A comprehensive review on bergenin, a potential hepatoprotective and antioxidative phytoconstituent

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Summary

Bergenin (1) is a C-glucoside of 4-O-methylgallic acid that has been reported to occur naturally in several genera. It exhibits a wide array of biological activities and also in several cases is responsible for the traditional use of its natural sources. It has been shown to exhibit various pharmacological activities, and thus has several possible applications in clinical research. This review presents a comprehensive literature search of different studies carried out on this secondary metabolite, especially its hepatoprotective potential, antioxidative and antiviral effects as well as antiulcerogenic and antiarthritic activities. Bergenin seems to be a potent phytotherapeutic agent as demonstrated by experimental data in animal models. Therefore, further investigations may help in exploiting its properties and developing phyto-pharmaceuticals based on it.

Key words: bergenin, phytoconstituent, pharmacology, hepatoprotective, antioxidant

INTRODUCTION

For decades, natural products have played an important role in the development of drugs and drug leads. Several recent reviews have discussed the importance of compounds derived from natural sources in modern medicine [1-7]. According to recent survey by Newman et al. [2], 61% of 877 small-molecule new chemical entities introduced as drugs worldwide in 1981–2002 can be traced to or were inspired by natural products. The secondary metabolites from natural sources are good candidates for drug development because being elaborated within
the living systems, they are perceived to exhibit more similarities to drugs and show more biological friendliness than totally synthetic drugs [3, 5, 8]. A recent review [9] describes over 20 new drugs derived from natural sources originating from terrestrial plants, terrestrial microorganisms, marine organisms as well as terrestrial vertebrates and invertebrates that have been launched in the six-year-lasting period (2000–2005). That includes galantamine hydrobromide, an alkaloid obtained from Galanthus nivalis – used for the Alzheimer’s disease treatment and arteether, an antimalarial agent developed from artemisinin – a sesquiterpene lactone isolated from Artemisia annua. Besides, the clinical trials on several drug candidates from natural sources were carried out in the same period. These approved substances, representative of a very wide chemical diversity, have been approved for the treatment of cancer, neurological, infectious, cardiovascular and metabolic diseases, immunological, inflammatory and related diseases as well as genetic disorders, which encompass many of common human diseases.

Thus, thousands of plants existing in nature are an enormous reservoir of bioactive molecules that can be developed as new chemical entities, analogs, derivatives, synthetic compounds with natural product derived pharmacophores or as natural product mimics. The identification of right chemical entity is the only requirement. Taking the above mentioned into consideration, an extensive literature search for bergenin, which has exhibited antiasthmatic, antitussive, anti-inflammatory, antifungal, anti-HIV and antihepatotoxic activities was carried out [10].

CHEMICAL STRUCTURE OF BERGENIN

Bergenin (1) is 4-methoxy-2-[(1S,2R,3S,4S,5R)-3,4,5,6-tetrahydro-3,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-2-yl]-α-resorcylic acid δ-lactone monohydrate, C_{14}H_{16}O_{9}H_{2}O [10]. It is a C-glucoside of 4-O-methylgallic acid. The molecule is composed of three six-membered rings: an aromatic ring, a glucopyranose ring and an annellated δ-lactone ring. The glucopyranose ring exhibits only small deviations from an ideal chair conformation. The annellated δ-lactone ring possesses the expected half-chair conformation. There is one intra- and six intermolecular hydrogen bonds which form an extensive hydrogen-bonding network within the crystal. Figure 1 gives the chemical structure of bergenin (1) and its two analogs norbergenin (2) and acetylbergenin (3).

DISTRIBUTION OF BERGENIN

Bergenin has been most abundantly reported in genera belonging to families Euphorbiaceae, Saxifragaceae and Myrsinaceae. It is present in substantial amounts in Ardisia sp. [11] and Mallotus [12] and also found in several other plants from other families. Table 1 gives a list of the different natural sources of bergenin [11-53].
Figure 1. Structures of bergenin, norbergenin and acetylbergenin

Table 1.

