Review Article

TIPS Trends in Pharmaceutical Sciences 2020: 6(1): 1-10.

A glance at *Berberis integerrima* pharmacological effects and its active constituents

Mahmoodreza Moein[1,2], Zahra Sabahi[1,*], Hasti Salim[1]

[1]Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Berberis integerrima (family: Berberidaceae) is broadly used in the pharmacological studies as a rich source of active compounds. Among different compounds, alkaloids are the most isolated ones from various parts of this plant. Research in available databases including Web of Science, Pub Med and Google Scholar was used to reveal the pharmacological effects and active compounds of *B. integerrima*. The present review attempts to give a short overview on biological effects and active components of *B. integerrima* with emphasis on some mechanisms of activities. Scientific evidences suggest possible therapeutic properties of this plant on diseases such as diabetes, inflammation, free radicals associated diseases, seizure and cancer. In this review, we evaluated the most correlated original articles to determine the effects of *B. integerrima* on different medical conditions. It seems that *B. integerrima* would be useful in managing various types of disease, however more studies, particularly pharmacokinetic and clinical trials, need to be considered to improve our knowledge about toxicity and possible side effects of this plant.

Keywords: Berberis integerrima, alkaloid, berberine, phytochemical constituents.

1. Introduction

According to different taxonomy reports, Berberidaceae family comprises about 14 genera and 700 species and *Berberis* is the major genus in this family (1).

Phylogeny-based study showed that this genus have two sources of diversity: Asia (or Eurasia) and South America. Taxonomists have described different classifications. Among them, *Berberis khorasanica* Browicz and Zielinski, *Berberi scrataegina* DC., *Berberis orthobotrys* Bienert ex Schneid, *Berberis integerrima* Bunge (*B. integerrima*) and *Berberis vulgaris* L. are grown in various regions of Iran.

B. integerrima is famous as integrifolious barberry, distributing in many regions of Iran (2, 3) , particularly in Tash valley, Shahrood, Semnan province (4). This plant has been known as an ornamental herb. Fresh fruits are used as food additive in syrups, jellies, jams, juices, etc.; also, the root and fruits have therapeutic applications (4).

This review focuses on phytochemical characteristics and the pharmacological and experimental studies conducted to *B. integerrima* (Figure 1).

Corresponding Author: Zahra Sabahi, Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. Email: sabahiz@sums.ac.ir

2. Active constituents

Alkaloids are the most isolated compound from various parts of this plant. The alkaloids derivates which were isolated from the leaves, stem and root have been listed below (Table 1).

The oil content of *B. integerrima* seed was extracted by the solvent method, contain fatty acids (linolenic, linoleic and oleic as well as ω-3 and ω-6 fatty acid), and phytosterols including

Recieved: 27/01/2020; Accepted: 01/03/2020

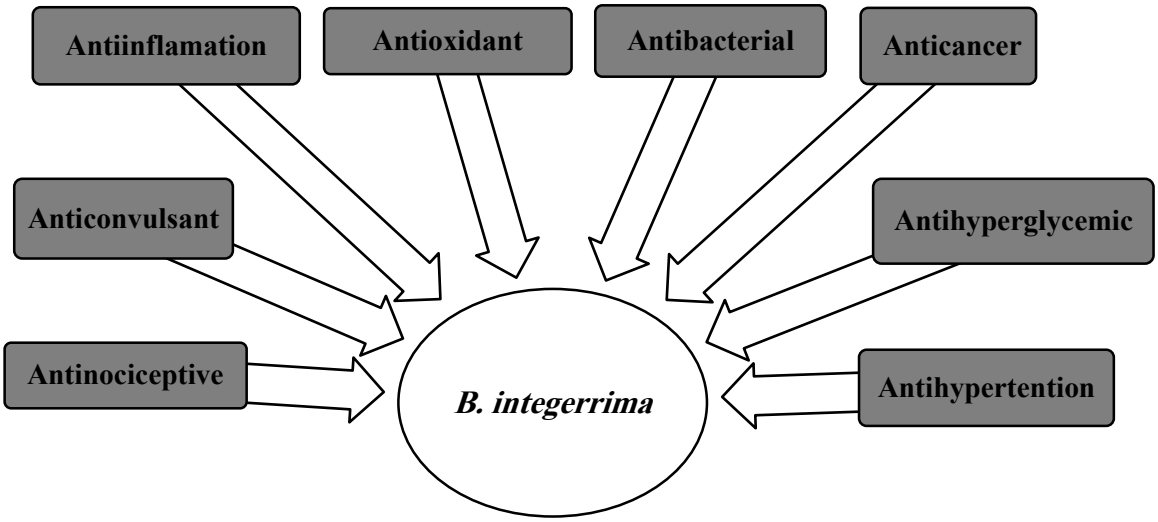


Figure 1. Schematic diagram of pharmacological activity of *B. integerrima*.

