Pharmacological Activities of Ribwort Plantain and its Key Constituents: a Review

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Abstract:

Ribwort plantain has shown considerable pharmacological properties that are related to its wide-range secondary metabolites. The present review is aimed to highlight the recent progress on the antimicrobial, anti-inflammatory, antioxidant, neuroprotective, anticancer and wound healing effects of acteoside, aucubin, catalpol, apigenin and luteolin as significant metabolites from this plant.

Functional evidences support their role in the neutralization of reactive oxygen species, inhibition of pathogen attack, wound healing, prevention of tumorigenesis and neuroprotection via anti-apoptotic pathways and blocking pro-inflammatory signaling cascades. By virtue of these actions, the plant is warranted as a candidate for treating several diseases. The bioactivity in these locations is maximized by the action of synergy occurring between constituents in the phytochemical matrix. Mechanistic studies reveal multi-target action such as enzyme inhibition, gene expression control and receptor modulation. Although preclinical evidences indicate the high polypharmacological potential of this plant, full-scale clinical translation will require more phytochemical standardization and human studies. The present work highlights the integrative therapeutic relevance of broadleaf plantain and its metabolites in the treatment of multifactorial diseases.

Keywords: Plantago, lanceolata, verbascoside, aucubin, catalpol, apigenin, luteolin, anticancer

مقالة عن الفعالية العلاجية لنبات لسان الحمل السهمي ومركباته الكيميائية

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الخلاصة:

بفضل ما تحتويه من مجموعة واسعة من المركبات الأيضية الثانوية، يمتلك نبات لسان الحمل السهمي تأثيرات طبية ملحوظة. تركز هذه المراجعة على الخصائص المضادة للبكتيريا، المضادة للالتهابات، المضادة الأكسدة، والواقية للاعتلال العصبي، المضادة للسرطان، والمُعززة لالتئام الجروح، والتي توفرها مركبات الأكتيوزيد، والأوكوبين، والكاتالبول، والأبيجينين، واللوتيولين، والتي تعتبر جميعها مركبات رئيسية في هذا النبات. تُظهر الدراسات العلمية قدرة هذه المركبات على تثبيط فعالية المواد المؤكسدة، تحييد مسببات الأمراض، تعزيز تجدد الأنسجة، منع نمو الخلايا السرطانية، وحماية الخلايا العصبية عبر

المسارات المانعة للموت المبرمج للخلايا وتثبيط الاشارات المحفزة للالتهابات. تجعل هذه الفعاليات الطبية نبات لسان الحمل السهمي خيارًا واعدًا لعلاج مختلف الأمراض.
تعمل المكونات الكيميائية النباتية بشكل تآزري لتعزيز الفعالية الطبية للنبات. وبالرغم من أن البيانات غير السريرية تؤكد الفعالية الطبية لهذا النبات، إلا أن نقلها للتطبيق السريري يتطلب توحيدًا معياريًا إضافيًا للمركبات النباتية وإجراء تجارب بشرية. تكشف الأبحاث حول هذا النبات عن فعاليات متعددة الأهداف مثل تثبيط الإنزيمات، تنظيم التعبير الجيني، وتعديل نشاط المستقبلات. تسلط هذه الدراسة الضوء على القيمة العلاجية لنبات لسان الحمل السهمي ومكوناته الكيميائية في معالجة الامراض متعددة العوامل.

الكلمات المفتاحية: لسان الحمل، السهمي، فيرباسكوسايد، أوكوبين، كاتلبول، أبجينن، لوتيولن، مضاد للسرطان

1. Introduction

The Plantain (*Plantago*) species are consumed as food and folk medicine in several countries. Old medicine has used plants, paving the way for new drugs. Ribwort plantain (*Plantago lanceolata L., Plantaginaceae*), is one such herb that has attracted attention for its healing properties. Native to Europe and grown throughout the world, this perennial herb has a rich history of use in wound healing, as well as treating inflammation and respiratory issues (1). Recent scientific studies have confirmed its pharmacological properties and attributed them to bioactive compounds including iridoids (catalpol, aucubin), flavonoids and phenolics. There is increasing attention on the development of natural products for health use, ribwort plantain (RP) is gaining attention as a potential candidate for therapeutic uses in modern as well as conventional medicine (2). This review focuses on recent research, highlighting the beneficial effects, phytochemistry and pharmacological efficiency of RP and its important metabolites.

