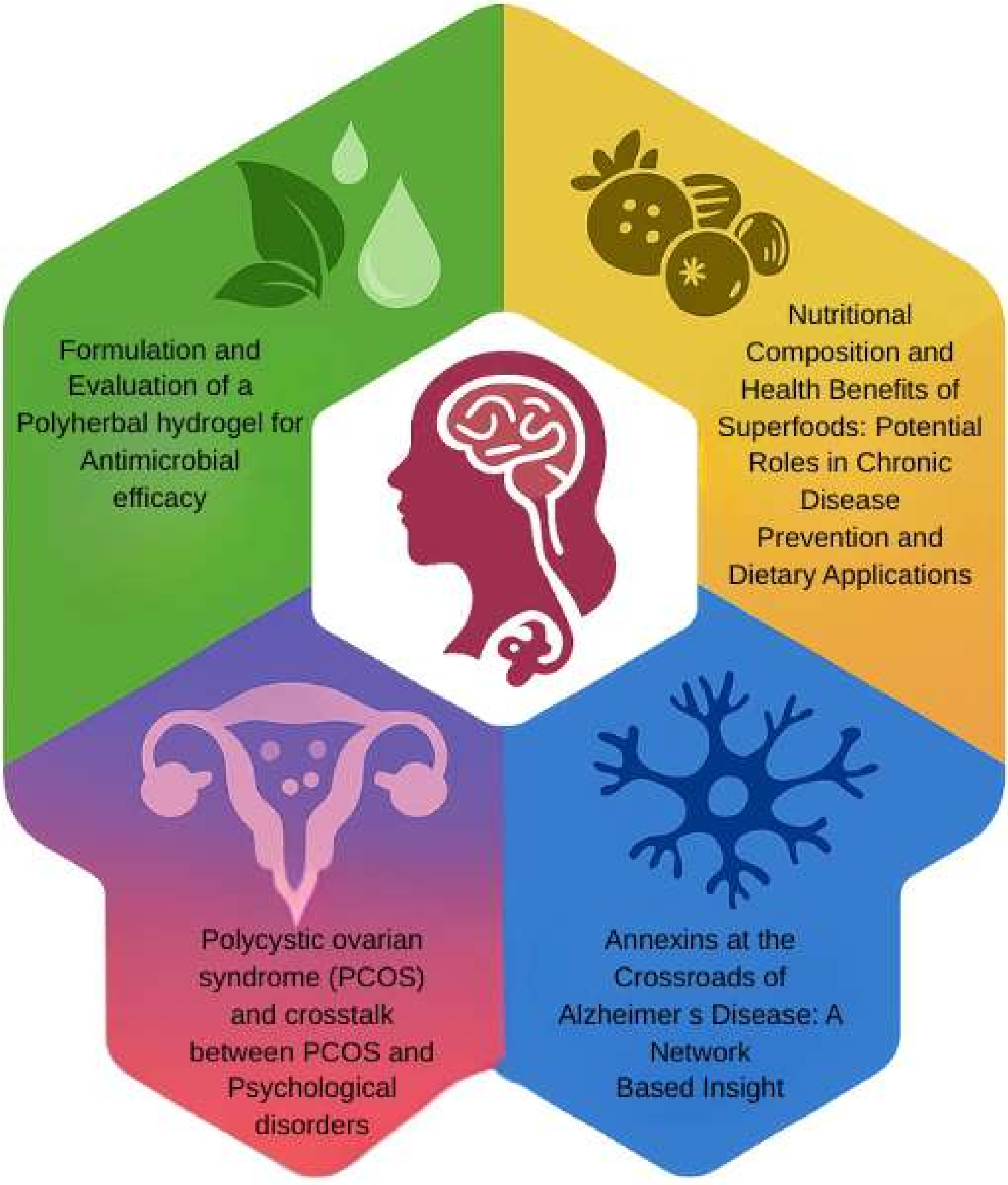
VITA SCIENTIA

VOLUME 1, ISSUE 2, SEPTEMBER 2025



ISSN: 3101-173X VITA SCIENTIA (MADRID)

Vita Scientia

Read Online

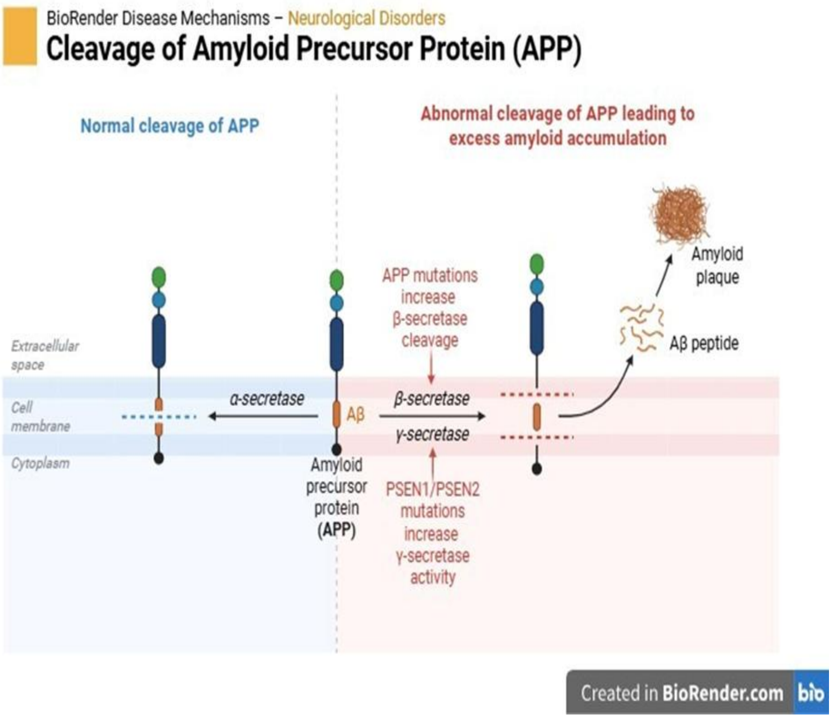
Research Article

Annexins at the Crossroads of Alzheimer's Disease: A Network-Based Insight

Anweya Mohanta, Sharmistha Dutta*

Department of Biotechnology, M S Ramaiah Institute of Technology, Bengaluru-560024.
*Correspondence: sharmistha@msrit.edu

