REVIEW ARTICLE

Phytopharmacological insights into *Pongamia pinnata* and *Rubia cordifolia:* Antibacterial and antioxidant activities with mechanistic perspectives

Sunil Tiwari[1], Prashant Singh[1], Bharat Mishra[2*], Archita Tiwari[3]

[1]Department of Pharmacognosy, Singhania University, Jhunjhunu, Rajasthan, India, [2]Department of Pharmacology, Institute of Pharmacy, Dr. Shakuntala Misra National Rehabilitation University, Lucknow, Uttar-Pradesh, India, [3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Khwaja Moinuddin Chishti Language University, Lucknow, Uttar-Pradesh, India

Abstract

Pongamia pinnata is a rich and bright tree that reaches heights of 35–50 feet. It belongs to the *Leguminaceae* family. This versatile tree has been traditionally utilized in India and neighboring countries for a wide range of purposes, including traditional medicine, green manure, wood, animal fodder, fuel, biopesticides, and even fish poison. *Rubia cordifolia* Linn, commonly known as Indian Madder, is a well-known Ayurvedic herb belonging to the *Rubiaceae* family. The root of *R. cordifolia* is used for treating various ailments such as cough, hepatic obstruction, freckles, indigestion, inflammation, ulcers, fractures, mental agony, urinary obstructions, and paralytic affections. Both *R. cordifolia* and *P. pinnata* are known for their antibacterial and anti-oxidant properties. *R. cordifolia* contains compounds like anthraquinones and glycosides, which contribute to its antibacterial and anti-oxidant activities by acting on mitogen-activated protein kinase, nuclear factor kappa-B, and Nrf-2 pathways. *P. pinnata* contains active constituents such as flavonoids and furano flavonoids, known for their antibacterial and anti-oxidant properties.

Key words: Anthraquinones, antibacterial, anti-oxidant, flurano-flavonoids, *Pongamia pinnata*, *Rubia cordifolia*, SAR

INTRODUCTION

Natural products, widely distributed in nature, are rich in various active constituents. These constituents have significant pharmacological potential, allowing for effective disease treatment with low or no side effects. Plants have long been used by humans as medicine to treat diseases and, due to its active constituents, continue to be a popular source for discovering new drugs, representing the oldest form of medical practice.[1] Karanj (*Pongamia pinnata),* indeed a versatile medicinal plant, is known for its rich phytochemical profile. The phytochemicals present in Karanj, such as flavonoids, furanoflavonoids, flavones, hydroxy flavones, and stearic acid, contribute to its wide range of therapeutic properties such as anti-plasmodial, anti-inflammatory, anti-nonciceptive, anti-lipidperoxidative, antihyperglycemic, anti-diarrhoeal, anti-hyperammonic, anti-ulcer, and anti-oxidant activity. These activities make Karanj an important plant in traditional and modern medicine for treating a variety of ailments.[2] The presence of furanoflavonoids enhances the therapeutic potential of *P. pinnata* as an antibacterial by inhibiting the growth of various bacterial strain and help in neutralizing free radicals, thereby protecting cells from oxidative stress and damage.[3] Similarly *Rubia cordifolia* is another medicinal plant which is known for its high medicinal value. Seven anthraquinones have been extracted from *R. cordifolia*. Among these, hydroxyanthraquinones are the predominant antioxidant phenolic constituents in the roots of *R. cordifolia*. The

Address for correspondence:
Dr. Bharat Mishra, Institute of Pharmacy, Dr. Shakuntala Misra National Rehabilitation University,
Lucknow - 226017, Uttar-Pradesh, India.
Mobile: +91-7311105552.
E-mail: bhartekansh@gmail.com

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presence of hydroxy groups on one benzene ring of the anthraquinone structure is essential for their activity, and the ortho-dihydroxy configuration in these molecules significantly enhances their radical scavenging effect.[4]