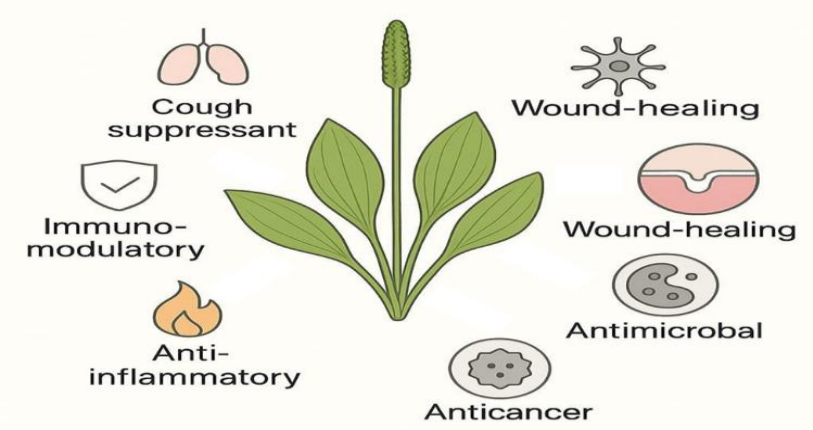

Figure (1): The main pharmacological activities of ribwort plantain

2. Phytochemical Composition

The five types of phytochemical groups included flavonoids, monoterpenoid, triterpenoids, iridoids and phenolics playing the crucial biological role in Plantago spp. RP's pharmacological activities are due to its broad spectrum of phytochemicals (3). These include some key classes of compounds, as elucidated by recent studies. Iridoid Glycosides, such as aucubin and catalpol, are the primary iridoids extracted from RP that exert anti-inflammatory, antibacterial and hepatoprotective effects. Flavonoid compounds, including luteolin and apigenin, present anti-inflammatory and antioxidant quality that protect cells from

oxidative damage. Phenylethanoid glycosides predominantly acteoside (verbascoside), are major bioactive phytochemicals of RP and essential to the pharmacological effects of this plant. It demonstrates strong antioxidant, antimicrobial and anti-inflammatory properties suggesting the effectiveness of ribwort as a remedy for respiratory and wound healing. Phenylethanoids are another important chemotaxonomic marker of RP in addition to iridoid glycosides (4).

Phenolic acids, especially chlorogenic, caffeic and ferullic acid abundantly present and known by their potential anti-inflammatory and antioxidant activity. Furthermore, RP contains polysaccharides that are high molecular weight compounds which facilitate the repair of the tissues and have an immunomodulatory effect. Antimicrobial and astringent properties of RP are attributed to saponins and tannins, which could be applied in wound healing. The advancements in metabolomics have aided researchers to identify a high number of secondary metabolites in RP, underlining its complexity and pharmacological activities (5,6).

3. Pharmacological Activities

3.1 Antimicrobial Activity

Different studies have shown that RP have broad-spectrum antimicrobial effects. Extracts have demonstrated effectiveness against both Gram-positive and Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* (7). There have also been reports of antiviral and antifungal efficacy (8,9). The agar disc diffusion method demonstrated that the methanol (70%) leaf extracts have high antibacterial effects against certain bacterial pathogens namely *Streptococcus*, *Monocytogenes*, *S. aureus*, *E. coli* and *Salmonella* species (10). Leaf extracts of RP shown antibacterial efficacy against *K. pneumoniae*, *S. pneumoniae*, methicillin-resistant *S. aureus* MRSA, *S. boydii*, *E. coli*, and *S. aureus* utilizing various extracts, including methanol, acetone, and water. The result exhibited that higher level of antimicrobial effect was detected with MIC and MBC values in the range of 6.2 to 26%, respectively (11). The antibacterial efficacy is influenced by the extraction solvent used to extract bioactive substances, the bacterial strain, and plant components (12). A recent study showed that the ethanolic extract of RP exhibits a potent antibacterial effect, mainly against drug-resistant pathogens like MRSA, *S.* MRSA+MDR, *S. mutans*, and *S. haemolyticus*, evidenced by low MIC values (0.174–0.179 mg/mL) and inhibition zones of 9–13 mm (13).

Three fungal pathogens (*Alternaria alternata, Penicillium expansum*, and *Mucor piriformis*) were tested using RP aqueous extracts. The result showed dose-dependent inhibition, with the highest efficiency against *M. piriformis* (71.91% inhibition at the higher concentration) and moderate activity against *A. alternata* and *P. expansum* (14). According to research in 2023, RP extracts in methanol and ethyl acetate showed a strong antifungal effect at MIC around 0.55 mg/mL against *M. globosa* and *C. albicans*, confirming its historic usage in the treatment of superficial fungal infections (15).

Recent in vitro studies have demonstrated that acteoside, RP's major component, is a potent sortase A inhibitor at 64 µg/mL, significantly reducing bacterial adhesion, invasion, and biofilm formation. Acteoside's significant protective effects on models of pneumonia and *G. mellonella* infection further establish its efficacy in vivo at 40 mg/kg [16]. Another study has shown that acteoside has an antifungal effect through the binding to specific proteins in *V. dahliae* and *P. infestans* (17). Acteoside exhibited extensive antibacterial activity, especially against *Staphylococci* spp., with a potent inhibitory effect at a dosage of 9.77 µg/mL.