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<th>family</th>
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<td>Caesalpinia digyna Rottl.</td>
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<td>Connarus monocarpus</td>
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<td>Moraceae</td>
<td>Ficus racemosa L.</td>
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PHARMACOLOGICAL ACTIVITIES OF BERGENIN

Different studies have shown bergenin to possess a wide range of biological activities, eg. antiulcer, hepatoprotective, antiviral, antidiabetic, anti-inflammatory, etc. These have been dealt with in greater details in the subsequent sections.

Bergenin and antiulcerogenic activities

Antiulcerogenic activities of bergenin have been reported [12, 26, 54, 55]. The effects of bergenin on various experimental ulcers were tested. It was effective in preventing stress-induced gastric ulcers (30 mg/kg i.v.). One of the mechanisms of its effectiveness may be inhibition of acetylcholine release, which induces gastric acid secretion and enhances gastric motility, both being well known ulcerogenic factors [55]. Oral administration of bergenin and norbergenin isolated from the leaves and roots of *Flueggea microcarpa* showed significant protection against pylorus-ligated and aspirin-induced gastric ulcers in rats and cold restraint stress-induced gastric ulcers in rats and guinea pigs. The study on prostaglandins release by human colonic mucosal incubates, indicated a concentration-dependent (1–10 µg/ml) stimulatory effect of bergenin and norbergenin. The results suggested that gastroprotective effects of bergenin and norbergenin could be due to increased prostaglandin production [26]. The aqueous extract of *Mallotus japonicus* showed an inhibitory effect on bovine adrenal tyrosine hydroxylase (TH), the rate-limiting enzyme in the biosynthesis of catecholamine. Bergenin and norbergenin, constituents of the aqueous extract of *M. japonicus* were studied to see their effect on bovine adrenal TH. Bergenin and norbergenin inhibited the TH activity by 29.0% and 53.4% at a concentration of 20 µg/ml, respectively, and exhibited noncompetitive inhibition of TH activity with the substrate L-tyrosine. The inhibition of TH activity and the inhibitory effect of norbergenin was more potent than that of bergenin. From these results, it can be presumed that bergenin and norbergenin may be the active components of *M. japonicus* responsible for the clinical use of *M. japonicus* in treating peptic ulcer [12].

Hepatoprotective potential of bergenin

The hepatoprotective effects of bergenin, the major constituent of *Mallotus japonicus*, were evaluated against D-galactosamine (GalN)-induced liver damage in rats [31] and against carbon tetrachloride (CCL₄)-induced liver damage in rats [56].
Bergenin (50, 100 and 200 mg/kg) was given orally once daily in 7 successive days and then GalN 400 mg/kg was injected intraperitoneally to rats at 24 and 96 h after the final administration of bergenin. Pretreatment with bergenin reduced the increased enzyme activities of alanine/aspartate aminotransferase, sorbitol dehydrogenase, γ-glutamyltransferase and elevated level of malondialdehyde induced by GalN. Bergenin restored the decreased hepatic contents of glutathione as well as decreased activities of glutathione S-transferase and glutathione reductase by GalN towards normalization, which suggested that hepatoprotective effects of bergenin may consist in maintaining adequate levels of hepatic glutathione for xenobiotics removal [31]. The hepatoprotective effects of bergenin were also evaluated against CCl₄-induced liver damage in rats [56]. Bergenin at a dose of 50, 100 or 200 mg/kg was administered orally once daily for successive 7 days and then a mixture of 0.5 ml/kg (i.p.) of CCl₄ in olive oil (1:1) was injected two times each at 12 and 36 h after the final administration of bergenin. The substantially elevated serum enzymatic activities of alanine/aspartate aminotransferase, sorbitol dehydrogenase and γ-glutamyltransferase due to CCl₄ treatment were dose-dependently restored towards normalization. Meanwhile, the decreased activities of glutathione S-transferase and glutathione reductase were restored towards normalization. In addition, bergenin also significantly prevented the elevation of hepatic malondialdehyde formation and depletion of reduced glutathione content in the liver of CCl₄-intoxicated rats in a dose-dependent fashion. The results indicated that bergenin had hepatoprotective effects against GalN-induced hepatotoxicity in rats and had a potent hepatoprotective action against CCl₄-induced hepatic damage in rats. The effects of bergenin against D-galactosamine-induced injury in primary cultured rat hepatocytes [57] and carbon tetrachloride (CCl₄)-induced cytotoxicity in primary cultured rat hepatocytes [58] were also evaluated. Bergenin (100 µM) decreased the release of glutamic pyruvic transaminase and sorbitol dehydrogenase by 62 and 50%, respectively, into hepatocyte medium incubated for 14 h with 1.5 mM galactosamine. Decreased RNA synthesis by 1.5 mM galactosamine was recovered 2.5 times compared to that of control hepatocytes at 100 µM bergenin. The results therefore suggested that bergenin showed hepatoprotective effects against galactosamine-intoxicated rat hepatocytes by inhibiting the release of glutamic pyruvic transaminase and sorbitol dehydrogenase as well as by increasing RNA synthesis [57]. Bergenin also significantly reduced the activities of glutamic pyruvic transaminase and sorbitol dehydrogenase released from the CCl₄-intoxicated hepatocytes. The antihepatotoxicity of bergenin was also evidenced by elevating the activities of glutathione S-transferase and glutathione reductase, and content of glutathione in the CCl₄-intoxicated hepatocytes. From these results, it is assumed that bergenin exerted antihepatotoxicity against CCl₄-induced cytotoxicity through glutathione-mediated detoxification as well as free radical suppressing activity [58].