Graphical abstract



Summary

The progressive neurodegenerative disease known as Alzheimer's disease (AD) is characterized by tau pathology, neuroinflammation, and β-amyloid buildup. Amyloid precursor protein (APP) and presenilin-1 (PSEN1) are the main targets of classical AD research, but new study indicates that Annexin family proteins may also influence the course of the illness by regulating inflammation, membrane repair, and protein aggregation. To investigate the connection between annexins and important AD-related proteins, we used a comparative in silico technique. To find conserved areas involved in amyloidogenesis, we first performed multiple sequence alignment (MSA) of APP across Rattus norvegicus, Pan troglodytes, and Homo sapiens. Then, using MSA, BLASTp, and domain annotation tools, we examined the functional relationships and sequence similarities between Annexins (A1, A2, A4, A5, and A6) and AD-related proteins amyloid precursor protein, presenilin-1 (PSEN1) and Formyl Peptide Receptor 2 (APP, PSEN1, and FPR2). Certain annexins and AD proteins were found to be evolutionary close by phylogenetic research, indicating a potential shared ancestral origin or functional convergence. Our findings underline the possible relevance of Annexin A1, A2, and A4 as molecular contributors to disease pathophysiology and as targets for additional experimental validation by suggesting that they may be functionally and evolutionarily associated with fundamental AD pathways.

Keywords

Alzheimer’s disease, Annexin, APP, PSEN1, FPR2, Multiple Sequence Alignment, Phylogenetics, Neurodegeneration.

Introduction

The most common type of dementia, Alzheimer's disease (AD), is typified by a buildup of abnormal protein aggregates such neurofibrillary tangles and β-amyloid plaques, as well as gradual cognitive decline and synaptic dysfunction [1]. Even though tau pathology and the amyloid cascade theory have dominated AD research for decades, new research shows that auxiliary proteins—such as the annexin protein family—that control neuroinflammatory signaling, membrane repair, vesicle trafficking, and apoptotic pathways are also significantly involved [2]. Recent research indicates that annexins, which are calcium-dependent phospholipid-binding proteins involved in a variety of cellular functions, may be important modulators in neurodegenerative pathways.

The ability to attach negatively charged phospholipids in a calcium-dependent way characterizes the structurally conserved family of proteins known as annexins. Each of the 12 known human annexins (ANXA1–ANXA11 and ANXA13) has a variable N-terminal region that confers functional specialization and a conserved C-terminal annexin repeat domain [3]. These proteins are involved in cytoskeletal architecture, endocytosis, vesicle aggregation, and membrane trafficking—processes that are becoming more and more linked to AD pathogenesis, especially in connection with synaptic degeneration, vesicle dysfunction, and membrane instability [4].

Annexins A1, A2, A4, A5, A13 and A6 have demonstrated distinct expression patterns and regulatory roles in the pathophysiology of Alzheimer's disease. By suppressing NF-κB signaling and lowering microglial activation, for example, Annexin A1 is becoming more well acknowledged for its anti-inflammatory properties, which may provide neuroprotection in AD models [5]. On the other hand, AD patients' brains have higher levels of Annexin A5, which is known for its function in apoptotic cell clearance and may be connected to plaque buildup and neuronal death [6]. Notably, Annexin A4 has been implicated in amyloidogenic pathways by being connected to membrane repair and the formation of amyloid fibrils on lipid surfaces [7].

Annexins play a significant role in AD through their impact on amyloid formation and processing. Studies have demonstrated that in pathological settings, particularly in the lipid-rich milieu of neuronal membranes, Annexin A5 and Annexin A6 can directly bind Aβ peptides and encourage fibril formation [8]. In addition to encouraging plaque nucleation, these interactions may compromise membrane integrity, resulting in calcium dyshomeostasis and neuronal damage. Furthermore, Annexin A4 seems to have two functions: it helps repair membranes under stress and, ironically, increases Aβ aggregation under chronic conditions. This could be because of oxidative stress and modifications in calcium influx [7].

Chronic neuroinflammation, which is primarily caused by activated microglia and astrocytes, is another key aspect of AD pathogenesis. Annexin A1 (ANXA1) has become a crucial anti-inflammatory mediator in this regard. It limits microglial overactivation, inhibits NF-κB signaling, and facilitates the removal of debris and dead neurons by acting through the formyl peptide receptor 2 (FPR2) [9]. Pharmacological mimetics of ANXA1 can restore homeostasis and enhance cognitive performance in AD models, while animal studies have shown that ANXA1 deficiency worsens neuroinflammation and speeds up cognitive decline [10]. This makes ANXA1 a promising therapeutic target in addition to being a crucial regulator of neuroimmune interaction.