Several bacterial strains, including MRSA, were successfully sensitive to acteoside (18). RP is known as an iridoid-containing plant, mainly aucubin. When given at concentrations ranging from 60 to 245 µg/mL, aucubin inhibited *C. albicans* overall growth, biofilm formation, metabolic activity, and cell surface hydrophobicity. At 244 µg/mL, it could start to have a fungicidal impact. Although the exact mechanisms underlying aucubin's antifungal action are yet unknown, it is thought to be due to the suppression of the cell surface hydrophobicity pathway (19). Moreover, aucubin exhibited antibacterial action on different gram-positive bacteria, including *S. aureus, S. epidermidis*, *B. subtilis* and *E. faecalis* and gram-negative bacteria including *E. aerogenes*, *P. vulgaris*, *E. aerogenes*, *C. diversus* and *P. mirabilis*, with MIC values from 8–129 µg/mL (20). Catalpol suppresses lipid mediators (PGE_2, LTB_4) and pro-inflammatory cytokines (TNF-α, MCP-1) to prevent influenza-induced inflammation in rat alveolar macrophages. Catalpol attain this effect by suppressing the TLR7/MyD88/NF-κB signaling cascade at the mRNA and protein levels. Catalpol also inhibits the formation of arachidonic acid metabolites by decreasing the activity of phospholipase A_2 (21).

Luteolin and apigenin work in concert with antibiotics to provide notable antimicrobial synergistic effects. Across several strains (e.g. *E. coli, K. pneumoniae, P. aeruginosa*, MSSA, and MRSA), luteolin showed wide or partial synergy with gentamicin, levofloxacin, and ampicillin, increasing antibiotic effectiveness, especially against MRSA by targeting cell walls and metabolism. Apigenin exhibited partial synergy in other bacteria, reversing resistance in certain infections, and synergistic interactions mostly with gentamicin and levofloxacin against *P. aeruginosa* and *Staphylococcus* strains (MSSA, MRSA). Both flavonoids increase the effectiveness of antibiotics against bacteria that are resistant to them (22). Luteolin reduced the production of viral mRNA and gB protein and hindered the virus throughout its replication stage in Pseudorabies-BALB/c infected mice. Luteolin at 100 mg/kg/day (three consecutive days) lowered the viral loads in the liver, brain, kidney, lung, and heart decreased brain lesions, delayed inflammation and oxidation processes, reduced the apoptosis of virus-infected cells, and increased the survival rate of mice following fatal challenge. These data point to luteolin's potential as a novel antiviral medication for PRV infection (23).

Through blocking the virus's ability to enter host cells—specifically, Vero E6 cells, luteolin at 10 µM has strong antiviral properties against SARS-CoV. It was shown that luteolin binds to the viral S2 subunit protein with high affinity. According to the suggested mechanism of action, luteolin interferes with the crucial virus-cell fusion process that the S2 protein mediates. The infection of host cells is successfully prevented by this disruption of binding and fusion (24).

3.2 Wound-Healing Properties

RP has a longstanding history in the treatment of the wounds, and current works validate its effects. The polysaccharides present in high levels in RP, are effective to stimulate fibroblast and collagen formation (25). Using 10 and 20% ointments of RP polar extracts (PLE) in 1 cm wounded mice on the back, promoting angiogenesis, collagen formation, and epithelialization in association with modulation of key growth factor levels, PLE strongly accelerates the process of wound closure in mice. PLE ointments accelerated wound closure in the trial with 10% dose leading to the best results. In PLE-treated wounds, vascular remodeling was preserved through reduced inflammation, increased TGF-β1 levels (which reached peak on day 14), and

regulation of ANGPT-2. Furthermore, PLE enhanced the fibroblast maturation and activation, leading to more adherent tissue healing. These results suggest that RP may be a useful natural medicine for wound treatment (26). RP is one of the plants that known for their high-mucilage. The mucilage-facilitated zinc oxide nanoparticles promoted the tissue regeneration in mice by collagen formation and antimicrobial activity, with a very high wound-healing potential (27). On the other hand, using scratch assays to promote cell migration in human dermal fibroblast, acteoside greatly enhances wound closure; the dose used was 20 µg/mL. It also suppresses UV-induced MMP-9 release from keratinocyte-fibroblast co-cultures, thus having anti-inflammatory and photoprotective effects. This makes extracts high in acteoside a viable treatment for protecting and repairing skin (28).

Iridoid glycosides showed ability to diminish inflammation and microbiological growth at wound site by targeting Ras/p53 MAPK/NF-κB signaling pathway. They play a crucial part in the biological process of wound healing by inducing M2 macrophage polarization early on, which facilitates the shift from inflammation to cell proliferation. By promoting collagen deposition, angiogenesis, and tissue remodeling, this process speeds up the healing and closure of wounds overall. Thus, by regulating macrophage activity, iridoids are essential for maximizing the wound healing process (29). Aucubin dramatically speeds up wound closure and shortens the healing period when given topically to hyperglycemic rats in a dose-dependent manner. Although not entirely explained, aucubin's proven antibacterial and anti-inflammatory properties via inhibiting NF-κB, may help explain its benefits on diabetic wound healing. It makes aucubin a viable option for treating diabetic wounds (30). Catalpol improves wound healing and greatly increases flap survival in Sprague-Dawley rats. By raising superoxide dismutase and reducing malondialdehyde, it lowers oxidative stress. Catalpol facilitates angiogenesis by increasing micro vessel density and upregulating vascular endothelial growth factor. It also inhibits the synthesis of tumor necrosis factor- α (TNF-α) and interleukin (IL-6) by downregulating TLR4/NF-κB signaling, which results in strong anti-inflammatory actions. Additionally, catalpol inhibits the creation of the NLRP3 inflammasome, which lowers the production of IL-1β and IL-18 and decreases cell pyroptosis (31).