The hepatoprotective effects of acetylbergenin were examined in carbon tetrachloride (CCl₄)-intoxicated rats [59] and against D-galactosamine (GalN)-intoxicated rats.
duced liver damage in rats [60] and compared with that of bergenin reported previously. Acetylbergenin was synthesized by acetylating bergenin, which was isolated from *Mallotus japonicus*. The hepatoprotective effects of acetylbergenin were examined against CCl₄-induced liver damage in rats by means of serum and liver biochemical indices. Acetylbergenin was administered orally once daily for 7 successive days, then a 0.5 ml/kg mixture of CCl₄ in olive oil (1:1) was intraperitoneally injected at 12 h and 36 h after the final administration of acetylbergenin. Pretreatment with acetylbergenin reduced the elevated serum enzymatic activities of alanine/aspartate aminotransferase, sorbitol dehydrogenase and γ-glutamyltransferase in a dose dependent fashion. Acetylbergenin also prevented the elevation of hepatic malondialdehyde formation and depletion of glutathione content dose dependently in CCl₄-intoxicated rats. In addition, the decreased activities of glutathione S-transferase and glutathione reductase were restored to almost normal levels. The results of this study strongly suggest that acetylbergenin has potent hepatoprotective activity against CCl₄-induced hepatic damage in rats by glutathione-mediated detoxification as well as having free radical scavenging activity. In addition, acetylbergenin doses of 50 mg/kg showed almost the same levels of hepatoprotective activity as 100 mg/kg of bergenin, indicating that lipophilic acetylbergenin is more active against the antihepatotoxic effects of CCl₄ than those of the much less lipophilic bergenin [59]. Acetylbergenin was administered orally once daily for 7 days and then GalN (400 mg/kg, *i.p.*.) was injected at 24 and 96 h after the final administration of acetylbergenin. Acetylbergenin reduced the elevated serum enzyme activities of alanine/aspartate aminotransferase, sorbitol dehydrogenase and γ-glutamyltransferase and the formation of hepatic malondialdehyde induced by GalN. Acetylbergenin also significantly restored towards normalization the decreased levels of glutathione and the decreased activities of glutathione S-transferase and glutathione reductase induced by GalN. Therefore, these results suggest that acetylbergenin has hepatoprotective effects against GalN-induced hepatoxicity by inhibiting lipid peroxidation and maintaining an adequate level of GSH for the detoxification of xenobiotics as underlying hepatoprotective mechanisms. In addition, lipophilic acetylbergenin showed more activity in the hepatoprotection than that of the much less lipophilic bergenin reported previously [60].