Furthermore, annexin function is closely related to the disruption of calcium homeostasis, a known factor in AD. As calcium buffers or sensors, annexins—especially A2 and A6—take role in exocytosis, endocytosis, and vesicle trafficking. In AD brains, disruption of these mechanisms has been linked to axonal degeneration and decreased synaptic plasticity [11]. Given that synaptic loss is the most reliable indicator of cognitive impairment in AD, annexins' pathogenic significance is highlighted by their function in preserving synaptic vesicle recycling and membrane integrity.

New proteomic and transcriptome research has revealed that a number of annexins exhibit varying levels of expression in the postmortem brain tissues and cerebrospinal fluid (CSF) of AD patients, indicating their potential use as diagnostic biomarkers. For instance, elevated levels of Annexin A5 and Annexin A2 have been identified in AD CSF and have been linked to Braak stage and Aβ load [12]. These results pave the way for the application of annexin-based markers in progression tracking or early diagnosis [13].

In addition to plaque buildup, annexins also have a role in mitochondrial dysfunction, oxidative stress, poor clearance, and persistent neuroinflammation, all of which contribute to the neurodegenerative development of AD. Their significance in disease development is further expanded by their interactions with tau protein aggregation, vesicle-mediated transport regulation, and microglial phagocytic capability [14].

When considered collectively, the annexin family proteins are now understood to be active regulators of the various cellular and molecular processes that underpin Alzheimer's disease rather than passive structural elements. Further investigation into their various roles could provide fresh perspectives on the pathophysiology of disease and aid in the creation of annexin-targeted treatment plans [15].

Results

The protein and sequence retrieval of Annexin A2 (ANXA2) gave its PDB number of 1BT6 and that of Annexin A4 (ANXA4) gave its PDB number as 1ANN. The proteins shown entirely in cartoon representation, with its alpha-helices prominently displayed in green, which dominate its secondary structure as represented in figure 1. This helical architecture is characteristic of annexins, forming a conserved core that facilitates calcium-dependent binding to phospholipid membranes. The absence of beta-sheets aligns with known structural profiles of both ANXA2 and ANXA4. The positively charged red markers indicate potential calcium-binding or structurally relevant regions contributing to membrane interaction or protein–protein interaction.

Annexin A2 (ANXA2) and Annexin A4 (ANXA4) are calcium-dependent phospholipid-binding proteins involved in membrane dynamics, vesicle trafficking, and cellular signaling. Their functional association with key neurodegeneration-related proteins APP, FPR2, and PSEN1 suggests potential regulatory roles in neuroinflammatory and amyloidogenic pathways. ANXA2 has been reported to influence APP processing and trafficking, possibly modulating amyloid-beta generation, while also participating in actin remodeling and endocytosis—processes disrupted in Alzheimer's disease. ANXA4, known for its membrane-stabilizing role, may contribute to cellular defense against oxidative and excitotoxic stress seen in neurodegeneration. Both annexins also intersect with inflammatory signaling: ANXA2 can regulate pathways linked to FPR2, a receptor involved in resolution of inflammation, while altered annexin expression may affect or reflect changes in PSEN1 function, a core component of the γ-secretase complexes that processes APP. Together, these interactions suggest that ANXA2 and ANXA4 could modulate or respond to amyloidogenic and inflammatory stress, linking membrane homeostasis to Alzheimer's-related signaling networks.

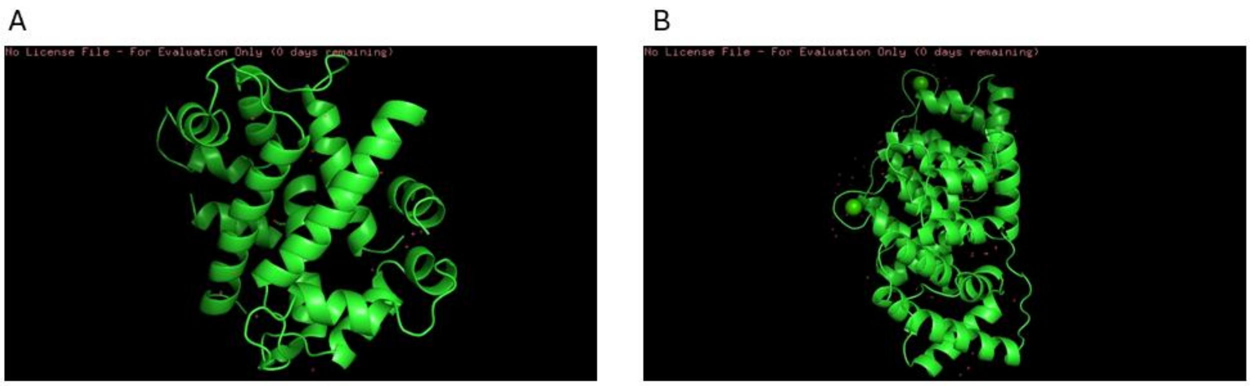


Figure 1. Three-dimensional conformation XRD structure of a) human Annexin A2 (ANXA2) and human Annexin A4 (ANXA4) visualized using PyMOL.

The Multiple Sequence Alignment studies of ANXA4 across species like rat, human and chimpanzee as shown in Figure 2 help us to understand high conservation across the sequences which reflect the evolutionary conservation of structural and functional domains in Annexin A4. The multiple sequence alignment presented corresponds to Annexin A4 (A4) from rat, human, and chimpanzee, as indicated by the sequence identifiers (A4_RAT, A4_HUMAN, A4_PANTR). The alignment reveals a high degree of sequence conservation across all three species, particularly in functional domains. Notably, residues from positions 121 to 180 are perfectly conserved, indicating a functionally important domain, likely contributing to calcium-dependent membrane binding or interaction with other proteins. These conserved motifs may reflect structural domains such as annexin repeats that are central to annexin function. Additional stretches, especially toward the C-terminal end (e.g., 180–240), also exhibit strong conservation with only minor substitutions, suggesting evolutionary pressure to maintain structural integrity and function. The high conservation across mammals emphasizes the crucial, possibly non-redundant, cellular roles of Annexin A4 in membrane-related processes such as exocytosis, inflammation, or calcium signaling.