Apigenin modifies the polarization of macrophages to improve wound healing in diabetics. It accelerates the transition from M1 to M2 phenotype by upregulating miR-21 expression in macrophages, which inhibits pro-inflammatory M1 factors (TNF-α, IL-1β) and increases anti-inflammatory M2 factors (IL-10, TGF-β). In co-culture, this miR-21-dependent macrophage reprogramming promotes the migration, proliferation, and VEGF production of endothelial cells (HUVECs). The mechanism of action entails the inhibition of the TLR4/Myd88/NF-κB signaling pathway (32). For regulated release, luteolin is integrated into pH- and ROS-responsive nanoparticles (PBE@Lut) in the hydrogel. When released, it has anti-inflammatory and antioxidant properties. In particular, luteolin encourages the polarization of anti-inflammatory macrophages. The hydrogel's overall therapeutic advantages in the healing of chronic wounds (33).

3.3 Anti-inflammatory and Immunomodulatory Effects

RP is particularly rich in mucilage, mainly in its seeds and leaves. The mucilage consists primarily of polysaccharides like arabinogalactan, rhamnogalacturonan, and glucan, which swollen in water forming a gel-like substances. This activity plays the major role in its traditional uses as a

demulcent, antitussive, and wound-healing agent (34). Aucubin mitigates inflammation by obstructing NF-κB/HMGB1 signaling and STAT3 phosphorylation (Ser727/Tyr705), decreasing TNF-α, IL-1β, and IL-6, while enhancing IL-10 levels. Aucubin prevents myocardial ischemia (MI)-reperfusion damage (RD) in MI/RD rat model by blocking HMGB1 release and NF-κB p65 nuclear translocation. In cardiac cells, these actions lessen oxidative stress and apoptosis. The regulation of the STAT3/NF-κB/HMGB1 pathway demonstrates aucubin's potential as a treatment for inflammatory diseases (35). Aucubin at 100 ng/mL lowers inflammation by blocking the TLR4/NF-κB pathway, changing macrophages from pro-inflammatory M1 to anti-inflammatory M2, using human THP-1 monocytes. This reduces cytokines like TNF-α and IL-6, relieving chronic inflammation. In GONFH rats induced by steroids, aucubin mitigates bone injury by regulating immunological responses at 100 mg/kg. The combined impact of aucubin on signaling and macrophage polarization indicates therapeutic potential (36).

In rheumatoid arthritis mice model, catalpol dramatically lowers the production of ROS and inhibits NF-κB-p65 activity. Additionally, catalpol significantly reduces nitrite release and the production of pro-inflammatory cytokines. Its anti-inflammatory and cellular protective properties are based on its multi-targeted suppression of oxidative stress, NF-κB signaling, and cytokine production (37). Catalpol provides pharmacological protection against retinal ischemia on many targets. Significant antioxidative effect against H_2O_2-induced stress and anti-ischemic effects against OGD in retinal ganglion cell-5 (RGC-5), which improve viability, are among catalpol important activities. Catalpol at 0.5 mM, preserves retinal function using electroretinography (ERG), in addition to promoting RGC survival in vivo (rat model) by exhibiting neuroprotective, anti-inflammatory (lowering MCP-1), and antiapoptotic impacts. Catalpol counteracts ischemia mechanistically by blocking downstream mediators HIF-1α, VEGF, and angiopoietin-2 and reducing the upstream Wnt/β-catenin pathway (38).

Acteoside and its isomer isoacteoside are shown to modify neutrophil immunological responses by controlling toll-like receptor-driven activation (39). In high glucose-treated HK-2 cells acteoside revealed inhibition of pro-inflammatory factors, IL-6, and TNF-α, as well as the expression of extracellular matrix proteins, fibronectin (FN) and collagen-IV (COLIV) at 8, 12 and 16 µM, demonstrating renoprotective effects through the activation of AMP-activated protein kinase (40). Luteolin and apigenin showed notable immunomodulatory effect, treating the immunosuppression caused by cyclophosphamide in a dose-dependent method (50 and 100 mg/kg) in rats. Both flavonoids boosted T-cell-mediated immunity, promoted macrophage phagocytosis, and recovered deficient hemoglobin, RBCs, WBCs, and leukocyte numbers, supporting their role as immunoenhancing agents (41).

