene	Leaves and flowers	[56]
Ursolic acid	Leaves and seeds	[30,51]
Monogalactosyldiacylglycerol	Leaves/seeds	[10]
Phytol	Leaves/seeds	[10]
4,7-dihydroxycoumarin	Seeds	[57]
Canarigenin	Seeds	[58,59]
Cannogenol-3-O-β-D-glucopyranosyl-(1→4)-O-β-D-boivinopyranoside	Seeds	[59]
Cannogenol-3-O-β-Dglucopyranosyl-(1 4)-O-β-D-digitoxopyranoside	Seeds	[58,59]
Chorchoroside	Seeds	[60]
Chorchoroside B	Seeds	[61]
Chorchorusoside A	Seeds	[62]
Chorchorusoside B	Seeds	[62]
Chorchorusoside C	Seeds	[62]
Chorchorusoside D	Seeds	[62]
Chorchorusoside E	Seeds	[62]
Coroloside (digitoxigenin-3-O-β-D- glucopyranosyl-(1→4)-O-β-D-boivinopyranoside)	Seeds	[58,63-65]
Corosin	Seeds	[31]
Corosolic acid	Seeds	[30]
Deglucocoroloside (digitoxigenin-3-O-β-D-boivinopyranoside)	Seeds	[58,61,64,65]
Digitogenin-3-O-β-Dglucopyranosyl-(1 4)-O-β-D-digitoxopyranoside	Seeds	[58,59]
Digitoxigenin3-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-(1→4)-O-β-D-digitoxopyranoside	Seeds	[58,64,65]
Erysimoside (strophanthidin 3-O-β-D-glucopyranosyl (1→4)-O-β-D-digitoxopyranoside)	Seeds	[58,64,65]
Evatromonoside (digitoxigenin-3-O-β-D-digitoxopyranoside)	Seeds	[58,64,65]
Glucoevatromonoside (digitoxigenin-3-O-β-D-glucopyranosyl - (1→4)-O-β-D-digitoxopyranoside)	Seeds	[58,64,65]
Helveticoside (strophanthidin 3-O-β-D—digitoxopyranoside)	Seeds	[58,64,65]
Olitoriside (strophanthidin 3-O-β-D-glucopyranosyl (1→4)-O-β-D -boivinopyranoside)	Seeds	[58,64,65]
Olotoriside	Seeds	[66]
Oxocorocin	Seeds	[30]
Periplagerin-3-O-β-D-glucopyranosyl-(1 4)-O-β-Ddigitoxopyranoside	Seeds	[58,59]
periplogenin-3-O-β-D-glucopyranosyl-(1→4)-O-β-D-digitoxopyranoside	Seeds	[59]
Strophanthidin trioside	Seeds	[63]

Figure 1: Molokhia (*Corchorus olitorius* L.)

Antidiabetic activity

A number of studies have evaluated the antidiabetic potential of molokhia leaves and seeds both as supplements and extracts. A stearic acid ethyl ester with potent antidiabetic activity has also been isolated from seeds of molokhia.[67]

Antidiabetic effect of methanolic extract from leaves at doses of 100 and 200 mg/kg caused a significant reduction in blood glucose levels in streptozotocin-induced diabetic rats. The study also evidenced that, oral administration of the extract led to notable reduction of serum cholesterol, triglycerides, total protein and transaminases (aspartate transaminase [AST] and alanine aminotransferase [ALT]) associated with an increase in high-density lipoprotein (HDL) and body weight. The observed antidiabetic was attributed to the presence of gallic acid.[68] In another study, molokhia leaf powder mixed with the diet was fed to alloxan-induced diabetic rats over 14 days significantly reduced blood glucose, serum cholesterol, triglycerides, and low density lipoprotein (LDL) levels. The study opined that the antidiabetic effect is due to the presence of flavonoids, alkaloids, terpenoids, steroids, and complex carbohydrates.[21] In a similar study, molokhia soup prepared from leaves was fed to streptozotocin-induced diabetic rats and the results revealed a significant reduction in plasma glucose, cholesterol, and triglycerides indicating antidiabetic and hypolipidemic effects.[69] Molokhia soup at a dose of 4.8 g/kg given by gavage for 14 days, reduced fasting blood glucose, total cholesterol, triglycerides, LDL, and thiobarbituric acid reactive substances in streptozotocin-induced diabetic rats. Furthermore, the levels of glutathione (GSH) and superoxide dismutase (SOD) were found to be significantly elevated in molokhia soup fed group indicating potential antidiabetic effect.[70] Oral glucose tolerance test conducted to evaluate the hypoglycemic potential of methanolic extract of molokhia in Swiss albino mice revealed a significant glucose lowering activity. The extract exhibited dose dependent reduction in blood glucose levels to an extent of 18%–51% at the dosage range of 50–400 mg/kg body weight. The observed reduction of blood glucose at 400 mg/kg of extract was better than that of glibenclamide at a dosage of 10 mg/kg body weight. The results support the use of molokhia as a supplement in diabetic subjects.[71]