Bergenin and 11-O-galloylbergenin, isolated from the leaves of *Allophyllus edulis* var. edulis and *Allophyllus edulis* var. gracilis were found to have remarkable antihepatotoxic activities against CCl₄ and galactosamine cytotoxicity in primary cultured rat hepatocytes [49].

**Antiviral activity against hepatitis C and HIV**

A broad-degree of anti-hepatitis C virus (HCV) activity was observed for bergenin (over 1000 µM) when its *in vitro* inhibitory activities (IC₅₀) against HCV NS3 serine protease were tested by enzyme-linked immunosorbent assay [23]. As a
part of the screening of anti-AIDS agents from medicinal plants, the methanolic extract of the aerial parts of *Ardisia japonica* was tested, and it showed moderate *in vitro* anti-HIV activity. Bergenin and norbergenin showed weak anti-HIV activity [38]. The methanolic extract of the stem-bark and roots of *Peltophorum africanum* exhibited strongest inhibition (IC$_{50}$ 3.5 µg/ml) against the RNA-dependent-DNA polymerase (RDDP) activity of RT when screened for inhibitory properties against HIV-1 reverse transcriptase (RT). However, bergenin was found to be inactive when tested for inhibitory properties against HIV-1 reverse transcriptase (RT) and on HIV-1 integrase (IN) [47].

**Bergenin as a PTP1B inhibitor (antidiabetic/antiobesity activity)**

Protein tyrosine phosphatase 1B (PTP1B) is a major negative regulator of both insulin and leptin signalling. In addition, evidence suggests that insulin and leptin action can be enhanced by the inhibition of PTP1B. Consequently, PTP1B has emerged as an attractive novel target for the treatment of both type 2 diabetes and obesity. The link between PTP1B and diabetes and obesity has led to an avalanche of research dedicated to finding inhibitors of this phosphatase [61]. In bioassay-directed isolation from the whole plant of *Ardisia japonica*, sixteen known compounds including bergenin and norbergenin were obtained. Bergenin showed moderate bioactivity against PTP1B *in vitro* with IC$_{50}$ value of 157 µM [37].

**Anti-arrhythmic activity of bergenin**

Anti-arrhythmic effects of bergenin were investigated. At concentrations of 0.2, 0.4 and 0.8 mg/kg, bergenin showed distinct therapeutic effects on barium chloride-induced arrhythmias in rats. At concentrations of 0.4 and 0.8 mg/kg bergenin significantly countered arrhythmias induced by ligation and reperfusion of the coronary artery. At 0.8 mg/kg, bergenin elevated the atrial fibrillation threshold in rabbits from 1.34 mV to 1.92 mV. The results suggested that bergenin has good potential to treat cardiac arrhythmias [25].

