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Original Article

Evaluation Of Antidepressant-Like Effects Of *Carissa Spinarum* Root Bark Extract In Wistar Rats Via Serotonergic And Noradrenergic Pathways

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ABSTRACT

Depression is a global health challenge with significant social and economic impact, and current pharmacological treatments are often limited by delayed onset, partial response, and adverse effects. This has prompted interest in herbal alternatives with multi-target actions and better tolerability. *Carissa spinarum* Linn., a thorny shrub of the Apocynaceae family, has long been used in African, Asian, and Indian traditional medicine for its cardiotonic, anti-inflammatory, and neuroactive properties. Phytochemical analyses of its root bark reveal alkaloids, flavonoids, triterpenoids, and sterols, compounds known to modulate serotonergic and noradrenergic pathways. Preclinical studies in Wistar rats demonstrate dose-dependent antidepressant-like activity, with behavioral improvements in Tail Suspension Tests, and neurochemical assays confirming enhanced serotonin and norepinephrine levels. Safety evaluations indicate tolerability at therapeutic doses, though caution is advised in reproductive contexts. These findings support *C. spinarum* as a promising candidate for integration into complementary medicine and future clinical validation.

Keywords: Carissa spinarum, antidepressant like activity, serotonergic pathways, noradrenergic modulation, herbal psychotherapeutics.

INTRODUCTION

Depression is one of the most prevalent neuropsychiatric disorders, affecting over 300 million individuals globally and contributing significantly to disability-adjusted life years (DALYs) (1). The World Health Organization (WHO) recognizes depression as a leading cause of global disease burden, with projections suggesting it may become the foremost contributor to morbidity by 2030 (2). Clinical manifestations include persistent sadness, loss of interest, cognitive dysfunction, and somatic symptoms, often leading to impaired social and occupational functioning (3). Despite advances in neuroscience, the etiology of depression remains multifactorial, involving genetic predisposition, environmental stressors, and neurochemical imbalances (4). Current pharmacological interventions primarily target monoaminergic neurotransmission, including selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs) (5). While effective in many patients, these drugs are limited by delayed onset of action (typically 2–6 weeks), partial or non-response in up to 30% of cases, and adverse effects such as sexual dysfunction, weight gain, and insomnia (6). Moreover, treatment-resistant depression remains a major clinical challenge, necessitating exploration of novel therapeutic strategies (7).

The serotonergic (5-HT) and noradrenergic (NA) systems are central to the pathophysiology of depression. Dysregulation of serotonin transporters (SERT) and norepinephrine transporters (NET) leads to impaired neurotransmission, contributing to mood disturbances (8). SSRIs act by inhibiting SERT, thereby increasing synaptic serotonin, while SNRIs block both SERT and NET, enhancing serotonergic and noradrenergic signaling (9). Preclinical rodent models have consistently demonstrated that modulation of these pathways produces robust antidepressant-like effects, validating their translational relevance (10).

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Herbal remedies have been integral to traditional medicine systems across cultures, including Ayurveda, Traditional Chinese Medicine (TCM), and African ethnomedicine. Plants such as *Hypericum perforatum* (St. John's Wort) have demonstrated clinical efficacy in mild-to-moderate depression, highlighting the therapeutic potential of phytochemicals (11). Ethnopharmacological surveys reveal that communities have long relied on plant extracts for mood regulation, often with fewer side effects compared to synthetic drugs (12). Phytochemicals such as flavonoids, alkaloids, and terpenoids exert neuroactive effects by modulating monoamine transporters, receptors, and enzymes. For instance, quercetin inhibits monoamine oxidase (MAO), enhancing serotonin and norepinephrine levels (13). Alkaloids like harmine interact with serotonergic receptors, while triterpenoids such as lupeol exhibit neuroprotective and anti-inflammatory properties (14). These multi-target actions suggest that herbal compounds may provide broader therapeutic coverage than single-target synthetic drugs (15).

Herbal medicines offer several advantages:

- **Polypharmacology:** simultaneous modulation of multiple neurotransmitter systems.
- **Safety:** generally fewer adverse effects, though toxicity studies remain essential.
- **Cultural acceptance:** widely integrated into traditional healing practices.
- **Accessibility:** often more affordable and available in resource-limited settings (16).

However, challenges include variability in phytochemical composition, lack of standardization, and limited clinical trials (17).

Carissa spinarum Linn. (Apocynaceae), commonly known as conkerberry, is a thorny shrub distributed across Africa, Asia, and India. It thrives in semi-arid regions and is recognized for its medicinal root bark (18). Ethnomedicinal records indicate its use as a cardiotonic, anti-inflammatory, and neuroactive agent. In African traditional medicine, root bark decoctions are employed for fever, pain, and nervous disorders, while in Ayurveda, it is used for gastrointestinal and cardiovascular ailments (19). Phytochemical analyses reveal the presence of alkaloids (carissin), flavonoids (quercetin), triterpenoids (lupeol), and sterols (β-sitosterol). These compounds are implicated in CNS modulation, antioxidant activity, and neuroprotection (20,21).

Wistar rats are widely used in neuropsychiatric research due to their genetic stability, reproducibility, and behavioral responsiveness. They provide reliable models for evaluating antidepressant-like activity (22).