Olusanya *et al.*[72] evaluated the antidiabetic potential of ethanolic leaf extract in alloxan-induced diabetic rats at doses of 200, 400, and 800 mg/kg after treatment with extract for 14 days, a significant reduction in fasting blood glucose was observed at all the doses tested and the antidiabetic effect at 800 mg/kg dose was comparable with that of glibenclamide (5 mg/kg) in addition to the hypoglycemic effect, the extract also reduced the levels of total cholesterol, triglycerides, LDL, bilirubin, transaminases (AST and ALT), urea, creatinine, and alkaline phosphatase while restoring the levels of total protein, albumin, globulin, and HDL. The observed effects were attributed to the presence of phytochemicals, including flavonoids, tannins, saponins, phenolics, phlobatannin anthraquinones, and cardiac glycosides. Mercan *et al.*[73] reported significant reduction blood glucose levels with 250 mg/kg dose of ethanolic extract of molokhia leaves in streptozotocin-induced diabetic rats. The researchers also reported potent testicular protective effect as indicated by restoration of testicular architecture destroyed by streptozotocin over the study period of 3 weeks. It was concluded that molokhia leaf could be used in the management of diabetes and its complications.

Ethanolic extract of *C. olitorius* seeds was studied for its effect on blood glucose and glycosylated hemoglobin in normoglycemic, glucose challenged and alloxan-induced diabetic Albino rats. Treatment with the extract at 500 mg/kg dosage for 14 days significantly lowered blood glucose and glycosylated hemoglobin levels associated with increased circulating insulin levels in all groups.[29] In continuation of exploring antidiabetic potential of molokhia seeds, the aqueous extract was portioned with hexane, chloroform, ethyl acetate and butanol to yield different fraction and their tested in alloxan-induced diabetic rats at 250 and 500 mg/kg doses. The results indicated that chloroform, ethyl acetate and aqueous fractions showed better antidiabetic activity at the dose of 500 mg/kg and the antidiabetic effect was attributed to the presence of flavonoids, alkaloids and saponins.[74] Further, the chloroform fraction was subjected to column chromatography followed by thin layer chromatography to isolate 3 pure compounds. The antidiabetic effect of the 3 isolated compounds was evaluated in alloxan induced diabetic and compared with that of glibenclamide (0.2 mg/kg) one of the three compounds with significant hypoglycemic activity comparable to that of glibenclamide was found to be stearic acid ethyl ester as confirmed by NMR and GC-MS.[67]

Antioxidant activity

A number of studies have reported different extracts of molokhia leaves containing phenolic and flavonoid compounds to exhibit strong antioxidant activity both *in vitro* and *in vivo*. Antioxidant activity of different extracts of molokhia leaves was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and β-carotene bleaching assay and correlated with total phenolic content of the extracts. All the extracts exhibited significant antioxidant activity in both DPPH radical scavenging assay and β-carotene bleaching assay and the resultant antioxidant activity was directly proportional to the total phenolics content of the extracts.[75] The mucilaginous polysaccharides were isolated from the leaves and were found to be rich in total polyphenols and flavonoids also exhibited significant antioxidant activity as reflected by the results of DPPH radical scavenging activity, lipid peroxidation inhibition and β-carotene bleaching assay. The polysaccharides also effective against hydroxyl radicals and DNA breakage.[76] Similar observations were reported by Hussien *et al.*,[77] wherein, antioxidant