3.4 Anticancer Potential

Emerging evidence shows that RP may have anticancer activity. The methanol extract of RP exhibited potent anticancer activity against U87-MG glioblastoma cells at low doses (3.125 µg/mL) with significant selectivity (SI = 23.97), indicating a preferential targeting of cancer cells over healthy cells. Crucially, the extract did not harm normal cells (HDF-a) DNA up to 200 µg/mL, indicating a safe profile. These findings underscore its promise as a natural anticancer drug, especially for brain cancer (42,43). Another research indicated that phenylethanoid glycosides (acteoside and plantamajoside) derived from RP

demonstrated significant, selective cytotoxicity against breast (MCF-7), ovarian (OVCAR-3), liver (HepG2), and glioblastoma (U138-MG) cancer cell lines, with acteoside exhibiting greater potency and less harmful to healthy cells (MCF-12A), which can be considered as a targeted anticancer treatment (44). Against colorectal cancer cells, RP root extracts exhibited selective anticancer action; the acetonic extract was the most potent (IC50 119.68 µg/mL). Potential bioactive substances with cytotoxic effects, such as fatty acids and phthalate derivatives, were discovered by GC-MS (2). In toxicity studies, the extracts showed outstanding safety characteristics. However, more refining is necessary to fulfill potency criteria (45). According to recent research, alcoholic extracts preferentially targeted cancer cells without causing toxicity to normal cells, whereas acetonic extracts demonstrated cytotoxicity against HCT-116 cancer cells (IC50: 185.04 µg/mL) while also injuring normal cells (HEK-293). Bioactive substances having anticancer activities, such as n-hexadecanoic acid, linoleic acid, and oleic acid, which may play an important role in this extract were discovered by GC-MS (46).

Different studies demonstrated the anticancer effects of acteoside through its activity on AKT/NF-κB, PI3K/AKT pathways, the expression of Bax, Bcl-2, caspase-9, TLR4, MyD88, also its involvement in different mechanisms of drug resistance (i.e. oncogenes, ABC transporters, cellular proliferation rates, tumor suppressor genes, and other parameters) (47), showing activities against ovarian (48), breast (49,50), esophageal (51), lung (52), cervical (53), liver (54) colorectal (45).

Aucubin, which is the major iridoid glycoside in RP, showed dose-dependent anticancer activity, which found to cause apoptosis in human osteosarcoma MG-63 cells via caspase-dependent mechanisms, also showed anticancer effect against breast cancer, through induction of apoptosis in tumor cell (55). Catalpol demonstrates anticancer activity by modifying the mesenchymal phenotypes of cancer cells, as indicated by decreased expression of markers (α-SMA/S100A4). It improves antioxidant defenses to lessen cellular damage linked to tumor growth by targeting oxidative stress pathways (Nrf2/HO-1) with highest activity at 50 µM. Although it hasn't been explicitly studied on skin cancer, earlier research has shown that it works well against tumor cells through these pathways. It is positioned as a possible anticancer drug due to its combined impact on redox balance and phenotypic regulation (56).

In preclinical models, luteolin and apigenin show anti-inflammatory, antioxidant, and broad-spectrum anticancer pharmacology, preventing the formation of tumors in skin, liver, lung, stomach, colon, breast, pancreatic, and prostate malignancies. Modification of important cellular signaling networks is one of their anticancer strategies. Clinical translation is yet unknown despite strong in vivo effectiveness, requiring more pharmacodynamic and delivery improvement (57). Using human neuroplastoma cell line, treatment with apigenin at 5 µM enhanced the phosphorylation of downstream CREB (Ser133) and TRKB (Tyr516, Tyr817). This decreased the expression of the pro-apoptotic protein BAX and increased transcription of the anti-apoptotic component BCL2. Apigenin therefore demonstrates anticancer effect by TRKB signaling to produce an anti-apoptotic change (58). By preventing STAT3 phosphorylation, luteolin and apigenin downregulate IFN-γ-induced PD-L1 production while directly suppressing the development of KRAS-mutant lung cancer and inducing apoptosis. Both flavonoids exhibit strong anticancer effect in vivo in models of genetically modified KRAS mutations, Lewis's lung cancer, and xenografts (59).

3.5 Antioxidant and Neuroprotective Effects

The antioxidant activity of RP is the result of its phenolic constituents at high level. These phenolics neutralize the free radicals and decrease oxidating stress, which is playing important role in aging and chronic diseases (60,61). Numerous experiments have shown that RP demonstrates strong antioxidant activity through a variety of routes. Water extracts performed exceptionally well in ferrous ion chelating (95% at 0.25 mg/mL) and β-carotene bleaching (71% at 0.4 mg/mL), whereas methanolic extracts had the strongest antiradical and reducing power actions. Strong total antioxidant capacity was found in methanol extracts (145 mg AAE/g extract) by the phosphomolybdenum test. These actions show the plant's potential as a natural antioxidant source for food and medicinal uses by correlating with its high phenolic and flavonoid content, especially acteoside (94.8 mg/g dry weight), chlorogenic acid, rosmarinic acid, and flavonoids (62). Another study showed that in DPPH radical scavenging tests, RP's aqueous crude extract and its biosynthesized silver nanoparticles (Ag NPs) showed strong antioxidant activity. In comparison to ascorbic acid (66.13 µg/mL), the IC50 values for the aqueous crude extract and silver nanoparticles were 369.6 µg/mL and 159.4 µg/mL, respectively. According to this study, RP is a promising natural antioxidant source that may find use in nanotechnology and biomedicine (63).