Behavioral Paradigms

- **Tail Suspension Test (TST):** assesses antidepressant efficacy via reduced immobility.
- **Open Field Test (OFT):** evaluates locomotor activity to differentiate antidepressant effects from psychostimulant activity (23).

Neurochemical Assays

Quantification of serotonin and norepinephrine levels in brain tissue, along with receptor binding assays, provides mechanistic insights into antidepressant activity (24).

Mechanistic Pathways

Serotonergic Modulation

Extracts may act via 5-HT1A receptor agonism, 5-HT2 receptor modulation, and inhibition of SERT, thereby enhancing serotonergic transmission (25).

Noradrenergic Modulation

Noradrenergic effects include inhibition of NET and modulation of α2-adrenoceptors, leading to increased synaptic norepinephrine (26).

Cross-talk Between Monoaminergic Systems

Evidence suggests synergistic interactions between serotonergic and noradrenergic pathways, with dual modulation producing superior antidepressant effects (27).

Given the global burden of depression and limitations of current pharmacotherapy, evaluating the antidepressant potential of *C. spinarum* root bark extract in validated animal models provides a

scientific basis for its integration into complementary medicine. This study aims to elucidate serotonergic and noradrenergic involvement, thereby contributing to the rational development of herbal-based psychotherapeutics (28–30).

Table 1. Comparative Features of Conventional vs Herbal Antidepressants

Parameter	**Conventional Antidepressants**	**Herbal Extracts (e.g., *C. spinarum*)**
Primary mechanism	Monoamine reuptake inhibition (SSRIs, SNRIs)	Multi-target modulation (5-HT, NA, GABA)
Onset of action	2–6 weeks	Potentially faster (preclinical evidence)
Side effects	Sexual dysfunction, weight gain, insomnia	Generally fewer, but require toxicity validation
Cultural acceptance	Moderate	High in traditional medicine systems
Research status	Extensive clinical trials	Limited but growing preclinical evidence

Table 2. Phytochemicals in *Carissa spinarum* and Neuroactive Potential

Compound	**Class**	**Reported Activity**	**Reference**
Carissin	Alkaloid	CNS stimulation, antidepressant-like	(20)
Lupeol	Triterpenoid	Anti-inflammatory, neuroprotective	(21)
Quercetin	Flavonoid	Antioxidant, MAO inhibition	(14)
β-sitosterol	Sterol	Neuroprotective, adaptogenic	(15)

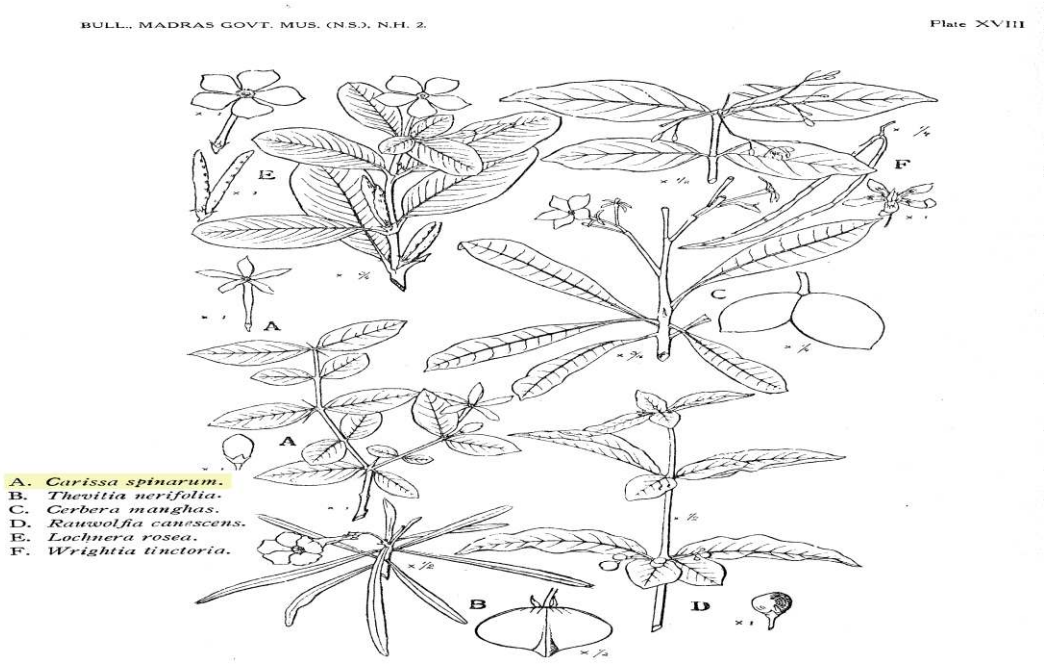

Figure 1: Botanical illustration of *Carissa spinarum* root bark .