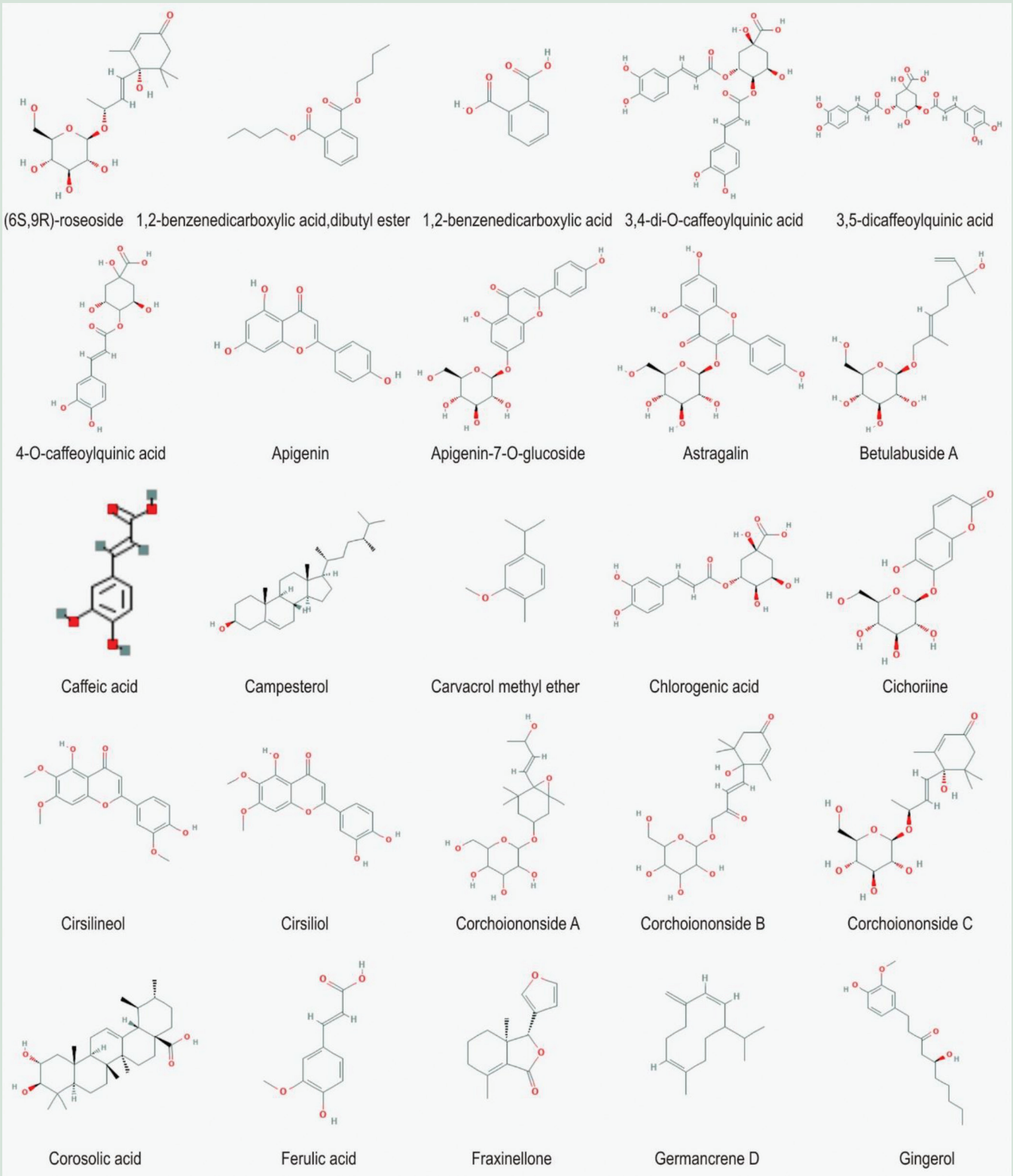

Figure 2: Bioactive compounds present in molokhia leaves

activity of petroleum ether, ethanol, and aqueous extracts of molokhia leaves was evaluated using DPPH radical scavenging assay and found ethanol extract with highest amounts of phenolics, alkaloids, and ascorbic acid to show highest antioxidant activity with lowest IC_{50} value of 0.0054 µg/mL compared to petroleum ether and aqueous extracts. The ethanolic extract has also been found to exhibit higher DPPH radical scavenging activity than butylated hydroxyl toluene (BHT); a synthetic antioxidant.[78] In another study, methanol extract containing phenolics, flavonoids, glycosides, steroids, and alkaloids exhibited significant antioxidant activity against DPPH free radical.[79,80] The essential oil obtained