β-sitosterol, campesterol, Δ5-avenasterol and stigmasterol as well as α- and γ-tocopherol are other components of this oil (5). Also, 1-methyl malate (butanedioic acid, hydroxy, 1-methyl ester) which is a simple organic acid, was isolated from fruits of *B. integerrima*. This compound has been used in sysnthesis of β-lactam rings, which is a part of different penicillin derivatives structure (6).

3. Pharmacological studies

3.1. Antioxidant effects

Potency of antioxidant capacity of the *B. integerrima* seed oil has been measured by evaluating 2,2 diphenyl-1-picryl hydrazyl (DPPH) scavenging activity (IC_{50}:5.47±0.01 mL/L) and ferric-reducing antioxidant power. Reducing power result was 5.68 µmol Fe (II) per gram of the oil.

Indeed, the seed oil was able to protect soybean oil against oxidation compared to commercial antioxidants (5). In another study, antioxidant activity of the fruit extract from four samples of *B. integerrima* (collected from various regions of Iran) were measured by DPPH and ferric reducing antioxidant power (FRAP).

The DPPH inhibition percentages were 74.72, 20.69, 48, 61%, the trolox equivalents (TE) were 70.39, 20.36, 40.98, 60.27 and Fe^{2+} chelating activity were 41.46, 18.56, 40.53, 46.21%. These results related to the high amounts of phenolic compounds, anthocyanins and flavonoids in these fruits. Also, they are the sources of antioxidant enzymes such as guaiacol peroxidase (G POD) and catalase (CAT)(10).

In another study, these fruits exhibited low TE in the oxygen radical absorbance capacity assay (ORAC) test and low EC_{50} in cellular antioxidant assay. Moreover, the fraction of these fruits strongly inhibits xanthine oxidase activity, which plays a critical role in free radical production. This fraction also protects the human lymphocyte s against H_2O_2-induced DNA damages (11). Since this fraction is a rich source of polyphenols, these activities can be due to their content (12).

Table 1. Alkaloids derivate isolated from *B. integerrima*.

Parts of plant	Ref No.	Isolated alkaloids
Leaves	(7)	reticuline, isoboldine, isocorydine, glaucine, armepavine, oxyacanthine, and heliamine, intebrinine, intebrimine
Stem	(8)	glaucine, thalicmidine, isocorydine, oxyacanthine, berberine, armepavine, berbamine, palmatine, jatrorrhizine, columbamine, 8-trichloromethyldihydroberberine, 8-trichloromethyldihydropalmatine
Roots	(9)	Palmatine, Berberine, Jatrorrhizine, columbamine

3.2. Antibacterial effects

The ethanol extract of *B. integerrima* fruits showed antibacterial activity against clinical isolates of *Staphylococcus aureus*. 1-Methyl malate which was isolated from these fruits enhanced the antibacterial activity of ampicillin against this strain. 1-Methyl malate (2 mg/ml) was able to reduce the MIC of ampicillin from 128 to 1 µg/ml (6). In another study, four isolated alkaloids from *B. integerrima* root (columbamine, palmatine, berberine and jatrorhizine) exhibited antimicrobial activity against *Brucella abortus;* 15 µg/ml of jatrorhizine and columbamine were comparative to 10 µg/ml of streptomycin (9).

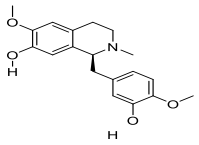
3.3. Antidiabetic effects

According to an ethnopharmacological study, *B. integerrima* has been known as an antidiabetic plant in persian folk medicine (13). Several investigations have evaluated antidiabetic effect of this plant and its mechanisms of action in animal models. These studies were focused on the antidiabetic effects of the fruits and root. Fallah *et al*. examined the effect of the aqueous ex-

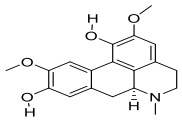
Table 2. Chemical structure of alkaloids from *B. integerrima*.