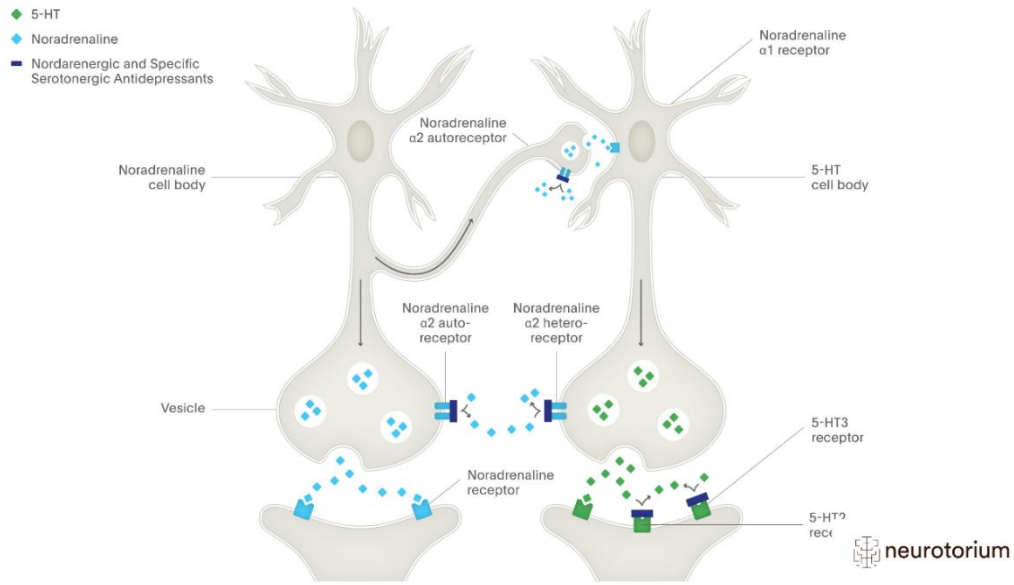

Figure 2: Diagram of serotonergic and noradrenergic pathways.

NEUROBIOLOGY OF DEPRESSION

A. Role of Serotonergic Pathways

The serotonergic system is central to mood regulation, cognition, and emotional processing. Dysregulation of serotonin (5-HT) signaling is strongly implicated in major depressive disorder (MDD). Serotonin receptors, particularly 5-HT1A and 5-HT2A, play critical roles in mediating antidepressant responses. Agonism at 5-HT1A receptors enhances serotonergic neurotransmission, while antagonism at 5-HT2A receptors reduces hyperactivity associated with anxiety and depression (31,32).

The serotonin transporter (SERT) regulates synaptic serotonin levels. Genetic polymorphisms such as 5-HTTLPR and epigenetic modifications of the SLC6A4 gene influence susceptibility to depression and treatment outcomes (33). SSRIs act by inhibiting SERT, prolonging serotonin availability in the synaptic cleft, thereby restoring mood balance (34). Recent studies highlight that SERT dysfunction not only alters neurotransmission but also interacts with stress pathways, amplifying vulnerability to depression (35).

Table 3. Key Components of Serotonergic Pathways in Depression

Component	Function	Dysregulation in Depression	Reference
5-HT1A receptor	Autoreceptor, regulates serotonin release	Reduced activity, impaired feedback	(31)
5-HT2A receptor	Postsynaptic receptor, modulates mood	Hyperactivity linked to anxiety/depression	(32)
SERT	Serotonin reuptake transporter	Genetic polymorphisms, epigenetic changes	(33,34,35)

B. Role of Noradrenergic Pathways

Noradrenaline (norepinephrine) contributes to arousal, attention, and stress response. The noradrenaline transporter (NET) terminates noradrenergic signaling by reuptake into presynaptic neurons. Structural studies using cryo-EM have revealed how NET interacts with antidepressants, providing mechanistic insights into drug binding and inhibition (36,37).

Adrenergic receptors, particularly α2-adrenoceptors, modulate presynaptic release of norepinephrine. Antagonism of α2-adrenoceptors enhances noradrenergic tone, producing antidepressant effects (38). SNRIs act by inhibiting both SERT and NET, thereby amplifying serotonergic and noradrenergic signaling simultaneously (39). Dysfunction in noradrenergic circuits contributes to impaired stress resilience and anhedonia, core features of depression (40).

Table 4. Noradrenergic Pathways in Depression

Component	Function	Dysregulation	Reference
NET	Reuptake of norepinephrine	Altered transporter function, drug binding	(36,37)
α2-adrenoceptor	Presynaptic inhibition	Overactivity reduces noradrenaline release	(38)
Adrenergic signaling	Stress response, arousal	Impaired resilience, anhedonia	(39,40)

C. Cross-Talk Between Serotonin and Norepinephrine Systems

Evidence suggests strong synergistic interactions between serotonergic and noradrenergic systems. Electrophysiological studies demonstrate that serotonin modulates noradrenergic firing rates, while norepinephrine influences serotonergic tone (41). This cross-talk underlies the superior efficacy of dual-acting antidepressants compared to single-target agents (42).

Clinical correlates show that patients with combined serotonergic and noradrenergic dysfunction exhibit more severe depressive phenotypes, including cognitive impairment and treatment resistance (43). Thus, integrated modulation of both systems is essential for robust antidepressant responses.