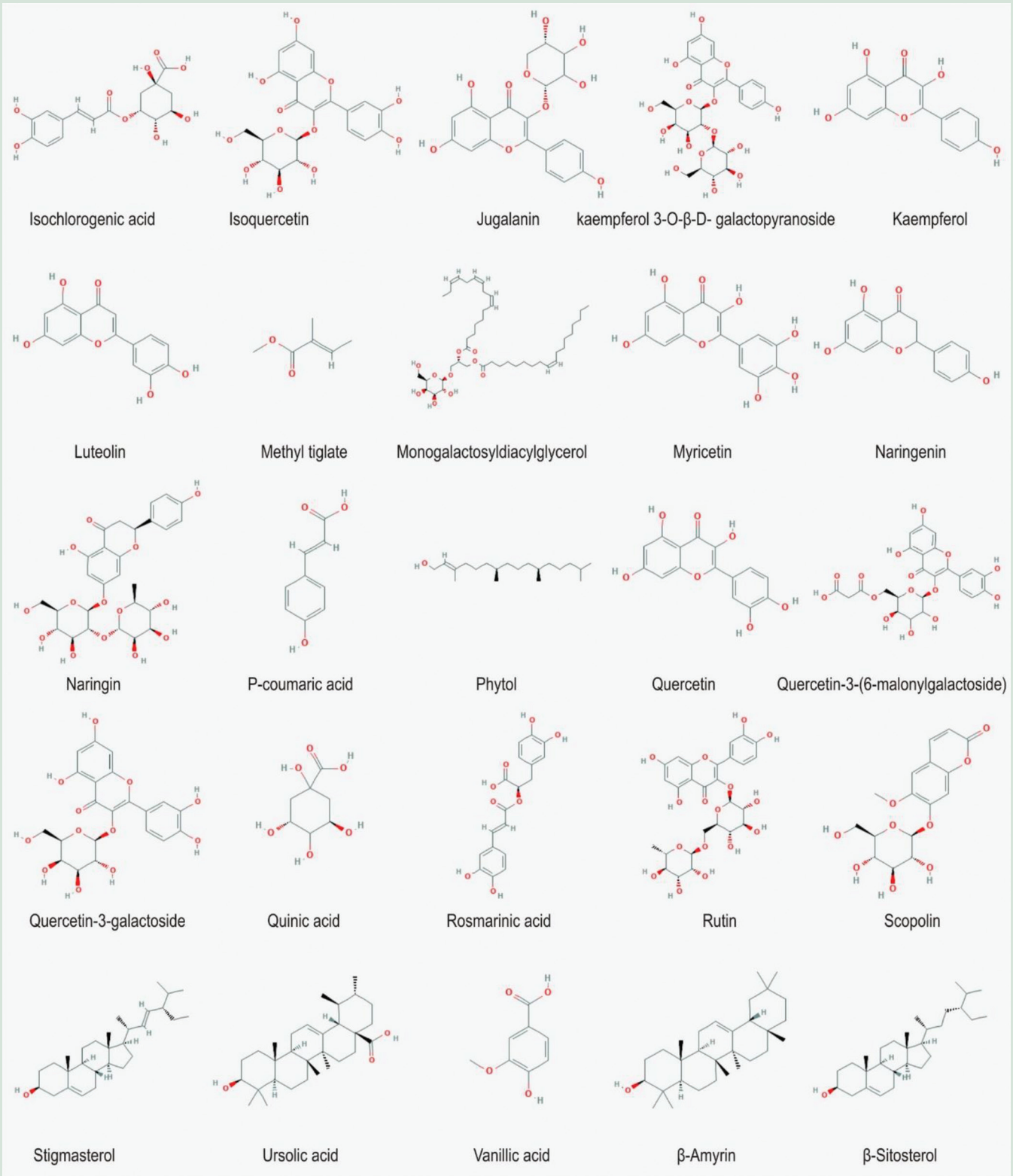

Figure 3: Bioactive compounds present in molokhia leaves

by hydro distillation of aerial parts (leaves and flowers) was found to exhibit strong DPPH radical scavenging activity and β carotene–linoleic bleaching inhibition activity. The oil showed an IC_{50} value of 0.49 mg/mL in DPPH assay.[56] Though the antioxidant activity of the oil was lower than that of BHT and BHA in DPPH assay and β carotene–linoleic bleaching inhibition assay, respectively, it is considered significant. The oil from seeds is also reported to strong antioxidant activity *in vitro*.[81]

Oboh *et al*.,[9] evaluated the antioxidant activity of hexane and aqueous extracts of molokhia leaves, wherein polar aqueous extract exhibited significantly higher DPPH radical scavenging activity, Fe^{2+} chelating