Multiple sequence alignment of the C-terminal region (residues 580–770) of APP (Amyloid Precursor Protein) from human, chimpanzee, and rat is represented in Figure 3. The alignment highlights the conserved Aβ region (residues ~672–713, shaded red) and the transmembrane domain (residues ~700–723, shaded purple). High conservation is observed across species, especially within the Aβ and transmembrane regions, emphasizing their crucial role in amyloidogenic processing and membrane insertion. Minor variations are present in the flanking sequences, while the core pathogenic region of Aβ remains highly conserved, underscoring its functional and pathological relevance across mammals.

The PPI (Protein-Protein Interaction) of the Annexin family proteins and Alzheimer's Disease related proteins like PSEN1, FPR2, APP show a strong correlation with ANXA1, ANXA4, ANXA2, ANXA5, ANXA6, ANXA13 as shown in Figure 4. This central positioning of ANXA2 suggests it plays a pivotal role in bridging annexin-regulated processes like vesicle trafficking, membrane repair, and calcium signaling with AD-related pathways such as amyloid precursor protein processing and γ-secretase activity. ANXA1 and ANXA6 also show direct links to APP, implicating them in modulating APP cleavage and neuroinflammatory responses. ANXA5, known for its role in membrane dynamics and apoptosis, and ANXA13, a less characterized but evolutionarily conserved annexin, interact within this network—likely contributing to structural and regulatory support. The interaction of APP and ANXA1 with FPR2 further highlights annexins' involvement in inflammation-related signaling. Overall, this network underscores the functional convergence of annexins on central AD mechanisms, particularly through ANXA2, and suggests that even underexplored members like ANXA13 may play supporting roles in neurodegenerative pathways.

```
CLUSTAL O(1.2.4) multiple sequence alignment


sp|P08592|A4_RAT        MLPSLALLLLAAWTVRALEVPTDGNAGLLAEPQIAMFCGKLNMHMNVQNGKWESDPSGTK    60
sp|P05067|A4_HUMAN      MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWDSDPSGTK    60
sp|Q5IS80|A4_PANTR      MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWDSDPSGTK    60
                        ***.**********.************************:************:*******

sp|P08592|A4_RAT        TCIGTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHTHIVIPYRCLVG    120
sp|P05067|A4_HUMAN      TCIDTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHPHFVIPYRCLVG    120
sp|Q5IS80|A4_PANTR      TCIDTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHPHFVIPYRCLVG    120
                        ***.******************************************** *:*********

sp|P08592|A4_RAT        EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR    180
sp|P05067|A4_HUMAN      EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR    180
sp|Q5IS80|A4_PANTR      EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR    180
                        ************************************************************

sp|P08592|A4_RAT        GVEFVCCPLAEESDSIDSADAEEDDSDVWWGGADTDYADGGEDKVVEVAEEEEVADVEEE    240
sp|P05067|A4_HUMAN      GVEFVCCPLAEESDNVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVAEVEEE    240
sp|Q5IS80|A4_PANTR      GVEFVCCPLAEESDNVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVAEVEEE    240
                        **************.:************************.**************:****
```


```
CLUSTAL O(1.2.4) multiple sequence alignment


sp|P08592|A4_RAT        MLPSLALLLLAAWTVRALEVPTDGNAGLLAEPQIAMFCGKLNMHMNVQNGKWESDPSGTK    60
sp|P05067|A4_HUMAN      MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWDSDPSGTK    60
sp|Q5IS80|A4_PANTR      MLPGLALLLLAAWTARALEVPTDGNAGLLAEPQIAMFCGRLNMHMNVQNGKWDSDPSGTK    60
                        ***.**********.************************:************:*******

sp|P08592|A4_RAT        TCIGTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHTHIVIPYRCLVG    120
sp|P05067|A4_HUMAN      TCIDTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHPHFVIPYRCLVG    120
sp|Q5IS80|A4_PANTR      TCIDTKEGILQYCQEVYPELQITNVVEANQPVTIQNWCKRGRKQCKTHPHFVIPYRCLVG    120
                        ***.******************************************** *:*********

sp|P08592|A4_RAT        EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR    180
sp|P05067|A4_HUMAN      EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR    180
sp|Q5IS80|A4_PANTR      EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR    180
                        ************************************************************

sp|P08592|A4_RAT        GVEFVCCPLAEESDSIDSADAEEDDSDVWWGGADTDYADGGEDKVVEVAEEEEVADVEEE    240
sp|P05067|A4_HUMAN      GVEFVCCPLAEESDNVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVAEVEEE    240
sp|Q5IS80|A4_PANTR      GVEFVCCPLAEESDNVDSADAEEDDSDVWWGGADTDYADGSEDKVVEVAEEEEVAEVEEE    240
                        **************.:************************.**************:****
```

```
sp|P08592|A4_RAT        EAEDDEDVEDGDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPC    300
sp|P05067|A4_HUMAN      EADDDEDDEDGDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPC    300
sp|Q5IS80|A4_PANTR      EADDDEDDEDGDEVEEEAEEPYEEATERTTSIATTTTTTTESVEEVVREVCSEQAETGPC    300
                        **:**** ****************************************************

sp|P08592|A4_RAT        RAMISRWYFDVTEGKCAPFFYGGCGGNRNNFDTEEYCMAVCGSVSSQSLLKTTSEPLPQD    360
sp|P05067|A4_HUMAN      RAMISRWYFDVTEGKCAPFFYGGCGGNRNNFDTEEYCMAVCGSAMSQSLLKTTQEPLARD    360
sp|Q5IS80|A4_PANTR      RAMISRWYFDVTEGKCAPFFYGGCGGNRNNFDTEEYCMAVCGSVMSQSLLKTTQEPLARD    360
                        *******************************************. ********.*** :*

sp|P08592|A4_RAT        PVKLPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEEAERQA    420
sp|P05067|A4_HUMAN      PVKLPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEEAERQA    420
sp|Q5IS80|A4_PANTR      PVKLPTTAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEEAERQA    420
                        ************************************************************

sp|P08592|A4_RAT        KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL    480
sp|P05067|A4_HUMAN      KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL    480
sp|Q5IS80|A4_PANTR      KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL    480
                        ************************************************************

sp|P08592|A4_RAT        QAVPPRPHHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER    540
sp|P05067|A4_HUMAN      QAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER    540
sp|Q5IS80|A4_PANTR      QAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER    540
                        *******:****************************************************

sp|P08592|A4_RAT        MNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTET    600
sp|P05067|A4_HUMAN      MNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTET    600
sp|Q5IS80|A4_PANTR      MNQSLSLLYNVPAVAEEIQDEVDELLQKEQNYSDDVLANMISEPRISYGNDALMPSLTET    600
                        ************************************************************

sp|P08592|A4_RAT        KTTVELLPVNGEFSLDDLQPWHPFGVDSVPANTENEVEPVDARPAADRGLTTRPGSGLTN    660
sp|P05067|A4_HUMAN      KTTVELLPVNGEFSLDDLQPWHSFGADSVPANTENEVEPVDARPAADRGLTTRPGSGLTN    660
sp|Q5IS80|A4_PANTR      KTTVELLPVNGEFSLDDLQPWHSFGADSVPANTENEVEPVDARPAADRGLTTRPGSGLTN    660
                        ********************** **.**********************************

sp|P08592|A4_RAT        IKTEEISEVKMDAEFGHDSGFEVRHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL    720
sp|P05067|A4_HUMAN      IKTEEISEVKMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL    720
sp|Q5IS80|A4_PANTR      IKTEEISEVKMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL    720
                        *************** ****:**:***********************************

sp|P08592|A4_RAT        VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN      770
sp|P05067|A4_HUMAN      VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN      770
sp|Q5IS80|A4_PANTR      VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN      770
                        **************************************************
```