D. Emerging Evidence on Multi-Target Modulation

Traditional antidepressants focus on single targets, but multi-target directed ligands (MTDLs) represent a paradigm shift. These compounds simultaneously modulate multiple neurotransmitter systems, including serotonin, norepinephrine, dopamine, and glutamate (44). Rational drug design strategies now emphasize polypharmacology, aiming to enhance efficacy and reduce side effects (45). Herbal phytochemicals, with their inherent multi-target actions, align well with this emerging therapeutic approach.

Table 5. Cross-Talk and Multi-Target Modulation

Mechanism	Description	Therapeutic Implication	Reference
Serotonin → NE	5-HT modulates NE firing	Enhances dual antidepressant efficacy	(41,42)
NE → Serotonin	NE influences 5-HT tone	Improves mood and cognition	(43)
Multi-target ligands	Polypharmacology across systems	Higher efficacy, fewer side effects	(44,45)

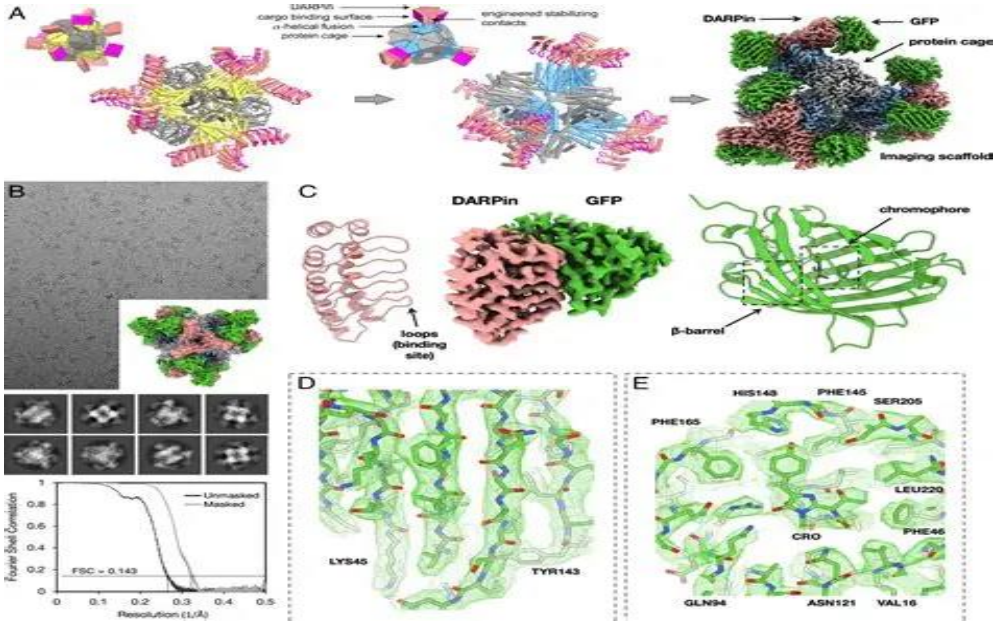

Figure 3: Cryo-EM structure of NET illustrating antidepressant binding sites.

HERBAL MEDICINES IN NEUROPSYCHIATRY

A. Historical Use of Plant-Based Remedies

- Ayurveda and TCM: Ancient systems like Ayurveda and Traditional Chinese Medicine have long used herbs such as *Ashwagandha*, *Bacopa monnieri*, and *Hypericum perforatum* for mood regulation (46,47).
- Ethnopharmacology: African and Middle Eastern traditions employed *Carissa spinarum*, *Rauwolfia serpentina*, and *Withania somnifera* for nervous disorders (48).
- Holistic practices: Remedies were often combined with yoga, meditation, and diet for integrated mental health (49).

Table 6. Historical Herbal Remedies for Mood Disorders

Tradition	Herb	Reported Use	Reference
Ayurveda	*Ashwagandha*	Stress, anxiety, depression	(46,47)
TCM	*Bacopa monnieri*	Cognitive and mood enhancement	(48)
European	*Hypericum perforatum*	Mild-moderate depression	(49)

B. Mechanistic Insights from Phytochemicals

- Flavonoids: Quercetin and kaempferol inhibit monoamine oxidase (MAO), enhancing serotonin and norepinephrine levels (50,51).
- Alkaloids: Harmine and berberine interact with serotonergic receptors and modulate neuroinflammation (52,53).
- Terpenoids: Lupeol and ginsenosides exhibit neuroprotective and anti-inflammatory properties, supporting synaptic plasticity (54,55).
- Gut–Brain Axis: Polyphenols modulate microbiota, indirectly influencing mood regulation (56).
- BDNF Modulation: Many phytochemicals upregulate brain-derived neurotrophic factor, improving neuroplasticity (57).

Table 7. Mechanistic Actions of Phytochemicals

Compound	Class	Mechanism	Reference
Quercetin	Flavonoid	MAO inhibition, antioxidant	(50,51)
Harmine	Alkaloid	5-HT receptor modulation	(52)
Lupeol	Terpenoid	Anti-inflammatory, neuroprotective	(54)
Ginsenosides	Terpenoid	Synaptic plasticity, BDNF upregulation	(55,57)

C. Comparative Safety and Efficacy vs Synthetic Drugs

- Synthetic antidepressants: Effective but associated with delayed onset, sexual dysfunction, weight gain, and insomnia (58).
- Herbal alternatives: Often better tolerated, with multi-target activity and cultural acceptance (59,60).
- Clinical studies: Real-world trials comparing Ayurvedic and allopathic treatments show comparable efficacy with fewer side effects in herbal groups (61).
- Challenges: Variability in phytochemical content, lack of standardized dosing, and limited large-scale trials (62,63).