ability and trolox equivalent antioxidant capacity than nonpolar hexane extract. The antioxidant activity of aqueous extract was attributed to the presence of phenolics, flavonoids and ascorbic. On the other hand, hexane extract exhibited higher hydroxyl scavenging activity due to the presence of high total carotenoids. Similar observations were reported by Biswas *et al*.,[82] wherein, aqueous and hydro-methanol leaf extracts exhibited significant antioxidant activity measured as DPPH and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity, ferric reducing antioxidant potential , and trolox equivalent antioxidant capacity. Methanolic extract of the leaves also exhibited strong antioxidant activity in terms of inhibition of hydroxyl radical and DPPH radical scavenging and Fe^{2+} induced lipid peroxidation *in vitro*.[83-87] Kinetic antioxidant studies using DPPH showed that molokhia extracts follow second order kinetics via hydrogen atom transfer mechanism.[88] (Yusuff *et al*., 2019). Extracts of the molokhia leaves grown with synthesized biogenic silver nanoparticles (AgNPs) exhibited significant higher free radical scavenging ability and ferric reducing ability.[89] Azuma *et al*.,[18] isolated six phenolic antioxidant compounds including chlorogenic acid, 3,5-dicaffeoylquinic acid, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-(6-malonylglucoside), and quercetin 3-(6-malonylgalactoside) from molokhia leaves. Of these isolated compounds, chlorogenic acid exhibited significant antioxidant activity *in vitro*. The DPPH and ABTS antioxidant activity of extruded products developed by incorporating by molokhia by up to 5% was evaluated. It was products containing molokhia exhibited higher radical scavenging activity which was directly proportional to the level of molokhia incorporation. Furthermore, the products had improved nutritional profile and sensory acceptability.[90]

Yabani and Adotey[78] evaluated the *in vivo* antioxidant activity of molokhia leaf aqueous extract at three dosage levels, namely, 45.57, 455.7 and 2278.5 mg/kg in mice. Feeding of extracts to the mice of both the sexes for 21 consecutive days resulted in a significant reduction in lipid peroxidation as evidenced by significantly low levels of malonaldehyde (MDA) in erythrocyte lysates compared to control group. The observed antioxidant effect was dose dependent and highest activity was found with 2278.5 mg/kg dose. On an interesting note, feeding extracts led to significant decrease in body weight in male mice, while the increase in mean body weight was in female mice. It was concluded that the extract could be beneficial in obesity treatment in males. The conclusion seems speculative and further studies are warranted in this direction. In another study oral feeding of molokhia leaf methanol extract to male Wistar rats for 21 consecutive days at 50, 100, 150, and 200 mg/kg dosage levels resulted in a dose-dependent reduction of MDA levels. The extract also increased GSH levels in a dose-dependent manner thereby substantiating *in vitro* antioxidant potential of molokhia leaf extract in biological systems.[85] The *in vivo* antioxidant effect of methanolic extract of molokhia leaves were evaluated in ethanol induced oxidative stress in rats. Treatment with the extract for 21 days at a dose of 4 mL/100 g restored the levels of AST, ALT, LDH, LPO, catalase, SOD, GSH peroxidase, and GSH toward normal.[91]

Hepatoprotective activity

Alhough various extracts of molokhia leaves have shown antioxidant activity both *in vitro* and *in vivo*, studies on hepatoprotective effects in various animal models report conflicting findings. Some of the studies report strong hepatoprotective effect, while some studies report adverse effects in hepatotoxicity models.

Ethanol extract of the leaves were evaluated for protective effect against CCl_4 induced hepatotoxicity in Wistar rats at dosage levels of 500, 750, and 100 mg/kg. Oral administration of the extract for 15 days showed dose-dependent reduction in serum transaminases (ALT and AST), alkaline phosphatase, and serum albumin toward control levels. Furthermore, a significant decrease in serum albumin, platelet, and white blood cell count, but no significant differences were observed with respect to serum bilirubin, hemoglobin, and packed cell volume. On the contrary, total protein concentration was found to be elevated in extract-treated groups.[92]

Pretreatment with aqueous leaf extract at a dose 50 and 100 mg/kg of showed significant restoration of key hepatic and renal biomarkers in sodium arsenite-induced toxicity in rats. The extract not only attenuated the effects of sodium arsenite as evidenced by increased levels of catalase, SOD, GSH reductase GSH-S-transferase, and GSH peroxidase but also reduced fragmentation of DNA in liver and kidney tissues. The biochemical findings were substantiated by histological studies.[93] Similar findings were reported by Haridy *et al*.,[94] wherein, aqueous extract at the dose of 500 and 1000 mg/kg exhibited significant hepatoprotection against CCl_4-induced hepatotoxicity in rats. The extract restored elevated levels of alanine transaminase, AST, alkaline phosphatase and malondialdehyde to normal levels. The activity of GSH peroxidase which was increased on administration of CCl_4 also restored to normal levels with extract treatment. The hepatoprotective effect was dose dependent and 1000 mg/kg dose showed higher effect mediated through strong antioxidant activity.