Figure 2. Multiple sequence alignment of Annexin A4 from rat, human, and chimpanzee using Clustal Omega (version 1.2.4).

The sequences of Annexin A4 from Rattus norvegicus (A4_RAT), Homo sapiens (A4_HUMAN), and Pan troglodytes (A4_PANTR) were aligned to identify conserved and variable regions. Asterisks (*) indicate positions with fully conserved residues across all three species, colons (:) denote conservation between groups with strongly similar properties, and periods (.) mark weaker similarities. High conservation across the sequences reflects evolutionary conservation of structural and functional domains in Annexin A4.

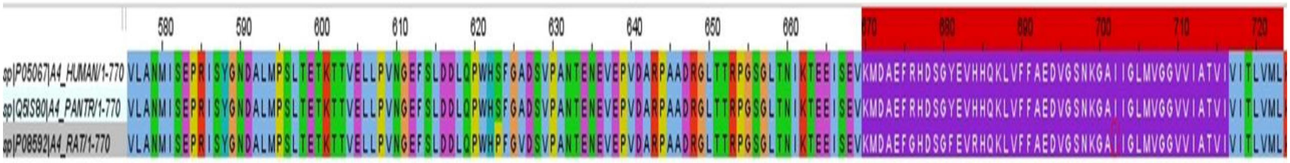


Figure 3. This figure shows a multiple sequence alignment of the APP protein across human, chimpanzee, and rat, highlighting the conserved Aβ and transmembrane regions. Highly conserved regions, especially from residues 670 to 720, are shaded consistently in purple indicating potential functional or structural importance, suggesting evolutionary conservation with slight species-specific divergence.