P. PINNATA

P. pinnata (Linn.) Pierre is a medium-sized glabrous tree, commonly referred to as Indian Beech in English, Karanja in Hindi, and Pongam in Tamil. The Pongam tree, is one of the richest and brightest trees of India, is scientifically named *P. pinnata.* The name "Pongamia" is derived from its Tamil name, and "pinnata" refers to its pinnate leaves. This tree belongs to the *Leguminosae* (*Fabaceae*) family, with its subfamily being *Papilionaceae*.[5] This tree is notable for its adaptability to various environmental conditions and has a wide range of uses, both medicinal and agricultural. *P. pinnata*, also known as *Derris indica*, is a monotypic genus that grows abundantly along the coasts and riverbanks in Myanmar. The tree's adaptability to coastal and riverine environments contributes to its widespread distribution in these areas.[6] It is a versatile tree traditionally utilized in India and neighboring countries for a wide range of purposes. Its various applications include traditional medicine, green manure, wood, animal fodder, fuel, biopesticides, and even fish poison.[7] More recently, interest in *P. pinnata* has surged due to its potential as a biofuel source, given that its seeds contain approximately 40% oil. This high oil content makes it a promising candidate for sustainable energy production, particularly biodiesel.[8] Its wide adaptability and diverse uses make it an important tree for environmental sustainability, economic development, and renewable energy.[9,10] *P. pinnata* leaves include a diverse range of phytoconstituents, such as alkaloids, flavonoids, tannins, glycosides, and fixed oils these compounds contribute to the medicinal properties of *P. pinnata*, including its antibacterial and anti-oxidant activities.[11]

MORPHOLOGICAL DESCRIPTION OF *P. PINNATA*

Growth and canopy: The tree typically grows up to 35–40 feet in height. It has a spreading canopy that casts moderate shade, providing a pleasant environment underneath.[12] Leaves: These are alternate and odd pinnately compound. Each leaf is wide, measuring up to 2–4 inches. The leaves are evergreen and hairless, maintaining their green appearance throughout the year.[13] Flowers: Flowers are lavender, pink, and white. They appear in clusters of 2–4 together. The flowers are short-stalked, pea-shaped, and measure 15–18 mm in length.[14] Pods and seeds: The pods are smooth, brown, hard, thick-walled, and indehiscent (they do not open on their own to release seeds). They are 3–6 cm long and 2–3 cm wide. The seeds are ovoid, compressed, or elliptical, oily, bean-like, and measure 10–15 cm in length.[15] Roots: Numerous and well-developed, contributing to the tree's stability and ability to thrive in various soil conditions. Thick and long, providing deep anchorage and accessing water from deeper soil layers. Bark: The bark changes from thin Gray to grayish-brown as the tree matures and is yellow on the inside. The bark consists of channeled, recurved, and slightly quilled pieces.[16] Phytopharmacological profile of *P. pinnata*shown in Table 1.

R. CORDIFOLIA

R. cordifolia Linn is a known ayurvedic herb popularly known as Indian Madder and related to the *Rubiaceae* family which is a coffee family.[17] *R. cordifolia* is a climber, that grows in the forests of India, Pakistan, China, Korea, Japan, and Mongolia. It is used in many Asian countries as a dye, for imparting shades of red, scarlet, brown, and mauve to cotton and woolen fabrics.[4] The *R. cordifolia* root is used for cough, hepatic obstruction, freckles of skin, indigestion, ulcers and fractures, inflamed parts, mental agony, obstructions in the urinary passage, and paralytic affections. It is also used to cure snake bite and scorpion sting.[18] Historically, traditional Chinese medicine has developed a wide range of drugs that have been widely spread and applied in many countries for treating a wide variety of diseases through the use of these drugs. It has been a key component in the treatment of COVID-19 and SARS-CoV-2 infection. As a result of the extensive chemical composition of *R. cordifolia*, it contains more than 100 substances, including anthraquinones, naphthoquinones, anthraquinone glycosides, naphthoquinone glycosides, bicyclic hexapeptides, triterpenoids, and polysaccharides that have multiple pharmacological effects.[19]