Alkaloides

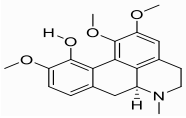
Reticuline



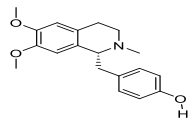
Isoboldine



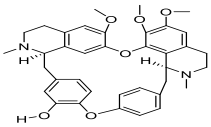
Isocorydine



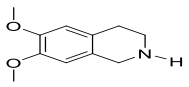
Armepavine



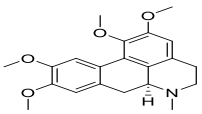
Oxyacanthine



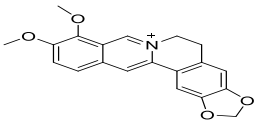
Heliamine



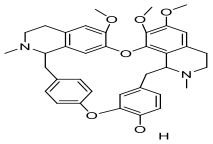
Glaucine



Berberine



Berbamine

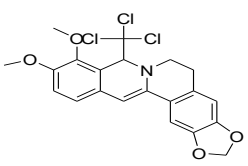


Palmatine

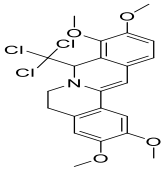
Jatrorrhizine

Columbamine

8-trichloromethyldihydroberberine



8-trichloromethyldihydropalmatin



tract of *B. integerrima* fruits on insulin resistance in high fructose-fed insulin-resistant rats. In this evaluation, rats were fed with a high-fructose diet and then the extract was administrated (1000 mg/kg). This treatment showed a significant decrease in the levels of insulin and blood glucose. The results show elevated levels of adiponectin, while in treated and non-treated groups there is no significant difference between mRNA and protein level of GLUT4 and PPAR-γ. Hence, this mechanism was not related to GLUT4 and PPARγ pathway. The suggested mechanism of this extract was insulin-like effect and an enhancement of adiponectin levels (14). Adiponectin is secreted by adipocytes and show a critical role in obesity-related diseases such as insulin resistance and type 2 diabetes (15-17). Also, the administration of adiponectin in humans and rodents leads to insulin-sensitization (15); therefore, if the probable mechanism of this extract is adiponectine enhancement it would be a proper candidate in the management of diabetic complications.

Ashraf *et al*. have examined the hypoglycaemic effects of fruit aqueous extract in Streptozotocin-induced diabetic Rats. In this research, while, treatment of diabetic rats with glibenclamide (0.6 mg/kg) for 42 days decreased hyperglycemia, but their treatment with the aqueous extract was not able to improve the glucose concentration in comparison to untreated ones (18). In another study, the administration of anthocyanin fraction from the fruits of *B. integerrima* (400, 1000 mg/kg) to streptozotocin-induced diabetic rats decreased the blood glucose, and increased the liver glycogen content and the body weight compared to the control: Though, they could not see any synergistic effects between this fraction and metformin or glibenclamide to improve these factors (13). In this study, the possible antidiabetic mechanisms of the fruit anthocyanin were attributed to its antioxidant activity, protection of pancreatic β-cells against oxidative stress, induction of insulin secretion, activation of phosphorylation of AMP activated protein kinase (AMPK) and the increase of glucose intake by skeletal muscles (13). Phenolic compounds like anthocyanins improve glucose metabolism, lipid profile, and regulates the hormones and enzymes. Consequently the molecular mechanisms in glucose and lipid metabolism would offer novel insights in antidiabete effects of herbal medicines (19, 20).

In another research the administration of the aqueous extract of *B. integerrima* root (500 mg/kg) for 6 weeks improve of the kidney parameters and renal function to near normal (serum creatinine, blood urea nitrogen, urine glucose, urine urea and urine creatinine, urine protein, urine albumin, and water intake) and increased the body weight as well. Also, histopathological studies confirmed the renal protective effect of this extract in diabetic rates (21).

Moreover, intra-gastric consumption of the root aqueous extract (500 mg/Kg) for 6 weeks (3 weeks before STZ injection and continued for three weeks) more resulted in a significant improvement in the levels of blood glucose, malondialdehyde (MDA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin. This treatment also increased the body weight, total protein, SOD, CAT and glutathione (GSH) in comparison to diabetic control rats. So, the administration of this extract can be considered as an ameliorating factor in diabetic liver complications. Berberine and alkaloids present in *B. integerrima* root can play critical roles as α-glucosidase inhibitors, glucose absorption inhibitors and peripheral glucose uptake inducers. Furthermore, SOD and CAT play critical roles in preventing ROS cellular damages in diabetes complications (22). It is also suggested that hypoglycemic effects of berberine can be mediated by other mechanisms such as improvement of gut-derived hormones, reduction of defect of mucosal barrier function and decrease of the pro-inflammatory fluctuations of intestinal immune cells and cytokines (23).