The hepatoprotective activity of molokhia leaf supplemented diet (100 mg/g) was evaluated in streptozotocin induced diabetic rats. The results indicated as significant increase in the activities of hepatic δ-aminolevulinic acid dehydratase (δ-ALAD), catalase, SOD and decreased serum transaminases (AST and ALT). The study concluded that restoration of hepatic δ-ALAD activity, strengthen antioxidant defense systems and modulating hepatic function biomarkers could be possible factors responsible for the hepatoprotective effects of molokhia leaves in diabetes.[95] Azeez *et al*.,[89] reported hepatoprotective activity of molokhia leaves grown with synthesized biogenic AgNPs restored hydrogen peroxide induced reduction in catalase concentrations and elevated malondialdehyde levels toward normal levels in the liver. It was concluded that molokhia leaves possess significant antioxidant and hepatoprotective effects. Ethanol extracts of molokhia leaves at 200 mg/kg dose significantly reduced the levels of serum transaminases, alkaline phosphatase, bilirubin, urea, and creatinine levels in thioacetamide induced hepatotoxicity in experimental rats. Histopathology revealed that the extracts restored tissue architecture of both liver and kidney tissues.[96]

In another study, hepatoprotective effects ethanol extract of molokhia leaves at three dosage levels, namely, 50, 100, and 200 mg/kg was evaluated in normal rats. Oral feeding of the extract for 28 days significantly reduced the levels of ALT, aspartate aminotransferase, and alkaline phosphatase. The extract also reduced total cholesterol levels at 50 and 100 mg/kg dose and resulted in a dose dependent increase in HDL levels. Since the extract at 200 mg/kg increased cholesterol levels, it was opined that the extract offers hepatoprotection with possible tendency to increase total cholesterol levels.[97] However, controlled experiments are required to arrive at meaningful conclusion in this regard because molokhia leaf supplemented diet resulted in a significant hepatotoxic effect in CCl_4 induced hepatotoxicity in rats.[98] Supplementation of molokhia leaves in the at 5% and 10% levels did not result in significant improvements in hepatic function biomarkers and also did not enhance antioxidant defense systems. However, histological study revealed a significant damage to the liver tissue in molokhia supplemented CCl_4 treated rats indicating potentiation of hepatotoxic effects of CCl_4.

Antimicrobial activity

The antimicrobial activity of different extracts of molokhia leaves were evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Geotrichum candidum,* and *Botrytis cinerea*. Although, all extracts showed varied levels of antibacterial or antifungal activity, petroleum ether extract was found to be most potent against *E. coli*, *S. aureus,* and *Y. enterocolitica*, while hydro-ethyl acetate extract exhibited potent activity *G. candidum* and *B. cinerea*.[12,99] Hayyawi[100] evaluated antibacterial effects of ethanolic extracts of leaves, aerial parts and roots of molokhia against *Klebsielle pneumoniae*, *E. coli*, *Proteus mirabilis*, *Serratia marcescuns*, *Aeromonas hydrophila S. aureus,* and *Streptococcus fecalis* at concentrations of 30–50 mg/mL. All extracts were found exhibit varying degrees of antibacterial activity against the bacterial strains tested in a dose-dependent manner. The root extract was found to be more potent than other extracts at 70 mg/mL concentration. Similar observations were reported in another study with ethanol, chloroform, and ethyl acetate extracts of the leaves were found to inhibit the growth of *E. fecealis*, *B. subtilis*, *E. coli*, *P. vulgaris* and *Serratia marcescen*.[83] In another study, antimicrobial activity of hexane, chloroform, methanol, and ethanol extracts of molokhia leaves was evaluated against *S. aureus, S. epidermidis, and B. subtilis, E. coli, Klebsiella* spp., *E. cloacae, C. albicans* and *E. faecalis* using disc diffusion assay. Results indicated that none of the extracts except hexane extract exhibited antimicrobial activity against *B. subtilis* and *S. aureus*. It was concluded that the extracts may be used as antimicrobial agents in infections arising from *B. subtilis* and *S. aureus*.[101] Methanolic extract of the leaves inhibited *S. aureus, E. coli, K. pneumonia* and *Citrobacter* sp. with inhibition ranging between 10.9 and 14.23 mm at 1 mg/mL concentration.[15] Eleven fractions obtained by column chromatographic separation of lipophilic extract of molokhia leaves were tested against *S. aureus* and *E. coli* using agar well diffusion assay. The fractions were found to exhibit significant antibacterial activity against *S. aureus* with 19 ± 2.80 mm zone of inhibition.[102] Similarly, the mucilaginous polysaccharides isolated from leaves showed significant antibacterial activity against *Klebsiella pneumoniae* and *Salmonella enterica* at 25 mg/mL concentration.[76] Essential oil obtained by hydro distillation of aerial parts (leaves and flowers) was found inhibit the growth of *B. subtilis, S. aureus, E. faecalis, B. thuringiensis, M. luteus, K. pneumoniae, E. coli, S. typhimurium, Enterobacter* sp, *Actinomyces* sp, and *P. aeruginosa* with zones of inhibition ranging from 12.5-16.7 mm at 6 mg concentration. the extract was even more potent than ampicillin against *B. thuringiensis, K. pneumoniae, S. typhimurium* and *P. aeruginosa*.[56] The oil from the seed showed strong antimicrobial activity against *S. aureus*, *A. fumigatus,* and *T. mentagrophytes*. The minimal inhibitory concentration was found to be 250 mg/mL.[81]