MORPHOLOGICAL DESCRIPTION OF *R. CORDIFOLIA*

Leaves: The leaves are cordate-ovate to ovate-lanceolate, meaning they vary from heart-shaped (cordate) to lance-shaped (lanceolate), with an intermediate ovate form. The base of the leaves is slightly cordate, indicating a heart-shaped base. The petioles (the stalk that attaches the leaf blade to the stem) are quadrangular (four-angled) and may be prickly along the angles. They are also glabrous (smooth, without hairs) and shining. Stipules (small leaf-like appendages at the base of leaf stalks) are absent.[20] Roots: The shape of roots is long, cylindrical, and flexuous which contain thin, red, brittle, and hard-to-break bark. The texture of the roots is splintery and hard. The external surface is longitudinally furrowed, the external bark peels easily, and inner surface furrowed and dark purple when peeled.[21] Flowers: The flowers are small and are either white or greenish in color. The flowers are arranged in terminal panicles or cymes, meaning they form clusters at the ends of the branches.[22] Fruit: Fruit is, 1–2 seeded, minute, glabrous, dark purplish or blackish. Fruit is

Table 1: Phytopharmacological profile of *Pongamia pinnata*

S. No.	Plant part	Chemical constituents	Pharmacological potential	References
1	Seed	Karanjin, pongamol, glabrin, pongagalabrone and pongapin, pinnatin and kanjone	Chronic skin diseases, painful rheumatic joints, hypotensive, inflammations, pectoral diseases, chronic fevers, hemorrhoids, anemia, antiviral effects and produce uterine contractions	[6,41,42]
2	Leaves	Quercetin, kaempferol, pongamol, karanjin, flavone derivatives	Anti-diarrhoeal, anti-oxidant, antiulcer, hepatoprotective, antihyperammonemic, anticonvulsant, antidiabetic activity	[6,42]
3	Stem bark	Pongaflavanol, tunicatachalcone	CNS sedative, antipyretic antidiabetic, analgesic and anti-inflammatory activity	[6,43]
4	Flowers	Aurantiamide acetate, luteolin, apigenin	Antihyperglycemic and antilipidperoxidative effect hypoglycemic and hypolipidemic activity renal protective activity	[43]
5	Fruits	Pongamol and karangin pongamosides A to C, furanoflavonoid glucosides, pongamoside D and a new flavonol glucoside.	Antidiabetic activity metabolic disorders	[42,44]

fleshy, succulent, and has red juice.[23] Phytopharmacological profile of *R. cordifolia* is shown in Table 2.

ANTIBACTERIAL ACTIVITY OF *P. PINNATA* AND *R. CORDIFOLIA*

The leaves of *P. pinnata* show antibacterial activity and can be used to treat enteric infectious diseases. It is hoped that this study will lead to the discovery of new pharmaceuticals.[5] Leaf extract of *P. pinnata* possesses significant antibacterial properties against both Gram-positive and Gram-negative bacteria.[24] Bajpai *et al*. tested the antibacterial effect of different *P. pinnata* leaf extracts of chloroform, methanol, and ethyl acetate at a concentration of 2500 µg/mL against several bacterial strains including *Staphylococcus aureus* ATCC6538, *Bacillus subtilis* ATCC6633, *Listeria monocytogenes* ATCC19118, *Pseudomonas aeruginosa* ATCC6432, *L. monocytogenes* ATCC19166, and *Salmonella typhimurium* ATCC2512. In comparison to streptomycin, chloroform, ethyl acetate, and methanol extracts all showed significant antibacterial activity.[25]

Certain morphological alterations were brought about by the application of Karanjin to the *S. aureus* and *Escherichia coli* enterotoxin bacterial strains. The bacterial cells showed signs of shrinkage, rupture of the membrane, and cell wall breakdown.[26]