In another study, STZ- injection in rats lead to significant increases in the biochemical factors such as blood glucose, triglycerides (TG), total cholesterol (TC), low density lipoprotein LDL-cholesterol (LDL-C), creatinine (Cr), urea, ALT, AST, ALP and total bilirubin. Diabetic rats were treated by 250 and 500 mg/kg of the root extract of *B. integerrima* for six weeks. Results exhibited significant reduction in blood glucose, TG, TC, LDL-cholesterol, ALT, AST, ALP, total bili-

rubin, creatinine and urea compared to untreated diabetic rats. This extract in a dose of 500 mg/kg is more effective on all parameters except blood glucose compared to glibenclamide (0.6 mg/kg) as the standard drug. This study suggested the root of *B. integerrima* as an antihypoglycemic, antihypolipidemic and antioxidant candidate in diabetes (24).

3.4. Antiinflamatory effects

In another research, the impact of *B. integerrima* fruit on lymphocytic immune responses was evaluated. So, splenocytes of Balb/c mice were exposed to the phytohemagglutinin and lipopolysaccharide as mitogens and aquatic and alcoholic extract of *B. integerrima* (0.001-000 µg/ml), simultaneously. Both extracts inhibited production of IFN-γ from splenocytes and boosted the release of IL-4, IL-10 and TGF-β. Other effects were the suppression of T-cell expansion and the increase of B-cell proliferation. This extract could be considered as a humoral immunity stimulant (25).

In another study, macrophages and lipopolysaccharide-stimulated macrophages were treated with alcoholic and aqueous extracts. Both extracts blocked nitric oxide production. Low dose of them were able to suppresse TNF-α production while aqueous extract induced it. Furthermore, the release of IL-6 was inhibited while the release of IL-12 was increased. These data suggested that the extract showed anti-inflammatory functions by altering the cytokine production and release (26). Previously, the anti-inflammatory activity of other *Berberis* genus has been reported for *B. aristata* (27), *B. vulgaris* (28) and *B. crataegina* (29).

3.5. Anticancer effects

The potency of anticancer effects of *B. integerrima* was carried out by Malayeri *et al*.. In this study, colon cancer was induced in male wistar rats by injecting 1,2-dimethyl hydrazine (DMH). They received *B. integerrima* fruit hydroalcoholic extract (50 and 100 mg/kg). To some extent, this treatment improved the levels of ferric reducing ability of plasma (FRAP), the hepatic glutathione S-transferase (GST), cytochrome P-450 (CYP450) and β-catenin. Also, aberrant crypt foci (ACF) formation in colon tissue of DMH-treated rats was reduced by this extracts. So, this extract could be a potent chemotherapeutic agent against colon carcancer in future studies (30).

3.6. Cytorotective effects

The *B. integerrima* root extract has been reported as an effective agent in protecting against CCl_4-induced testicular damages in wistar rats. This extract improved different factors such as serum testosterone level, testis weight, seminiferous tubules diameter, thickness of the epithelium, tubule differentiation index, spermiogenesis index and catalase activity. Other parameters such as interstitial tissue thickness and malondialdehyde were reduced, significantly. Adose of 500 mg/kg of the extract was more effective than silymarin (50 mg/kg) as a reference drug. Since carbon tetrachloride induce toxicity by tissue oxidative damage and *B. integerrima* roots are rich in alkaloids hence, these effects would be related to antioxidant activity of this natural compounds (31).

Other studies have confirmed that anthocyanin fraction of *B. integerrima* fruits protected $HepG_2$ and MCF7 cells against hydrogen peroxide (H_2O_2) induced cytotoxicity. In this assay, the cells were pre-exposed (24 h) to anthocyanin fraction then cytotoxic concentration of H_2O_2 was added.

Anthocyanin fraction (200 and 400 µg/ml) increased viability of MCF7 cells compared of the control. But there is no difference between viability of treated and non-treated $HepG_2$ cells. In another test, the cells were exposed to anthocyanin fraction and toxic concentrations of H_2O_2, simultaneously. The results showed that anthocyanin (25-400 µg/ml) protected MCF7 cells against H_2O_2-induced cytotoxicity, and 100, 200 and 400 µg/ml of the fraction increased $HepG_2$ cells viability (32). Interaction of the H_2O_2 and the superoxide ($O_2^{\bullet-}$) resulted in formation of hydroxyl radicals, which are highly reactive free radical (33, 34). Subsequently, this protective effect would be associated with antioxidant activities of anthocyanin in this fraction. Meanwhile, flavonoids are considered to reduce the risky effects of free radicals by different mechanisms: scavenging free radical through dihydroxy groups in their structure and conjugation with transition metals (12, 35).