**Antioxidant effects**

Postulations from indirect evidences from histopathological studies suggested that the extract of *Sacoglottis gabonensis* bark and bergenin protected the brain against alcohol intoxication and trapped free radicals of 2,4-DNPH and ethanol from crossing the blood-brain barrier. It was also shown that the extract and bergenin complemented ascorbate utilization in the brain during oxidant-induced oxidative stress [62-64]. The effect of bergenin on membrane lipid peroxidation and tissue ascorbic acid level was studied using rat as the experimental animal and 2,4-dinitrophenyl as the experimental oxidant. Pretreatment with bergenin
significantly reduced but did not completely abolish DNPH-induced lipid peroxidation in the liver, brain and red blood cell as evidenced from the levels of the two intermediates, lipid hydroperoxide and aldehydes measured. Bergenin also protected against DNPH-induced depletion of tissue ascorbic acid and had a sparing effect in untreated animals. The results suggested that bergenin may be responsible for the anti-lipid peroxidizing effect of the bark extract of *Sacco-glottis gabonensis*. An investigation was also designed to study its influence on the metabolic and cytotoxic side effects of 2,4-dinitrophenyl hydrazine (2,4-DNPH) on the brain and blood using male weaving rats as the experimental model [65]. Lipid peroxidation was induced experimentally with a single intraperitoneal phenylhydrazine (2,4-DNPH) administration at the end of 3 days exposure to the bark extract or bergenin in drinking water. Three hours later, the brain, liver and red blood cells of the experimental animals were analyzed for glucose level and the blood was analyzed for selected key indices of oxidative stress: red blood cell (RBC) count hemoglobin (Hb), packed cell volume (PCV) and white blood cell (WBC) count (total and differential). The bark extract exhibited a protective action on brain glucose, significantly inhibiting the glucose-depleting action of both 2,4-DNPH and ethanol. It also inhibited the lowering action of DNPH and ethanol on PCV, RBC and Hb concentration of rat blood, but inhibited proliferation of white blood cells (total and differential). The data on the effect of bergenin, on the side effects of 2,4-DNPH experimental lipid peroxidation and on ethanol followed an essentially similar trend to those of the bark extract on brain glucose. Bergenin, similar to the bark extract, exerted a protective action on the brain tissue, though to a lesser extent, against the oxidants, 2,4-DNPH and ethanol. It is evident that aqueous ethanol extract of *S. gabonensis* stem bark has biological antioxidant properties against 2,4-DNPH and ethanol-induced tissue damage exerting its action on the hematological and metabolic side effects of the oxidants. By virtue of its essentially similar activity under the same conditions, bergenin appears to be the phytochemical constituent that is largely responsible for the observed action of the bark extract [65].

Antioxidant activity of *Caesalpinia digyna* root (CDM) and bergenin, its major compound, was studied in different in vitro models [51]. Bergenin exhibited good antioxidant activity in hydrogen peroxide, ABTS, DPPH and inhibition of lipid peroxidation assays with IC$_{50}$ values 32.54±1.78, 75.06±0.97, 165.35±1.60 and 365.12±2.78, respectively. It showed low antioxidant activity in scavenging of nitric oxide method and deoxy ribose method with IC$_{50}$ values 785.63±2.03 and 815.63±2.95, respectively. Bergenin, however, was found to be inactive in scavenging hydroxyl radical by p-NDA and superoxide radical by alkaline DMSO methods. Thus, it could be the relevant contributor for the synergistic activity of antioxidant metabolites of CDM extract.

Norbergenin, which is the O-demethyl derivative of bergenin, the main component of *M. japonicus*, was found to show moderate antioxidant activity (IC$_{50}$ 13 µM in DPPH radical scavenging; 32 µM in superoxide anion scavenging) [30]. Different
derivatives were prepared by coupling the sugar part of norbergenin with a variety of fatty acids for increasing its antioxidant activity. Selective esterification of hydroxyl groups on the sugar part greatly enhanced the antioxidant activity. The most potent one was norbergenin 11-caproate, which not only exhibited stronger antioxidant activity than that of catechin but also prevented neuronal death at 10 \( \mu \text{M} \) on the primary culture of rat cortical neurons in Dulbeco’s modified Eagle’s medium (DMEM) supplemented with \( \text{N}_2 \).

A compound, 11- \( \text{O}-(4′-\text{O-methylgalloyl})\)-bergenin isolated from the methanolic extract of \textit{Crassula cv. ‘Himaturi’} inhibited arachidonic acid-induced platelet aggregation more efficiently than acetylsalicylic acid and showed an anti-oxidative effect (\( \text{EC}_{50} \), 23.9 \( \mu \text{M} \)) equivalent to that of L-ascorbic acid or quercetin [45].

**Anti-arthritis activity**

Bergenin and nor-bergenin exhibited potent anti-arthritis activity which had a good correlation with their immunomodulatory activity through balancing of Th1/Th2 cytokine production [20]. Flow cytometric study showed that their oral administration at doses of 5, 10, 20, 40 and 80 mg/kg per oral dose inhibited the production of proinflammatory Th1 cytokines (IL-2, IFN-\( \gamma \) and TNF-\( \alpha \)) while potentiate anti-inflammatory Th2 cytokines (IL-4 and IL-5) in the peripheral blood of adjuvant-induced arthritic balb/c mice. The oral \( \text{LD}_{50} \), was more than 2000 mg/kg body weight of the mice. At the dose as high as 2000 mg/kg \( p.o \). neither any sign of mortality nor any observable negative symptom in general behavior of mice over a period of 1 week were observed, showing its apparent safety over long-term administration.