Karanjin is a 3-methoxy-2-phenylfuro-(2,3-h-chrome-4-ol furanoflavonoid compound. Karanjin belongs to the flavone subgroup of compounds of flavonoid and possesses benzoyl and cinnamoyl backbone with a furan ring and methoxy substituent groups, as shown in Figure 1. Antibacterial drugs are used to treat bacterial infectious diseases. *R. cordifolia* exhibits excellent antibacterial ability and can inhibit different bacteria, including *Bacillus pumilus*, *Bacillus cereus*, *B. subtilis*, *Mycobacterium luteum*, *Micrococcus luteus*, and *M. luteum*.[19] Alizarin-type anthraquinones have hydroxyl groups distributed on one side of the benzene ring, and includes alizarin, hydroxyalizarin, purpurin, and pseudo hydroxyl alizarin is the content of *R. cordifolia* is responsible for antibacterial activity, as depicted in Figure 2.[27,28] Flavonoids have a structure comprising three rings: A, B, and C multiple hydroxyl groups, especially at the 3', 4', and 5' positions on the B ring, increase anti-oxidant activity. Adding a methoxy group at the C3 position of the C ring enhances electron density, increasing hydrophobicity and improving bacterial cell membrane adhesion. A double bond between C2 and C3, along with a C4 keto group in the C ring, enhances planarity and conjugation, boosting anti-oxidant and antibacterial activity. An electron-rich furan ring attached to a ring can donate electrons to neutralize free radicals, enhancing anti-oxidant properties [Figure 1].[29-31]

ANTIBACTERIAL MECHANISM OF *P. PINNATA* AND *R. CORDIFOLIA*

P. pinnata (Karanjin) and *R. cordifolia* (purpurin/alizarin) have shown potential in modulating key signaling pathways, such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, which are critical for inflammatory and immune responses in both bacterial and host cells. Karanjin, by preventing the binding of tumor necrosis factor-alpha (TNF-α) with the TNF-α receptor, inhibits phosphorylation of extracellular signal-regulated kinase, Jun kinase, and p38 MAPK, leading to reduced inflammatory cytokine production. Purpurin prevents activation and phosphorylation of MAPK components, reducing cellular stress and inflammatory responses.[32] Karanjin also stabilizes

Table 2: Phytopharmacological profile of *Rubia cordifolia*

S. No.	Plant part	Chemical constituents	Pharmacological activity	References
1	Leaves	Anthraquinones, flavonoids	Anti-diabetic activity	[20,45]
2	Roots	Purpurin, small amounts of xanthopurpurin, munjistin, and alizarin, pseudopurpurin mollugin, rubimallin, βsitosterol and daucosterol	Antibacterial, anti-inflammatory, anti-diabetic activity, anti-cancer property, anti-oxidant activity	[19,20,46]

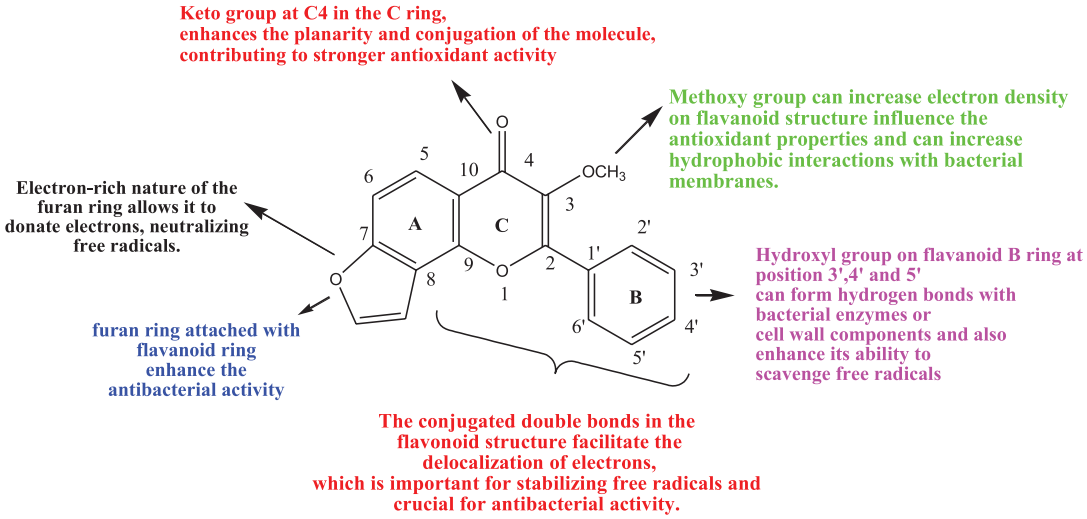

Figure 1: Structure-activity relationship of Karanjin; a flavonoidfor antibacterial and antioxidant potential