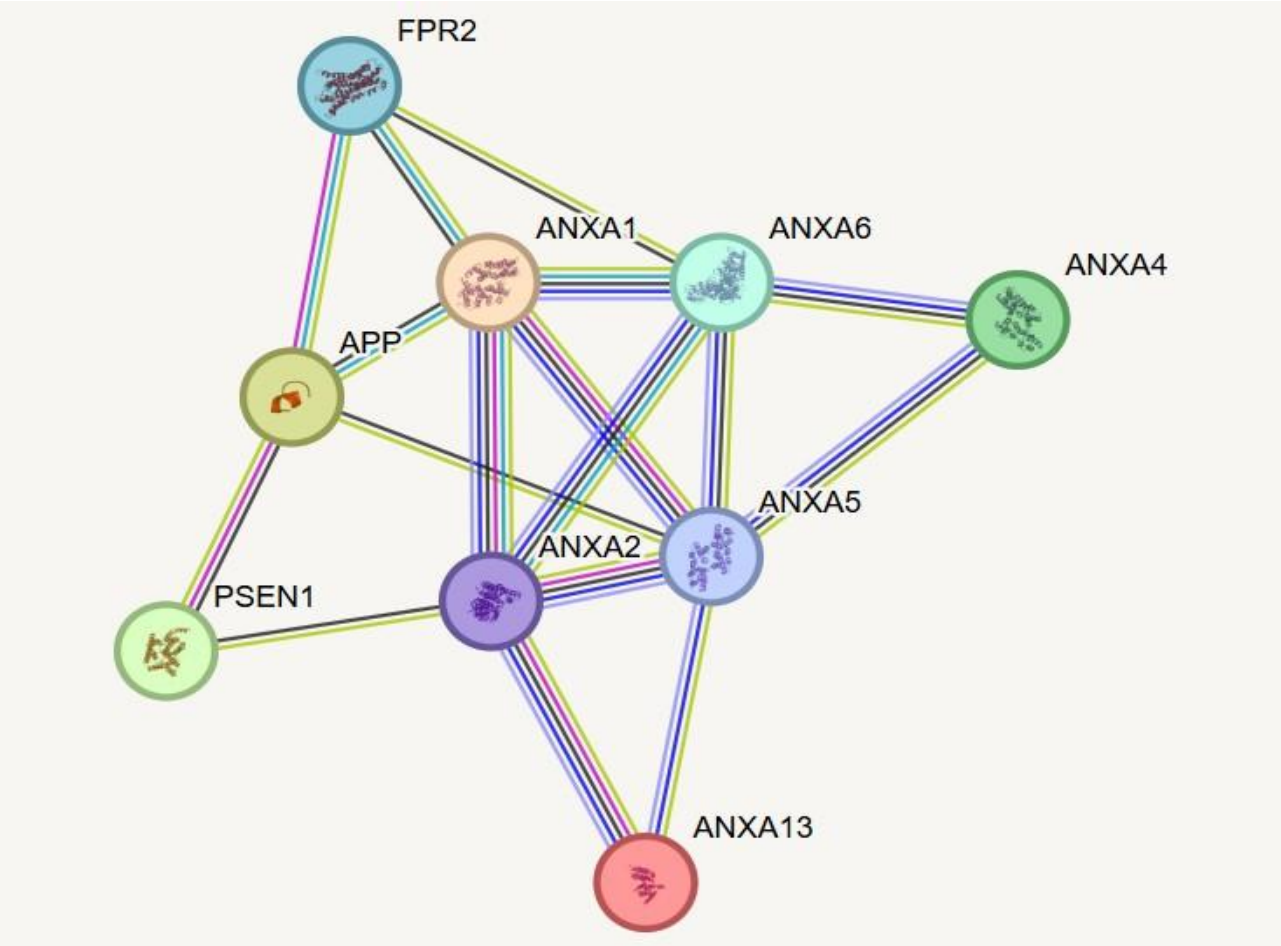


Figure 4. The protein-protein interaction (PPI) network reveals a tightly connected annexin subnetwork with ANXA2 (Annexin A2) at its core, interacting extensively with other Annexins (ANXA1, ANXA4, ANXA5, ANXA6, and ANXA13) as well as key Alzheimer's disease (AD) proteins APP and PSEN1.

The Multiple Sequence alignment studies of Annexin family proteins (ANX) related to AD revealed a high degree of conservation across the annexin protein family, particularly in the core annexin repeat regions known to mediate calcium-dependent phospholipid binding. Conserved residues are evident across all sequences, suggesting functional importance in membrane-related processes. Variable regions, typically located in the N-terminal or loop domains, may account for isoform-specific functions and interactions with disease-associated proteins such as APP and PSEN1. ANXA2, which shows strong centrality in the PPI network, shares substantial identity with ANXA1, ANXA5, and ANXA6, supporting their overlapping roles in Alzheimer's disease pathways. ANXA13 retains many conserved motifs but also exhibits unique sequence features, reflecting its evolutionary divergence and potential for distinct cellular roles. Annexins are characterized by conserved core domains comprising four homologous repeats (I–IV), which form the structural backbone essential for their calcium-dependent membrane-binding activity. These repeats contain signature motifs such as GAGTDE, DTSGDF, and KGLGT, which are highly conserved across the annexin family, highlighting their critical functional roles. In contrast, the N- and C-terminal regions display considerable variability among different annexins, contributing to isoform-specific functions, localization, and regulatory mechanisms. For instance, ANXA6 uniquely possesses additional repeats (V–VIII), granting it extended functional capabilities compared to other annexins. Meanwhile, ANXA13 exhibits distinct sequence insertions and deletions, identifying it as the most evolutionarily divergent member of the family. The phylogenetic tree in Figure 5 reveals two main clades: ANXA1/ANXA2 and ANXA4/ANXA5/ANXA6, indicating close evolutionary and functional relationships within each group. ANXA13 branches separately with the highest divergence, reflecting its distinct evolutionary path and specialized roles. ANXA6 shows moderate divergence, positioning it between the two clusters as a possible evolutionary intermediate.

Phylogram (ancestral relationship) studies of the Annexin family proteins involved in AD

The annexin family members group into distinct clades, as shown Figure 5 with ANXA1 and ANXA2 and ANXA4 and ANXA5 showing the highest similarity within their respective pairs, while ANXA13 is the most evolutionarily distinct. This suggests functional conservation within clades and potential specialization in ANXA13

Analysis of functional relationship using PROSITE

Prosite analysis of individual Annexin members helps identify conserved domains, functional motifs, and signature sequences unique or shared among the family. This allows you to understand differences in calcium-binding sites, membrane-binding regions, and isoform-specific functional elements, ultimately revealing how each Annexin may contribute differently to cellular processes such as signaling, vesicle trafficking, or stress response.