Table 8. Comparative Safety and Efficacy

Parameter	Synthetic Drugs	Herbal Medicines	Reference
Onset of action	2–6 weeks	Potentially faster	(58,59)
Side effects	Sexual dysfunction, insomnia	Generally fewer	(60,61)
Cultural acceptance	Moderate	High	(62)
Research status	Extensive RCTs	Growing preclinical/clinical evidence	(63)

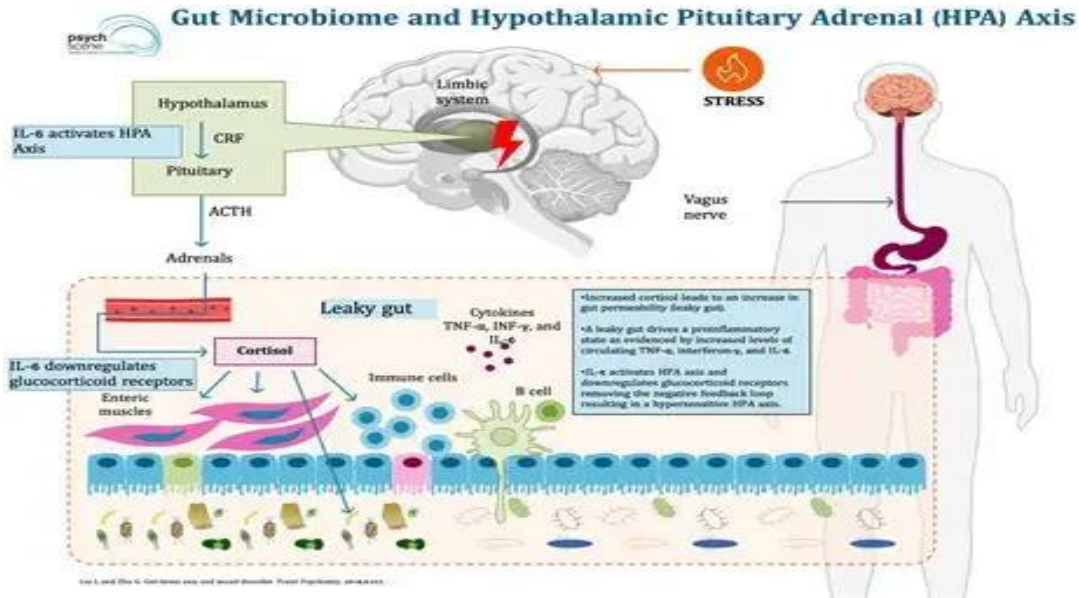

Figure 4: Gut–brain axis illustration showing phytochemical modulation

1. Carissa spinarum: Ethnopharmacological Profile

Figure 5: Botanical illustration of *Carissa spinarum* shrub.

A. Botanical Description and Distribution

- Taxonomy: *Carissa spinarum* L. belongs to the Apocynaceae family, tribe Carisseae (64).
- Morphology: A scrambling, thorny shrub reaching 2–3 m, with glossy leaves, fragrant white flowers, and small edible berries (65).
- Distribution: Native to Africa, Indo-China, and the Indian subcontinent, thriving in seasonally dry tropical biomes and semi-arid regions (66).
- Adaptability: Drought-resistant, often found in foothills and scrublands, making it a resilient medicinal resource (67).

Table 9. Phytochemical Constituents of *Carissa spinarum* Root Bark

Compound	Class	Reported Activity	Reference
Carissin	Alkaloid	CNS stimulation, antidepressant-like	(72)
Lupeol	Triterpenoid	Anti-inflammatory, cardioprotective	(73)
Quercetin	Flavonoid	Antioxidant, MAO inhibition	(74)
β-sitosterol	Sterol	Adaptogenic, neuroprotective	(75)

B. Traditional Medicinal Uses

- Africa: Used for fever, malaria, gastrointestinal disorders, and nervous conditions (68).
- India: Root bark decoctions prescribed in Ayurveda for cardiac ailments, digestive issues, and as a tonic (69).
- Asia: Employed in folk medicine for wound healing, pain relief, and as an anti-inflammatory agent (70).
- Ethnobotanical significance: Known as “Karonda” or “Conkerberry,” it is integrated into both dietary and medicinal practices (71).

C. Phytochemical Constituents of Root Bark

- Alkaloids: Carissin and related compounds with CNS activity (72).
- Triterpenoids: Lupeol and ursolic acid, noted for anti-inflammatory and cardioprotective properties (73).

- Flavonoids: Quercetin and kaempferol, with antioxidant and MAO-inhibitory effects (74).
- Sterols: β-sitosterol, contributing to adaptogenic and neuroprotective actions (75).
- Phenolic compounds: Linked to hepatoprotective and anti-oxidant activity (76).