**Bergenin as burn wound healing accelerator**

The ethanol extract of \textit{Astilbe thunbergii} rhizomes, traditionally used for the treatments of a sword cut, wound bitten by animals, frost-bite, burn, suppurative dermatitis or skin inflammatory diseases from the Tang period (about 8\(^{th}\) century) in China, was studied for its effects on burn wound healing in mice. The topical application at a dose of 100 mg ointment per wound of 70% ethanol extract (0.5 or 1.0% (w/w) ointment) of this drug promoted the healing of burn wounds. Bergenin was isolated from it as one of the promotional effectors of burn wound healing in mice. The effective dose (\( \text{ED}_{50} \)) of bergenin on burn wound healing 190 \( \mu \text{g/wound} \) [17].

**Trypanocidal activity**

Bergenin showed an inhibitory effect on the growth of the bloodstream form of \textit{Trypanosoma brucei} with an \( \text{IC}_{50} \) value of 1 \( \mu \text{M} \) [24].
CONCLUSIONS

Natural products have played a significant role in the area of drug discovery and are the source of numerous therapeutic agents. Over the past 75 years, natural product derived compounds have led to the discovery of many drugs to treat human disease. These natural products are being developed to improve cancer therapy, to treat resistant bacterial and viral infections and to expand immunosuppressive therapy to diseases such as multiple sclerosis. In the areas of cancer and infectious disease, 60 and 75%, respectively, of new drugs, originate from natural sources [66].

As discussed above, bergenin exhibits a wide array of biological activities and in several cases is also responsible for the traditional uses of its source eg. the inhibition of tyrosine hydroxylase activity exhibited by bergenin and norbergenin in vitro might also occur in vivo, and thus it may be partially responsible for the traditional uses of Mallot Cortex in treating peptic ulcer by reducing the availability of DOPA/dopamine [12]. In other cases there is still need for further investigations eg. a simplified in vitro screening static model was used to study the growth, inhibition and dissolution of urinary stones by the putative litholytic medicinal plant, Bergen ligulata, popularly known as Paashaanbhed in the Indian system of medicine, and the statistical analysis showed a highly significant correlation of data [67]. However, the antiurolithiatic activities of bergenin, its major component, need to be studied. Besides, Bergenin has demonstrated significant hepatoprotective activity when studied in different models and seems to hold good promise for becoming a useful chemical entity.

Thus, from the present review it can be concluded that although its full potential has not been fully explored, bergenin shows possible applications in clinical research and seems to be a potential phytotherapeutic agent especially as a hepatoprotective and an antioxidant. It is present in abundant yields in Ardisia pusilla and A. japonica (59%) [68] and in Mallot Cortex (Mallotus japonicas; 11–18%) [12] which can be used as good sources of bergenin. Further investigations may, therefore, help in exploiting its properties and developing phyto-pharmaceuticals based on it.

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w medycynie tradycyjnej w miejscach swojego występowania. Wykazano szereg jej właściwości farmakologicznych i dlatego istnieje kilka możliwości stosowania jej w badaniach klinicznych. Niniejsza praca to porównawcze studium prac ukazujących badania nad różnymi właściwościami bergeniny, szczególnie jej działania hepatoprotekcyjnego, antyoksydacyjnego i przeciwwirusowego, a także hamującego rozwój wrzodów i przeciwreumatycznego. Jak wykazano na modelach zwierzęcych, bergeninę można uznać za roślinny czynnik leczniczy. Kolejne badania mogą pomóc w określeniu sposobu wykorzystania jej właściwości i wynalezieniu leków roślinnych z jej udziałem.

_Słowa kluczowe: bergenina, składnik rośliny, farmakologia, hepatoprotekcja, antyoksydanty_