Prosite analysis of ANXA1 revealed four conserved regions matching known annexin domains, all aligned with its functional role in calcium-dependent membrane binding. The strongest match was observed between positions 114–185 (score: 31.258), indicating a highly conserved annexin repeat likely involved in calcium binding or structural stabilization. Additional conserved segments at positions 42–113 and 273–344 also suggest domains critical for phospholipid interaction and regulatory functions. A moderately conserved region from 197–269 may contribute to protein–protein interactions or structural integrity. Together, these matched regions confirm ANXA1’s role in membrane dynamics and signalling through well-preserved annexin domains.

Prosite analysis of ANXA2 reveals four key segments, each contributing uniquely to its structure and function. The region spanning residues 105–176 shows the highest conservation (score: 32.899) and corresponds to the core annexin domain essential for calcium-dependent phospholipid binding, underscoring its central role in membrane dynamics and signaling. The N-terminal region (33–104) is enriched in charged residues and likely aids in initial membrane association. In contrast, the segment from 189–261, with a lower score, may represent a flexible or regulatory region involved in protein-protein interactions or post-translational modifications. The final segment (265–336) contributes to oligomerization and cytoskeletal interactions. Together, these domains reflect ANXA2's functional versatility in processes such as endocytosis, exocytosis, inflammation, and potentially neurodegenerative diseases like Alzheimer's.

Prosite analysis of the ANXA4 protein reveals four key conserved domains contributing to its structural and functional integrity. The region from residues 14–85 (score: 28.874) appears moderately conserved and may be involved in early folding or membrane association. The segment from 86–157 shows a slightly higher score (29.548), likely corresponding to part of the annexin core domain important for calcium and phospholipid binding. Residues 169–241 (score: 28.567) maintain structural continuity within the core repeats, supporting calcium-dependent membrane interactions. The most conserved region, spanning residues 245–316 with the highest score (30.806), likely includes a critical portion of the C-terminal domain responsible for membrane affinity and potential interaction with other cellular components. Together, these conserved motifs suggest a consistent annexin repeat architecture essential for ANXA4's membrane-binding and regulatory functions.

Prosite analysis of ANXA5 highlights four conserved segments critical to its structure and Alzheimer's disease (AD)-related functions. The highest-scoring region, residues 15–86 (score: 30.767), encompasses the annexin core domain essential for calcium-dependent phospholipid binding and membrane association, aligning with ANXA5's role in apoptosis and vesicle trafficking. The adjacent segment, 87–158 (score: 29.249), also shows strong conservation, rich in charged residues that likely facilitate Ca^{2+} coordination and early apoptotic recognition—key during neuronal stress in AD. The moderately conserved region from 170–242 (score: 25.393) may support protein-protein interactions, possibly with Aβ or cytoskeletal partners, while the C-terminal segment (246–317; score: 27.747) likely contributes to membrane stabilization or vesicle fusion. These conserved domains collectively reflect ANXA5's involvement in apoptosis, Aβ interaction, membrane repair, and neuroinflammation—processes central to AD pathogenesis.

Prosite-based domain analysis of ANXA6 reveals a structurally and functionally diverse protein with eight conserved segments supporting its unique dual-core architecture. The N-terminal segments (residues 20–163; scores ~29.6) form the first annexin core, enriched in calcium-binding motifs critical for membrane association and curvature sensing—vital for neuronal vesicle trafficking. The central regions (175–322) show moderate conservation, likely mediating protein interactions or isoform-specific regulation. A strikingly conserved second core spans residues 363–506, with scores >31, highlighting its essential role in membrane fusion, calcium buffering, and neuroprotection. The less conserved region (521–595; score: 22.533) may serve regulatory functions, while the C-terminal segment (599–670; score: 30.53) reinforces membrane stabilization and cytoskeletal anchoring. These features support ANXA6's multifaceted roles in neurophysiology and its potential contribution to Alzheimer's disease mechanisms.

Although ANXA13 lacks direct associations with Alzheimer's disease (AD), its evolutionary ancestry and conserved core domains suggest potential indirect roles. It may act as a functional surrogate for annexins like ANXA2 or ANXA5 under cellular stress, contributing to membrane repair, endocytosis/exocytosis, or calcium regulation—processes disrupted in AD. Additionally, its involvement in lipid raft dynamics and compensatory expression during neuroinflammation could influence neuronal stability and survival in disease contexts.

Each ribbon diagram in Figure 6 illustrates the conserved α-helical architecture characteristic of annexins, with ANXA6 and ANXA13 displaying extended structures due to additional or divergent repeats. ANXA6 shows two tandem core domains, while ANXA13 reveals structural features indicative of evolutionary divergence. These models highlight both structural conservation across annexins and isoform-specific adaptations relevant to their cellular functions, including membrane interaction and signaling in neurodegenerative contexts such as Alzheimer's disease.

Limitations

These findings are purely based on computational results and in-silico studies which require validation through applied research. In-silico studies offer valuable support for drug discovery, toxicological assessment, prediction of physicochemical properties of proteins and ligands, it thus enhances our prediction score of the biological activities of genes, proteins and their functional dependencies. However, these predictions cannot completely be relied on keeping in mind the complexity of physiological mechanisms of biological systems. Thus, integrated workflows of using in-silico approaches together with wet lab strategies like genetic knockdown and chemical knockdown approaches on cell lines and animal models enhance efficient and effective target identification using minimal time, effort and resources. Web based systems integrating literature mining across authentic published work and curated databases accelerate the process of hypothesis testing on gene-protein relation and function, which needs an extensive. These computational tools complement wet lab experiments offering efficient means to explore and challenge complex mechanisms of biological systems.