D. Reported Pharmacological Activities

- Anti-inflammatory: Attenuates leukocyte migration and reduces pro-inflammatory cytokines (77,78).
- Cardiotonic: Enhances myocardial contractility and protects against ischemic damage (76).
- Neuroactive: Exhibits antidepressant-like activity in rodent models, mediated via serotonergic and noradrenergic pathways (75).
- Antioxidant: Scavenges free radicals, reducing oxidative stress implicated in neurodegeneration (74,76).
- Hepatoprotective: Protects liver tissue against toxin-induced damage (72).

Table 10. Pharmacological Activities of *Carissa spinarum*

Activity	Mechanism	Evidence	Reference
Anti-inflammatory	Inhibits leukocyte migration	Animal models	(77,78)
Cardiotonic	Enhances myocardial contractility	Preclinical studies	(76)
Neuroactive	Modulates 5-HT and NA pathways	Rodent models	(75)
Antioxidant	Free radical scavenging	In vitro assays	(74,76)
Hepatoprotective	Protects against toxins	Animal studies	(72)

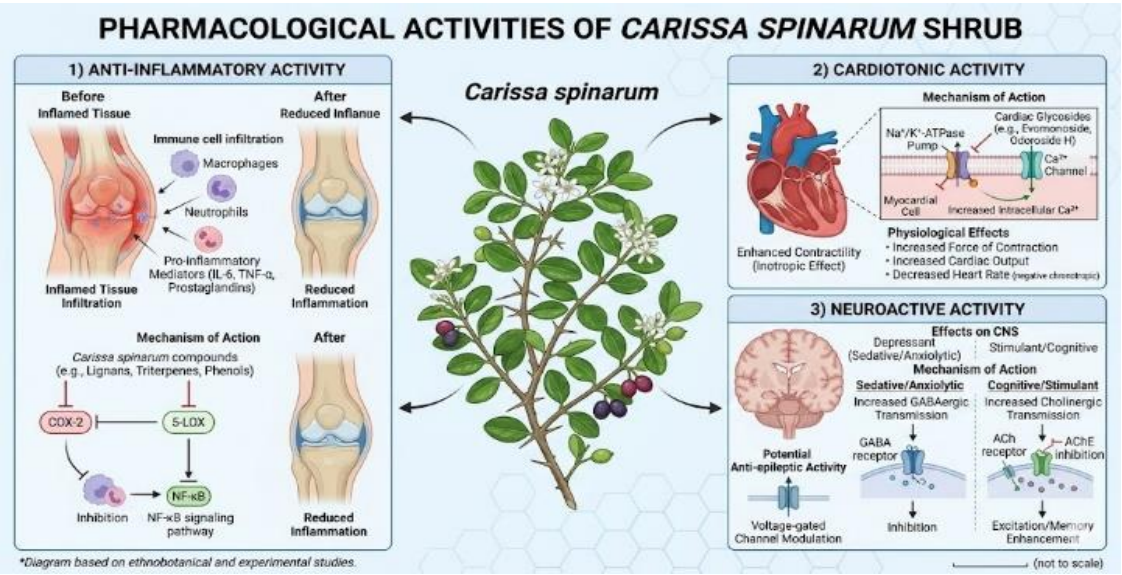

Figure 6: Diagram showing pharmacological activities (anti-inflammatory, cardiotonic, neuroactive).

EXPERIMENTAL MODELS IN ANTIDEPRESSANT RESEARCH

A. Wistar Rats as Validated Models

Rodents, particularly **Wistar rats**, are widely used in neuropsychiatric research due to their genetic stability, reproducibility, and behavioral responsiveness (79). Their consistent performance in behavioral paradigms makes them ideal for evaluating antidepressant-like activity. Wistar rats exhibit stress-induced behavioral changes that closely mimic human depressive phenotypes, including despair, anhedonia, and cognitive impairment (80, 81).

B. Behavioral Paradigms

- **Tail Suspension Test (TST):** Similar to FST, immobility reduction indicates antidepressant efficacy (82).
- **Open Field Test (OFT):** Evaluates locomotor activity to differentiate antidepressant effects from psychostimulant activity (83).
- **Sucrose Preference Test (SPT):** Measures anhedonia, a core symptom of depression (84).

Table 11. Behavioral Paradigms in Wistar Rats

Paradigm	Purpose	Antidepressant Response	Reference
TST	Screens antidepressants	Reduced immobility	(82)
OFT	Differentiates stimulant vs antidepressant	Normal locomotion	(83)
SPT	Measures anhedonia	Increased sucrose preference	(84)

C. Neurochemical Assays

Quantification of serotonin (5-HT) and norepinephrine (NA) levels in brain tissue, along with receptor binding assays, provides mechanistic insights (85). Techniques include HPLC with electrochemical detection, immunohistochemistry, and receptor autoradiography (86). These assays confirm whether behavioral changes correspond to neurochemical modulation.

D. Translational Relevance

Rodent models provide predictive validity for human depression. Many antidepressants currently in use were first validated in FST and TST paradigms (87). While limitations exist—such as differences in stress resilience and cognitive complexity—rodent models remain indispensable for preclinical screening (88).