A recent study emphasized the neuroprotective properties of RP, namely its methanol extract, which showed the capacity to prevent amyloid-β (Aβ) fibrillogenesis, damage mature fibrils, and diminish reactive oxygen species (ROS). The extract showed considerable inhibition of the enzyme's acetylcholinesterase and butyrylcholinesterase at doses of 100 and 500 µg/mL respectively, which are associated with the development of Alzheimer's disease (42). Further research emphasized the neuroprotective potential of RP, attributing this effect mostly to its bioactive components, such as acteoside, which demonstrate antioxidant and anti-inflammatory effects (64).

Recent data indicated that microRNAs (miRNAs), including miR-132, miR-34a, and miR-124, are integral to the pathogenesis of Alzheimer's disease (AD), affecting amyloid-beta (Aβ) aggregation, tau phosphorylation, neuroinflammation, and oxidative stress. The data highlighted the activity of catalpol as a potential modulator of miRNA expression, which may provide neuroprotective advantages through multi-target methods (65). Recent research showed that catalpol enhances axonal development, using C57BL/6J mice with a dose of 10 mg/kg, establishing a biological foundation for facilitating neurological recovery following ischemic stroke by directly targeting IGF-1 (66). Additional findings on cerebral organoid from human stem cells emphasized catalpol's capacity (10 µM) to enhance the production of cerebral organoids characterized by augmented populations of outer radial glia cells and elevated neurogenic potential via STAT3 activation, providing novel insights into the modeling of neocortical development (67). Another studies focus on aucubin, which has potential as an effective agent for relieving diabetic neuropathic pain by preventing expression of AKR1B1 (68). Aucubin also showed to be a viable therapeutic option for relieving airway inflammation by decreasing the level of nitric oxide (NO), TNF-α, and IL-6 levels (69). According to data obtained from the Web of Science Core Collection (WoSCC) database A collection of 152 publications were produced between 2013 and 2023, antioxidant and anti-inflammatory effects of aucubin were the most commonly recorded, and they may serve as the main avenues for future research (70).

Apigenin exhibits strong antioxidant properties via two different pharmacological pathways. Its phenolic hydroxyl groups, which transfer electrons, are responsible for its reducing power (IC_{50}= 8.5 µg/mL) and direct scavenging of free radicals (IC_{50}= 2.43 µg/mL), as demonstrated by its strong DPPH and NORSA test performance. Through specific binding interactions, molecular docking demonstrates apigenin capacity to inhibit pro-oxidative enzymes such as xanthine oxidase and iNOS. Apigenin retains strong antioxidant activity by interfering with oxidative cascades at the radical and enzymatic levels (71). In a rat model of LPS-induced neuroinflammation, luteolin-loaded micelles showed notable neuroprotective pharmacology. The formulation demonstrated neuroinflammation reduction by successfully lowering oxidative stress (MDA) and important pro-inflammatory cytokines (i.e, IL-6 and TNF-α). This antioxidant and anti-inflammatory effect resulted in a discernible enhancement of cognitive performance (72).

3.6 Osteoprotective effect

The major phytochemicals in RP (e.g. acteoside and aucubin) have exhibited an activity as osteoprotective agents. Therefore, RP may be a viable option for supporting bone health naturally, especially in cases of osteoporosis and inflammatory bone disorders. To verify effectiveness in humans, more clinical research is required. In MG63 human osteoblast-like cells aucubin at 5 µM rises the expression of cytokines in differentiating osteoblasts such as osteocalcin, osteopontin, osterix and collagen I, in bone tissues, hence improves bone formation, structural integrity and mineralization (73). In bone marrow mesenchymal stem cells (BMSCs), aucubin at 10 µM upregulates osteogenic markers via activating the signaling of bone morphogenetic protein 2 (BMP2)/SMAD family proteins. By scavenging iron and ROS, it prevents ferroptosis and maintains the survival of BMSCs. Additionally, aucubin at 30 mg/kg increasing mineralization and density, which helps ovariectomized rats regain bone mass. Its dual activity demonstrates its promise as an osteoporosis treatment (74). Aucubin enhances osteonecrosis and maintains trabecular integrity, hence preventing bone loss linked to glucocorticoids. In glucocorticoid-induced osteonecrosis of the femoral head rat models, aucubin at 100 mg/kg decreases trabecular separation and increases bone mineral density in a dose-dependent manner. By blocking TLR4/NF-κB signaling, the chemical causes macrophages to change from the destructive M1 phenotype to the reparative M2 phenotype. These processes illustrate aucubin's dual function in regulating inflammation and protecting bones (36).