Anti-tumor/Anti-cancer effects

The antitumor-promoting activity of compounds (phytol and monogalactosyldiacylglycerol) isolated from different cultivars of jute examined by immunoblotting analysis showed significant antitumor-promoting activity. It was interesting to note that hot water treatment similar to cooking increased the detectable levels of the bioactive compounds, thereby indicating the beneficial effects of cooking in promoting anti-tumor effects of molokhia.[10] Since the mechanism of anti-tumor activity of molokhia was unclear, Li *et al*.,[103] undertook an investigation on ethanolic extract of molokhia on the growth of human hepatocellular carcinoma (HepG2) cells to deduce the underlying mechanism of action. Results indicated that the extract at a concentration of >12.5 μg/mL significantly reduced the viability of HepG2 cells without affecting the viability of normal FL83B hepatocytes. It was opined that the extract could be effective against hepatocellular carcinoma as it induces apoptosis via mitochondria-dependent pathway by increasing the release of cytochrome c from mitochondria with decreased membrane potential. The extract also activated procaspases-3 and-9 and initiated cleavage of poly ADP-ribose polymerase, followed by downregulation of the inhibitor of caspase-activated DNase signaling. The ethanolic extract is reported to significantly suppressive effect on cytosolic aryl hydrocarbon receptor transformation induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat hepatic cytosol. The extract also suppresses aryl hydrocarbon receptor transformation in mouse hepatoma Hepa-1c1c7 cells, human colon adenocarcinoma Caco-2 cells, and human hepatoma HepG2 cells. Oral administration of the extract to the rats at a dose of 100 mg/kg decreased aryl hydrocarbon receptor transformation induced by 3-methylcholanthrene to control levels via inhibition of aryl hydrocarbon receptor translocation from cytosol into the nucleus in hepatocytes. It was concluded that molokhia could be an important source for novel phytochemical compounds having aryl hydrocarbon receptor transformation antagonist activity.[8]

The methanolic extracts of molokhia leaves were evaluated for cytotoxic and genotoxic effects using multiple myeloma-derived ARH-77 cells *in vitro*. Results indicated that the extract expressed its cytotoxic effects on cell after 48 h with an IC_{50} value of 151 mg/mL. The extract also exhibited significant dose-dependent DNA damage in ARH-77 cells as indicated by the findings of comet assay. It was concluded that molokhia leaves possess significant cytotoxic and genotoxic effects against ARH-77 cells.[104] However, further research is warranted for their utilization as anticancer agents. Ibrahim *et al*.[105] evaluated protective effects of aqueous extracts of molokhia against aflatoxin B1 and fumonisin B1-induced hepatocellular damage in H4IIE-*luc* rat hepatoma cells. Cell viability and disruption of DNA integrity were also measured. Results indicated that mycotoxins reduced cell viability associated increased DNA damage. Treatment with extract offered significant protection against cytotoxicity induced by the mycotoxins and increased cell viability and reduced DNA fragmentation. It was concluded that molokhia contains water-soluble natural chemopreventive agents that could be isolated and utilized as anticancer agents.

Cardioprotective effects

Das *et al*.[106] evaluated the cardioprotective effects of aqueous extract of molokhia leaves at a dose of 50 and 100 mg/kg against sodium arsenite-induced cardiotoxicity in rats. Exposing animals to sodium arsenite (10 mg/kg, p. o.) for 10 days resulted in a significant increase in serum total cholesterol, and cardiac tissue concentrations of arsenic, MDA, protein carbonyl and oxidized GSH while reduced serum HDL, SOD, catalase, GSH -S-transferase, GSH peroxidase, GSH reductase, and reduced GSH levels in myocardial tissues. Pretreatment with molokhia extracts restored the levels of all these parameters in blood and myocardial tissues towards normal levels. In addition, the extract also reversed DNA fragmentation caused by sodium arsenite in myocardial tissues. The biochemical findings were substantiated by histopathological studies wherein, extract pretreatment prevented tissue damage caused by sodium arsenite. It was concluded that molokhia leaf extract offers significant protection against sodium arsenite-induced cardiotoxicity by boosting antioxidant defense mechanisms.