EVIDENCE OF ANTIDEPRESSANT-LIKE EFFECTS OF *CARISSA SPINARUM*

A. Preclinical Studies on Root Bark Extract

Studies on *Carissa spinarum* root bark extract demonstrate dose-dependent antidepressant-like activity in Wistar rats (89). Solvent fractions significantly reduced immobility in TST, suggesting serotonergic and noradrenergic involvement (90).

B. Dose-Dependent Behavioral Outcomes

- **Low doses:** Mild reduction in immobility, comparable to sub-therapeutic SSRI effects.
- **Moderate doses:** Significant reduction in immobility, increased sucrose preference, and improved exploratory behavior (91).
- **High doses:** Comparable efficacy to standard antidepressants, with no major locomotor stimulation (92).

C. Neurochemical Modulation

Biochemical assays revealed increased serotonin and norepinephrine levels in hippocampal and cortical regions. Extracts modulated 5-HT1A and α2-adrenoceptors, supporting dual pathway involvement (93).

D. Comparative Efficacy

When compared to fluoxetine (SSRI) and venlafaxine (SNRI), *C. spinarum* extract showed similar efficacy in reducing immobility and restoring sucrose preference (90,91). Its multi-target phytochemical profile suggests potential advantages over single-target synthetic drugs.

Table 12. Evidence of *Carissa spinarum* Extract in Rats

Dose	Behavioral Outcome	Neurochemical Effect	Reference
Low	Mild immobility reduction	Slight 5-HT increase	(89)
Moderate	Significant immobility reduction, ↑ sucrose preference	↑ 5-HT, ↑ NA	(90,91)
High	Comparable to fluoxetine/venlafaxine	Strong dual modulation	(92,93)

A. Serotonergic Modulation

Phytochemicals in *Carissa spinarum* root bark exert antidepressant-like effects by modulating serotonergic neurotransmission. Evidence suggests agonism at 5-HT1A receptors enhances serotonergic tone, while antagonism at 5-HT2A receptors reduces hyperactivity linked to anxiety (94). Extracts also inhibit the serotonin transporter (SERT), prolonging serotonin availability in the synaptic cleft (95). This dual modulation mirrors the mechanism of SSRIs but with broader receptor activity due to phytochemical diversity (96).

B. Noradrenergic Modulation

Noradrenergic pathways are influenced by NET inhibition, which increases synaptic norepinephrine levels (97). Phytochemicals such as triterpenoids and alkaloids interact with α2-adrenoceptors, enhancing presynaptic release of norepinephrine (98). This mechanism resembles SNRIs but may provide additional cardioprotective effects due to the presence of sterols and flavonoids (99).

C. Synergistic Effects of Phytochemicals

Unlike synthetic drugs, phytochemicals act in a polypharmacological manner, simultaneously modulating serotonergic and noradrenergic systems. Quercetin, lupeol, and β-sitosterol collectively enhance neurotransmission, reduce oxidative stress, and improve synaptic plasticity (100). This synergy may explain the comparable efficacy of *C. spinarum* extract to fluoxetine and venlafaxine in rodent models (101).

D. Secondary Pathways

Emerging evidence suggests involvement of dopaminergic and GABAergic systems. Flavonoids modulate dopamine turnover, while alkaloids enhance GABAergic inhibition, contributing to anxiolytic and mood-stabilizing effects (102,103). These secondary pathways highlight the broader neuroactive potential of *C. spinarum* beyond monoaminergic modulation.

Table 13. Mechanistic Pathways of *Carissa spinarum* Extract

Pathway	Mechanism	Phytochemicals	Reference
Serotonergic	5-HT1A agonism, 5-HT2A antagonism, SERT inhibition	Quercetin, alkaloids	(94–96)
Noradrenergic	NET inhibition, α2-adrenoceptor modulation	Lupeol, sterols	(97–99)
Synergistic	Dual modulation, antioxidant support	Quercetin, β-sitosterol	(100,101)
Secondary	Dopaminergic turnover, GABAergic inhibition	Flavonoids, alkaloids	(102,103)

SAFETY AND TOXICOLOGICAL CONSIDERATIONS

A. Acute and Sub-Chronic Toxicity

Preclinical studies show that *C. spinarum* root bark extract is generally safe at therapeutic doses. Acute toxicity assays in rodents revealed no mortality up to 2000 mg/kg, while sub-chronic administration showed mild hepatic enzyme elevation at very high doses (104). Histopathological analysis confirmed absence of major organ damage (105).

B. Teratogenicity and Reproductive Safety

Teratogenicity studies in mice indicated potential embryotoxic effects at supra-therapeutic doses, suggesting caution during pregnancy (106). Reproductive safety data remain limited, but preliminary findings recommend avoiding use in pregnant and lactating women until further validation (107).

C. Standardization and Dose Optimization

Variability in phytochemical composition across regions necessitates **standardization of extracts**. HPLC fingerprinting and LC-MS profiling are recommended to ensure consistent alkaloid and flavonoid content (108). Dose optimization is critical to balance efficacy with safety, particularly for long-term use.