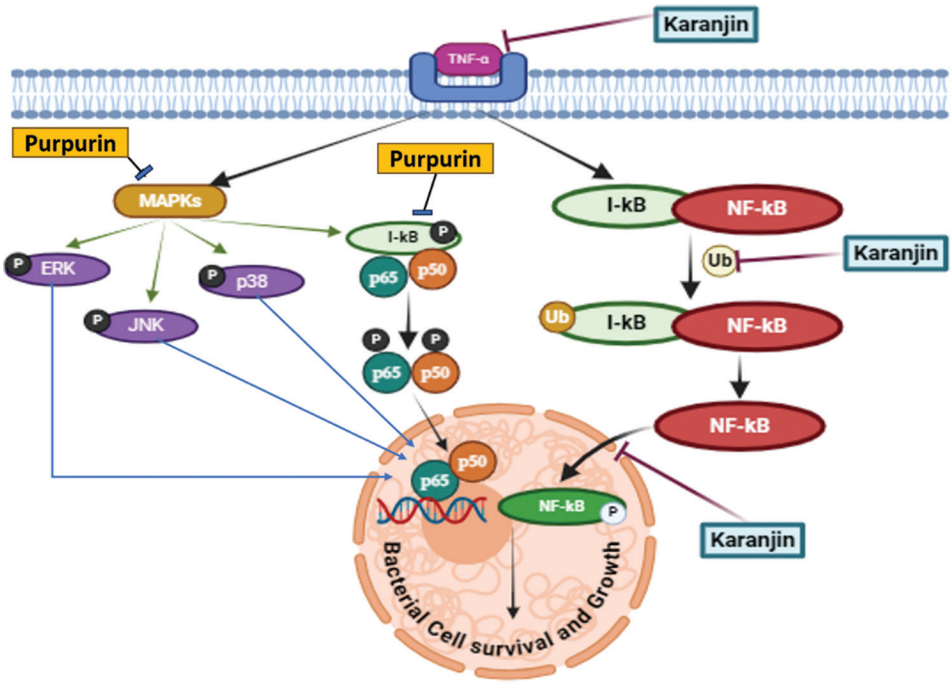

Figure 2: Inhibition of signalling pathway mitogen-activated protein kinase and nuclear factor kappa-B by karanjin and purpurin

inhibitor of κB (IκB) and prevents NF-κB translocation to the nucleus, reducing pro-inflammatory gene transcription, and purpurin inhibits IκB degradation and NF-κB activation, lowering expression of inflammatory mediators, as shown in Figure 2.[33,34]

ANTIOXIDANT ACTIVITY OF *P. PINNATA* AND *R. CORDIFOLIA*

Reactive oxygen species (ROS) can interact with DNA, causing mutations and potentially leading to cancer.

Karanjin, a compound found in the *P. pinnata* plant, has shown a protective effect on DNA. At physiological pH, karanjin interacts non-covalently with DNA via intercalation, offering protection even at low concentrations (as low as 10 µg/mL).[35] In addition, karanjin exhibits mild anti-oxidant properties, demonstrated by its 15.22% scavenging activity against the 2,2-diphenyl-1-picrylhydrazyl radical. This dual functionality makes Karanjin a compound of interest for its potential protective effects against DNA damage and oxidative stress [Figure 3].[28]