Recent research has shown that acteoside at 50 µg/mL inhibits caspase-3 by decreasing LPS-induced inflammation in MLO-Y4 osteocyte-like cell to prevent apoptosis, and restoring oxidative equilibrium by increasing SOD, CAT, and GSH levels. By downregulation of resorption signals (RANKL, MEPE) and upregulation of osteogenic markers (PHEX, RUNX2, OPG), it encourages the production of new bone. Moreover, acteoside improves cell survival and mineral homeostasis in these cells, therefore counteracting LPS-induced bone loss. Its promise as a treatment for inflammatory bone diseases is demonstrated by these activities (75).

Through reducing oxidative stress and cadmium buildup, one of the RP constituents, chlorogenic acid (CGA), lessens the damage that cadmium causes to bones of female SD rats at 42 mg/kg. CGA shown to repair the microstructure of bone by preventing adipocyte development and encouraging osteogenic activity. In addition, while inhibiting signs of bone resorption, it also promotes bone mineralization. These effects

revealed CGA protective role against Cd-induced osteoporosis (76). CGA in Ca-CGA nanoparticle has dual anti-inflammatory and osteogenic properties by polarizing macrophages to the M2 phenotype and promoting BMSC development. The nanoparticles disintegrate in lysosomes, liberating CGA and Ca^{2+} to inhibit pro-inflammatory reactions and facilitate bone healing. In vivo, Ca-CGA nanoparticles expedite the healing of skull defects by synergistically influencing immune and stem cells. This technique demonstrates CGA's potential for bone regeneration through tissue engineering and immunomodulation (77).

Apigenin exerts an osteoprotective effect via various mechanisms. It reduces oxidative stress by eliminating ROS, thus enhancing an osteoblast activity and reducing bone resorption. At 5 μM, it promotes osteoblast proliferation and differentiation by increasing levels of osteogenic markers such as ALP and collagen I, while at the concentration at 10 μM inhibits the generation of osteoclasts. In vivo, a 10 mg/kg dosage inhibits trabecular bone loss in ovariectomized mice, showing its anti-osteoporotic activity (78).

3.7 Activity against Polycystic Ovary Syndrome

Yet there is no specific study that clarifies the activity of RP against polycystic ovary syndrome (PCOS). In contrast, the phytochemicals of this plant showed interesting activities against PCOS. Acteoside, a crucial phenolic acid in *Plantago ovata* ethanol (70%) extract (POEE), contributes to the extract's pharmacological effects through a variety of pathways, according to research conducted in rats with letrozole-induced PCOS. POEE at 100 mg/kg/day markedly increased estrogen and decreased testosterone and LH levels, suggesting that the hypothalamic-pituitary-gonadal axis was modulated. Additionally, it decreased HOMA-IR and increased insulin sensitivity while reducing TOS, MDA, IL-1β, and NF-κB, which are indicators of ovarian oxidative stress and inflammation. Histologically, POEE increased the corpora lutea and decreased cysts to restore ovarian morphology (79). Catalpol helps in PCOS by lowering estrogen and testosterone levels and enhancing ovarian histology. By reducing MDA, increasing GSH, and enhancing antioxidant enzymes (SOD, CAT, GSH-Px), it effectively fights oxidative stress. Pharmacologically, catalpol suppresses the NF-κB signaling pathway in human granulosa cells and rat ovaries by upregulating SIRT1 expression. Importantly, this SIRT1-dependent suppression of NF-κB mediates the antioxidant, anti-proliferative, and pro-apoptotic actions of catalpol (80).

In the female wistar rats with PCOS, apigenin at 20 mg/kg increases corpora lutea, decreases cysts and collagen density, and restores follicular development, all of which enhance ovarian histology. Importantly, by inhibiting the proliferation of endothelium and periendothelial cells and ovarian production of VEGF and its receptor KDR (VEGF R2), apigenin has an antiangiogenic effect. Together, these effects imply that apigenin reduces PCOS ovarian changes in part by inhibiting the VEGF signaling pathway (81). According to the zebrafish study, apigenin uses a variety of pharmacological mechanisms to counteract the symptoms of PCOS induced by bisphenol A (BPA). Via lowering LDH levels and restoring SOD and CAT activity, it strengthens antioxidant defenses. Through inhibiting follicular disruption, collagen deposition, and hypertrophy, apigenin lessens ovarian damage. Additionally, it decreases the buildup of BPA in tissues and suppresses the production of TNF-α and genes linked to PCOS (82,83). Luteolin effectively suppresses the production of estrogen in human ovarian granulosa cells induced by follicle-stimulating hormone (FSH). This is accomplished by reducing the

MKK3/6-p38 MAPK-CREB signaling pathway, which lowers the production of CREB-mediated aromatase, by specifically targeting tumor progression locus 2 (TPL2). Significant disruption of the MAPK pathway is confirmed by transcriptional analysis. Additionally, luteolin showed promise as a treatment by successfully reducing PCOS symptoms in a rat model (84).