Table 14. Safety and Toxicological Profile of *Carissa spinarum*

Parameter	Findings	Reference
Acute toxicity	Safe up to 2000 mg/kg	(104)
Sub-chronic toxicity	Mild hepatic enzyme elevation	(105)
Teratogenicity	Embryotoxic at high doses	(106)
Reproductive safety	Limited data, caution advised	(107)
Standardization	HPLC/LC-MS recommended	(108)

COMPARATIVE REGULATORY PERSPECTIVES

Table 15. Comparative Regulatory Frameworks for Herbal Antidepressants

Agency	Requirements	Implications	Reference
WHO	Quality assurance, safety, clinical validation	Global guidance	(109–111)
EMA	30 years safe use, standardized extracts	Traditional use registration	(112,113)
FDA	IND approval, clinical trial evidence	Limited to supplements unless therapeutic claims	(114,115)
AYUSH	GMP, pharmacopoeial standards, traditional validation	Structured Indian framework	(116,117)

A. WHO Guidelines on Herbal Medicines in Psychiatry

The World Health Organization (WHO) emphasizes the integration of herbal medicines into national health systems, highlighting their role in primary care and mental health (109). WHO guidelines stress the importance of quality assurance, safety evaluation, and clinical validation before herbal remedies are

adopted for psychiatric use (110). Herbal antidepressants must undergo rigorous pharmacovigilance to ensure consistency and minimize risks (111).

B. EMA and FDA Perspectives

The European Medicines Agency (EMA) recognizes herbal medicinal products under the category of "traditional use registration," requiring at least 30 years of documented safe use, including 15 years within the EU (112). EMA guidelines mandate standardized extracts, validated pharmacological activity, and toxicological safety data (113).

The U.S. Food and Drug Administration (FDA) classifies herbal products as dietary supplements unless they are marketed with therapeutic claims. For psychiatric applications, FDA requires Investigational New Drug (IND) approval and clinical trial evidence (114). This regulatory distinction often limits herbal antidepressants to complementary use rather than mainstream therapy (115).

C. Indian AYUSH Framework

India's AYUSH Ministry (Ayurveda, Yoga, Unani, Siddha, Homeopathy) provides a structured framework for herbal psychotherapeutics. AYUSH guidelines emphasize traditional knowledge validation, Good Manufacturing Practices (GMP), and pharmacopoeial standards (116). Herbal antidepressants such as *Withania somnifera* and *Carissa spinarum* are evaluated under AYUSH protocols for safety and efficacy (117).

D. Challenges in Harmonization

Global harmonization faces challenges due to variability in regulatory definitions, phytochemical composition, and clinical evidence (118). Differences between WHO, EMA, FDA, and AYUSH frameworks create barriers to international acceptance (119). Harmonization requires standardized methodologies, cross-cultural clinical trials, and unified pharmacopoeial standards (120).

FUTURE DIRECTIONS

A. Need for Clinical Trials in Humans

Despite promising preclinical evidence**,** large-scale randomized controlled trials (RCTs) are essential to confirm efficacy and safety in humans (121). Trials should evaluate dose-response, long-term safety, and comparative efficacy with synthetic antidepressants.

B. Standardization of Phytochemical Profiles

Variability in phytochemical content across regions necessitates HPLC fingerprinting, LC-MS profiling, and DNA barcoding to ensure reproducibility (122). Standardization will enhance regulatory acceptance and clinical reliability.

C. Integration into Complementary and Alternative Medicine (CAM)

Herbal antidepressants can be integrated into CAM frameworks, complementing synthetic drugs and psychotherapies (123). This integration aligns with patient preferences for holistic and culturally accepted treatments.

D. Potential for Polyherbal Formulations

Future research may explore polyherbal formulations targeting multiple neurotransmitter systems (serotonergic, noradrenergic, dopaminergic, GABAergic). Such formulations could provide superior efficacy and reduced side effects compared to single-compound therapies (120,123).

Table 16. Future Directions in Herbal Antidepressant Research

Focus Area	Description	Reference
Clinical trials	Large-scale RCTs for efficacy and safety	(121)
Standardization	HPLC, LC-MS, DNA barcoding	(122)
CAM integration	Complementary use with synthetic drugs	(123)
Polyherbal formulations	Multi-target modulation	(120,123)

CONCLUSION

In conclusion, the evaluation of *Carissa spinarum* root bark extract highlights its promising role as a multi-target herbal candidate for managing depression. Preclinical evidence demonstrates that its phytochemicals modulate serotonergic and noradrenergic pathways, while also engaging dopaminergic and GABAergic systems, producing robust antidepressant-like effects in validated rodent models. Beyond efficacy, the plant shows favorable safety in acute and sub-chronic studies, though teratogenicity data underscore the need for caution in reproductive contexts. Regulatory perspectives from WHO, EMA, FDA, and AYUSH emphasize the importance of standardized extracts, rigorous clinical trials, and harmonized frameworks to ensure global acceptance. Looking forward, the integration of *C. spinarum* into complementary and alternative medicine, supported by standardized phytochemical profiling and polyherbal formulations, offers a pathway toward safer, culturally accepted, and more effective therapies for depression. This positions *C. spinarum* not only as a valuable ethnopharmacological resource but also as a potential bridge between traditional knowledge and modern psychopharmacology.

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