3.7. Anticonvulsant effects

Epilepsy is a neurological disorder with abnormal brain activity, seizures or unusual behavior.

Hosseinzadeh *et al.* evaluated the anticonvulsant activity of methanolic extract, hydromethanolic and chloroform fraction of *B. integerrima* root.

Pentylenetetrazole (PTZ) and maximal electroshock (MES)-induced seizure models were used in this study. In the PTZ test, extract and fractions improved the onset time of hind limb tonic extensions (HLTEs). while in the MES test, these samples were unable to reduce HLTE duration significantly. So, it seems that *B. integerrima* can be an anticonvulsant factor in PTZ-induced seizures and may be useful in petit mal epilepsy. As the methanolic extract showed better results in comparison to chloroform fraction in petit mal epilepsy, it can be related to the presence of alkaloids and tannins, which have been found in its methanolic extract (36). Other studies confirmed anticonvulsant activity of some alkaloids and berberine which were detected in root and stem bark of *Berberis* species (37, 38). These compounds are effective in mental depression and anxiety. The anticonvulsant effect of this plant may be related to the mentioned effects on central nervous system (36). Sadeghnia *et al*. used 4-aminopyridine (4-AP) as a convulsant factor by inducing the neurotransmitter (glutamate) release which lead to seizures. According to the results berberine reduces the release of hippocampal aspartate and glutamate and plays an anticonvulsant role, properly (38). Other studies also suggested modulation of neurotransmitter systems as a mechanism of berberine anticonvulsant effect (39, 40).

3.8. Antinociceptive effect

Hajhashemi *et al*. studied the antinociceptive effect of the total extract and its alkaloid fractions of *B. integerrima* root. According to the results of various tests, probable antinociceptive mechanism was mediated by suppressing pains with inflammatory origins and anti inflammation mechanisms (41).

3.9. Anti hypertensive effect

To evaluate *B. integerrima* fruits effects on hypertension, monocrotaline was injected to rats; after two weeks they received aqueous fruit extracts (50, 100, and 200mg/kg) or sildenafil (30 mg/kg/d) for 2 weeks. The extract (200 mg/kg) and sildenafil significantly reduced the right ventricular systolic pressure, right ventricular hypertrophy and the medial wall thickness. It seems that *B. integerrima* extract was more effective than sildenafil on improvement of the monocrotaline-induced pulmonary hypertension (42).

In another study, the effects of aqueous extract of this plant on hemodynamic and electrocardiogram (ECG) indices of rat were evaluated. The rats received 50, 100, and 200 mg/kg/day of fruit extract for two weeks and after the 15th days, data were collected. Electrocardiogram evaluation showed that the administrations had no significant effects on heart rate, RR interval, P duration, and Q wave amplitude of electrocardiogram as well as blood pressure. The doses of 100 and 200 mg/kg increased the QRS interval but decreased the QTc interval, the JT interval and TpTe interval compared to the control and 50 mg/kg dose. According to these results, high doses of extract extended the depolarization phase and decreased the repolarization phase of the ventricular muscle so this extract would play an antiarrhythmic role and in some cases could be even arrhythmogenic. It seems that these pharmacological effects are not associated with berberine and other components (43).

4. Discussion

As discussed previously, the most considered bioactive compounds isolated from this plant are alkaloids.Isoquinoline alkaloids were isolated from stem and quaternary benzylisoquinoline alkaloids were isolated from the root. Some other alkaloids are isolated from leaves.

Based on *in vitro* and *in vivo* pharmacological studies, *B. integerrima* can be considered as a natural source in pharmaceutical development in order to treat various diseases such as diabetes, cancers, hypertension, inflammation, and bacterial infections. Antidiabetic effects were the most investigated followed by antioxidant and cyto-

protective effects. Moreover, the antioxidant and chemopreventive properties of this herb may reveal the potential of *B. integerrima* as an adjuvant therapy for free radicals associated diseases.

Further investigations should be performed to improve our information about toxicity, probable side effects and herb-drug interactions. Other drawback of this field is lack of pharmacokinetic data to reveal absorption, distribution, and metabolism of this plant. These types of studies help to increase our knowledge about metabolism and choose proper formulations for products from this plant.

Conflict of Interest

None declared.

5. References

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