Table (1): Pharmacological activities of different extracts of RP

Pharmacological Activity	Mechanism of Action	Extract	Reference
Antimicrobial	Reduced Exotoxin A expression, biofilm disruption	Aqueous, ethanolic	(85) (86)
Wound-healing	Reduce inflammatory cell infiltration, modulation of ANGPT-2 expression, Stimulation of collagen synthesis, Regulation of TGF-β1, and increased organization of collagen I	Aqueous	(26)(87)
Antioxidant	NO-scavenging activity, Inhibition of iNOS expression, NF-κB pathway modulation	Hydroalcoholic	(88)
Anti-inflammatory	Suppressing NF-κB and MAPK pathways, blocking pro-inflammatory enzymes (COX-2, LOX) and cytokines, and by antioxidant capacity	Methanolic	(42)
Neuroprotection	Inhibition of Amyloid-β Fibrillogenesis, Antioxidant Protection, Cholinesterase Modulation	Methanolic	(42)(89)
Hepatoprotection	Hepatoprotection is mediated by antioxidant phytochemicals (e.g., phenylethanoids, flavonoids) that stabilize hepatocyte membranes, inhibit toxin-induced enzyme leakage, and block inflammatory cascades	Aqueous	(90)
Anticancer	Cancer cell death via mitochondrial disruption, Suppression of DNA damage and mutagenesis, Antioxidant, pro-apoptotic, and anti-mutagenic actions, starving tumors due to the content of isoleucine	Aqueous, methanolic	(91) (42)
Antispasmodic	Inhibits ACh (muscarinic) and histamine (H_1) receptor signaling in the ileum, Suppresses Voltage-Gated Ca^{2+} Channels	Aqueous, ethanolic	(92)

4. Safety and Toxicity

RP is typically regarded as safe when appropriately utilized. Acute and sub-chronic toxicity investigations have indicated no notable deleterious effects, even at elevated doses (93). Acute oral toxicity assessments in mice indicated no mortality or notable weight alterations at dosages up to 2000 mg/kg for methanolic and ethanolic extracts, categorizing them as non-toxic according to the Hodge and Sterner scale. After being exposed to alcoholic extracts (25–400 µg/mL) for 4 hours, hemolysis tests showed no harm to red blood cells. Lethality experiments on *Artemia salina* showed ≤ 2% death at the highest dose and very low toxicity with LC_{50} >1000 µg/mL. In vitro, alcoholic extract exhibited biocompatibility and selective toxicity against cancer cells, whereas acetonic extract showed cytotoxicity to both normal (HEK-293) and cancer (HCT-116) cells (94-96). However, allergic responses have been stated in rare cases. Potential drug-herb interactions, especially with immunosuppressive medications, require more research. Another study demonstrated low hemolytic activity (<1% at 400 µg/mL), indicating good blood biocompatibility by the methanolic root extract. The extract showed little toxicity in brine shrimp lethality assays with only ≤3% death at 1000 µg/mL. Low systemic toxicity was confirmed by acute oral delivery in rats, which showed no mortality or behavioral problems at dosages up to 2000 mg/kg after one week. Based on the combined in vitro and in vivo findings, RP root extracts are deemed non-toxic by the OECD and Hodge-Sterner scales (97).

5. Future Directions

Deep clinical research is necessary to discover new therapeutic uses of RP with benefits and validate traditional treatments such as combined treatment with herbs or common drugs. Moreover, progression in the advanced drug delivery system as nanoparticles can address the issue of bioavailability and increase its pharmacological efficacy which connect traditional wisdom to modern scientific developments.

6. Conclusion

The combination of acteoside, aucubin, catalpol, apigenin and luteolin that works synergistically to produce the pharmacological effects of RP. Collectively taken, these compounds influence some crucial biological processes such as inhibition of microbial pathogenesis modification of cellular differentiation improvement of oxidative stress via Nrf2 mediated antioxidant defences and suppression of inflammation through NF-κB and MAPK. Preclinical evidence is nevertheless compelling to support traditional uses in wound healing, which are underpinned by HIF-1α-driven angiogenesis and TGF-β/Smad-mediated extracellular matrix remodelling. Anti-inflammatory and immunomodulatory characteristics are well-described amongst different experimental models.Although they have mostly been confirmed in vitro or in preclinical settings, emerging mechanistic findings also show encouraging neuroprotective, osteogenic, and anti-polycystic ovarian syndrome benefits.

There are still significant translational problems, especially concerning the fast metabolism of flavonoid aglycones and the poor systemic bioavailability of glycosidic components. The creation of standardized extracts with distinct phytochemical profiles, sophisticated delivery methods to improve bioavailability, and thorough human clinical trials must be the top priorities of future research, particularly for endocrine and chronic degenerative diseases, where the available data clearly demonstrates mechanistic alignment. Although the polypharmacological framework of RP has great therapeutic potential, overcoming basic

pharmacokinetic constraints and producing solid human effectiveness data are necessary for its transfer from ethnopharmacological usage to evidence-based clinical integration.

Conflict of interests

No conflict of interest was declared by the authors.

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