There is a lengthy history of ethnopharmacology, including natural anti-oxidants, which are present in many possible plant medicine sources. As seen in Figure 4, the hydroxyl group on the benzene ring in *R. cordifolia* was essential in scavenging free radicals, and the hydroxyl structure in hydroxyanthraquinone could also significantly improve the capacity to scavenge free radicals. Shilpa *et al*. discovered that the methanol extract of *R. cordifolia* could lower levels of serum marker enzymes in a rat model of hepatocellular carcinogenesis, and Lodi *et al*. discovered that the ethanolic extract of *R. cordifolia's* roots could reverse lead nitrate-induced toxicity on oxidative stress and immunological parameters. In the aforementioned investigations, the presence of multiple anti-oxidant indices indicated *R. cordifolia's* anti-oxidant activity; yet, more research is

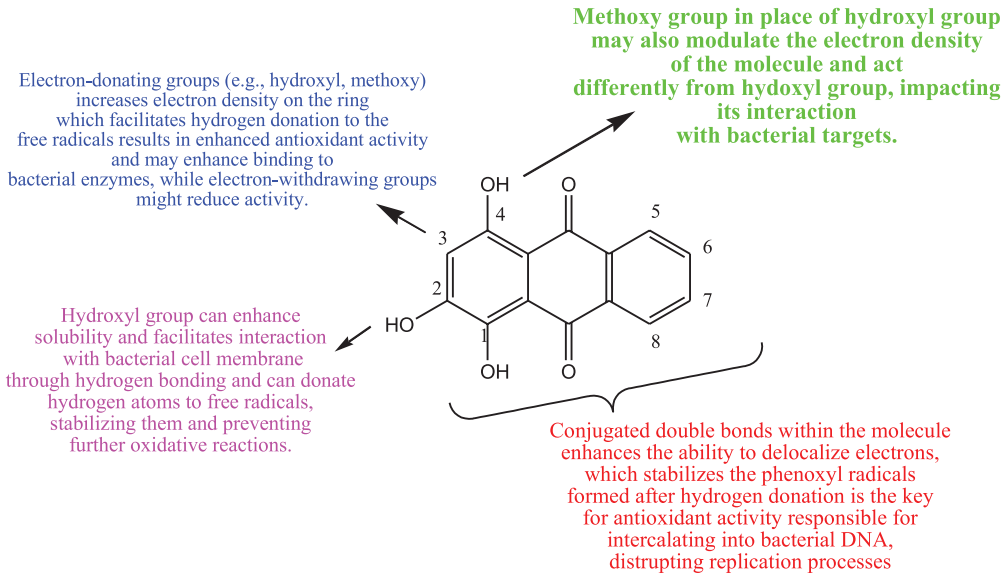

Figure 3: Structure-activity relationship of purpurin/alizarin; a anthraquinone for Antibacterial and antioxidant potential