Neuroprotective effects

The neuroprotective effect of 1,5-dicaffeoylquinic acid isolated from hydro-ethanol extract of molokhia leaves was evaluated in lipopolysaccharide-induced neuroinflammatory mouse model. The isolated compound offered significant protection of microglia

against hydrogen peroxide-induced cytotoxicity. Also reduced the expression of astrocytic marker, glial fibrillary acidic protein, and cyclooxygenase-2. In addition, cognitive functions were improved. Histopathological studies revealed reduction in lipopolysaccharide-induced neurodegeneration in brain tissues. It was concluded that 1,5-dicaffeoylquinic acid offers significant protection against neurodegeneration and cognitive impairment caused by neuroinflammation and glial cell activation.[107]

Analgesic activity

The analgesic activity of methanolic molokhia extract was evaluated against acetic acid induced writhing in mice. Animals were dosed with the extract at 50, 100, 200, and 400 mg/kg body weight and writhing were induced by intraperitoneal injection of acetic acid. Aspirin 200 and 400 mg/kg was used as reference. The extract showed a dose-dependent inhibition of abdominal constrictions induced by acetic acid. Abdominal contractions were decreased by 20%–58% at the dosage range tested. It was observed that the extract at 100 mg/kg exhibited higher analgesic activity than aspirin at 200 mg/kg indicating significant analgesic properties to molokhia.[71] Further research is warranted to identify and isolate the compounds responsible for the analgesic activity.

Wound healing Property

The wound healing effect of molokhia leaf powder and aqueous extract was evaluated in excision wound model in rats. Results indicated that both powder and extract showed significant wound healing activity compared to control. 100% wound contraction was achieved on the 18th day by 5% ointment of powder and 100 mg/ml extract. It was concluded that molokhia possess significant wound healing activity and has the potential to be developed as an alternative treatment for wound healing as it also reduces microbial load effectively.[14,87] In another study, skin hydration capacity of molokhia extract without high-molecular-weight compounds was evaluated in an experimental atopic dermatitis mice model. The extract (0.2%) was mixed with a stable base cream and applied on the dorsal skin of the mice. The observations included skin hydration, transepidermal water loss, atopic dermatitis scores, and plasma immunoglobulin E (IgE) levels for a period of 14 days. The treatment significantly increased skin hydration and reduced transepidermal water loss and atopic dermatitis scores. No changes in plasma IgE were observed. The study indicated that molokhia has superior ability to maintain skin hydration and prevent transepidermal water loss resulting in faster healing. Authors suggested the use of molokhia as an adjunct in the treatment for atopic dermatitis.[108] Further in an extended experiment, authors used the extract applied the extract on the rostral skin of specific pathogen-free mice and conventional mice for 14 days and measured plasma IgE levels. While the mice under specific pathogen-free conditions were not affected by the extract cream, the mice housed under conventional conditions showed lowered levels of plasma IgE, atopic dermatitis scores and expression of tryptase and MMP-9. Furthermore, degradation of collagen type IV at the basement membrane area was not observed in extract treated group. It was concluded that molokhia extracts can be used in the formulation of therapeutics for atopic dermatitis as it suppresses plasma IgE levels and degranulation of mast cells.[109]

TOXICITY STUDIES

Acute toxicity studies conducted in Swiss albino mice using aqueous extract of molokhia leaves up to 3.2 g/kg resulted in no signs of toxicity. The animals did not show any mortality, breathing difficulties, irritation, vomiting, diarrhea, paralysis, bleeding, restless, convulsions, and abnormal posture over the study period. The LD_{50} value for the oral administration of the extract was deduced as >3.2 g/kg body weight.[106] Acute toxicity studies carried out according to OECD-423 guidelines using methanolic molokhia leaf extract up to 2 g/kg dosage did not produce any toxic effects in rats. No mortality was reported during the study period.[53] In another acute toxicity study, mice were administered with methanolic extract of molokhia up to 3 g/kg body weight dosage and observed for 8 h. The extract did not show any signs of toxicity at the dosage levels tested. No changes in behavioral pattern and mortality were observed.[71]

CONCLUSION

The leaves of molokhia are rich sources of essential micronutrients and a diverse class of bioactive compounds exhibiting potent antidiabetic, antioxidant, anti-inflammatory, anticancer, antimicrobial, hepatoprotective, cardioprotective, neuroprotective, analgesic, and wound healing effects. Molokhia is safe to consume as reflected by the toxicity studies and hence, it has potential to be developed as a nutraceutical product for promoting general health and well-being. Further research is warranted in this direction to develop novel nutraceutical supplements and food products using molokhia leaves.

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Conflicts of interest

There are no conflicts of interest.

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