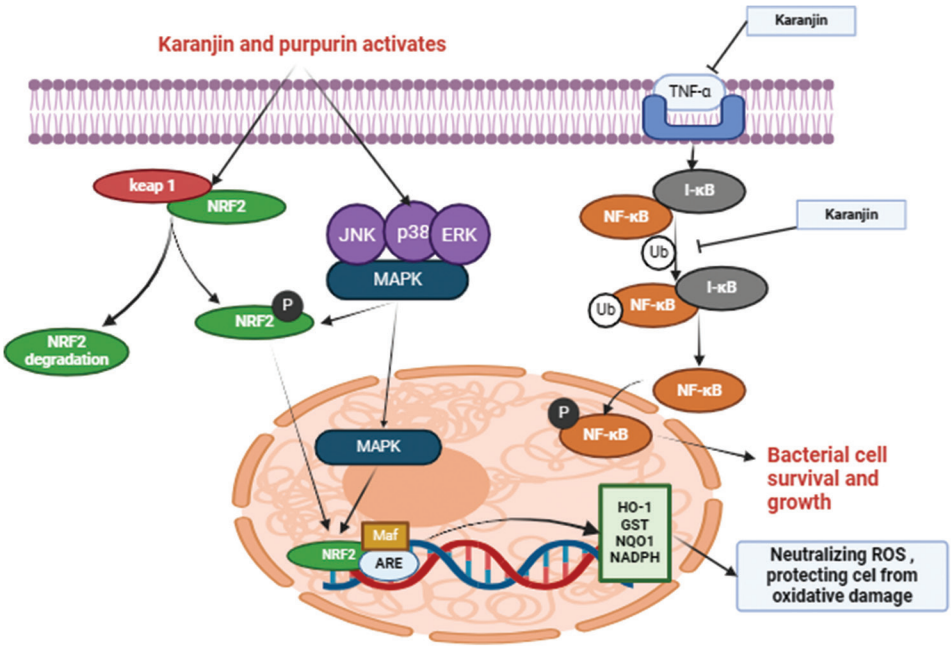

Figure 4: Activation of molecular signalling pathway Nrf2 and mitogen-activated protein kinase pathway and inhibition of nuclear factor kappa-B pathway to neutralize the reactive oxidative species by karanjin and purpurin

needed to determine why *R. cordifolia* has this capacity.[19] Purpurin, an anthraquinone analog, has hydroxy groups at positions 1, 2, and 4, enabling hydrogen donation to free radicals, thus reducing oxidative stress. These groups also interact with bacterial membranes, proteins, and enzymes, enhancing antibacterial potency through hydrogen bonding. The 1,2-hydroxyl configuration is particularly effective for scavenging free radicals. Methoxy or halogen substitutions can enhance antibacterial activity by increasing lipophilicity and membrane permeability, interacting with bacterial targets differently than hydroxyl groups [Figure 3].

ANTIOXIDANT MECHANISM OF *P. PINNATA* AND *R. CORDIFOLIA*

Karanjin and Purpurin neutralize ROS and mitigate oxidative stress by activating the Nrf2 pathway through dissociation of Nrf2 from Keap1 and translocation of Nrf2 to the nucleus. Nrf2 binds to the anti-oxidant response element in the promoter regions of target genes, inducing the expression of anti-oxidant proteins glutathione S-transferase, NAD(P)H quinone dehydrogenase 1, Heme oxygenase-1, superoxide dismutase. These anti-oxidant proteins enhance the cellular capacity to neutralize ROS, reducing oxidative damage. These drugs also activate the MAPK pathway and the activation of MAPK pathways can synergize with Nrf2 activation to amplify the cellular anti-oxidant response.[21,36-38] The NF-κB pathway is another major regulator of inflammatory responses and is also activated by oxidative stress. Inhibiting NF-κB can reduce inflammation and oxidative damage. Inhibition of NF-κB involves stabilizing IκB, which sequesters NF-κB in the cytoplasm and prevents its translocation to the nucleus. By preventing NF-κB activation, the transcription of pro-inflammatory cytokines (e.g., TNF-α, interleukin [IL]-1β, IL-6) and enzymes (e.g., COX-2, iNOS) responsible for ROS production is reduced. NF-κB inhibition can enhance Nrf2 activity depicted in Figure 4.[39,40]

CONCLUSION

Both *P. pinnata* and *R. cordifolia* possess significant antibacterial and anti-oxidant properties. Their efficacy is largely due to their rich phytochemical profiles, which include flavonoids, glycosides, tannins, and fatty acids. These compounds work through various mechanisms, such as disrupting microbial cell membranes, inhibiting nucleic acid synthesis, enhancing insulin sensitivity, and modulating lipid metabolism. Through understanding the basic structure-activity relationship it was found that some modification can be done in the structure of active constituents of *P. pinnata* and *R. cordifolia,* which may result in a more potent compound. Further research is warranted to fully understand the therapeutic potential and safety profiles of these plants in clinical settings. The active constituents present in the *P. pinnata* and *R. cordifolia* shows anti-oxidant activity by activating the Nrf2 and MAPK pathway and inhibiting the NF-kB pathway to neutralize the reactive oxidative species and protects the cells from oxidative stress and these constituents also act as antibacterial agents by inhibiting the Nf-kb and MAPK pathway.

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