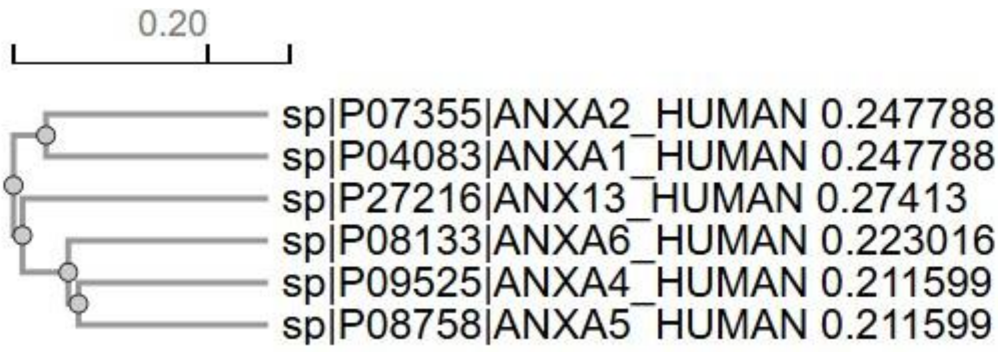


Figure 5. Phylogenetic tree of selected human annexin (ANXA) proteins. The tree shows the evolutionary relationships among six ANXA family members: ANXA1, ANXA2, ANXA4, ANXA5, ANXA6, and ANXA13, with their corresponding UniProt accession numbers. Branch lengths are proportional to sequence divergence, and the scale bar represents a genetic distance of 0.20. The numerical values indicate the pairwise evolutionary distances for each sequence. The decimal numbers (e.g., 0.247788) are branch lengths, which indicate the evolutionary distance (typically based on % sequence divergence).

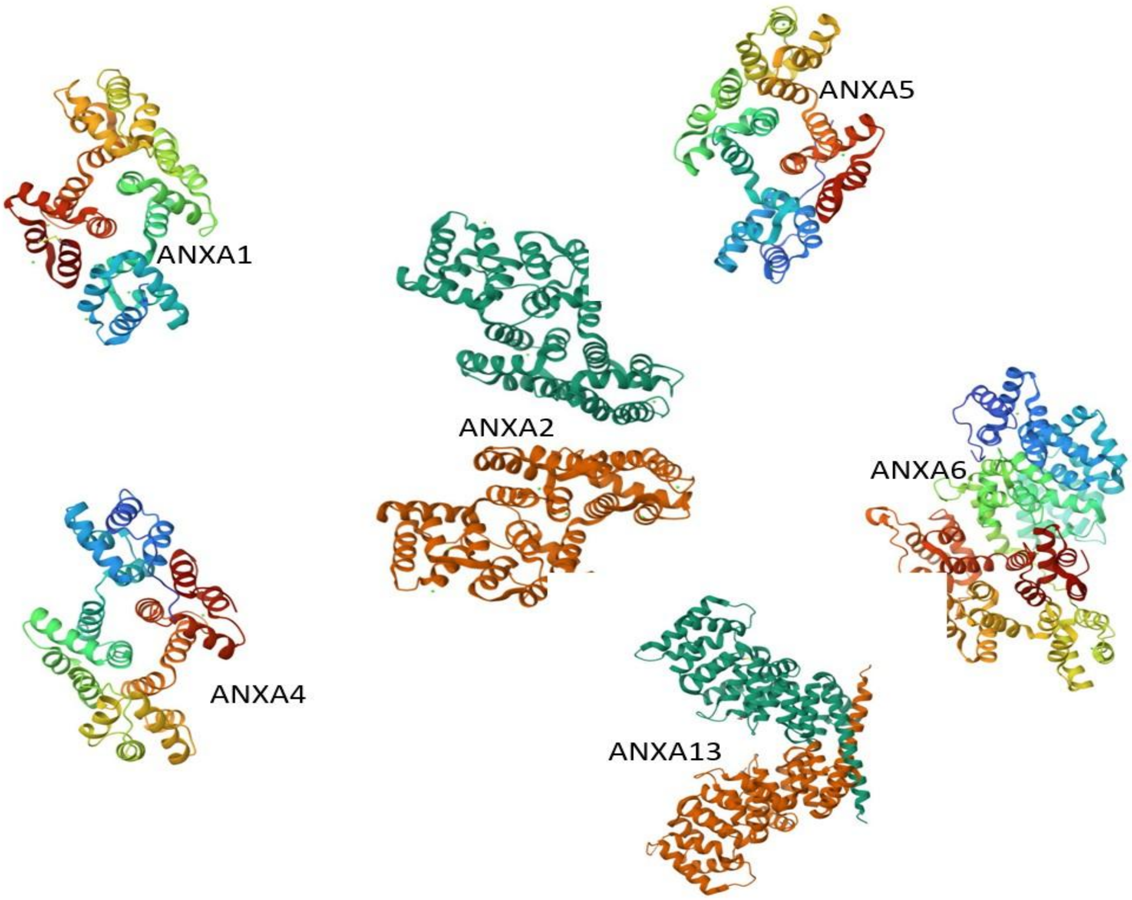


Figure 6. Comparative 3D structural representations and analysis of selected annexin family members (ANXA1, ANXA2, ANXA4, ANXA5, ANXA6, and ANXA13).

Conclusion

Previous research has mostly linked annexins to neuroinflammation and cellular balance. However, our results show that annexins and core AD proteins have always had a conversation with each other, which may be what sets off the first signs of the disease. According to the co-alignment and phylogenetic links found in this study, annexins may be upstream modulators affecting APP processing and plaque dynamics rather than only downstream effectors. Crucially, conserved domains and similar motifs suggest that annexin and AD proteins may interact directly, which calls for structural and biochemical confirmation. These revelations raise the possibility of annexin-based molecular therapies that focus on the early stages of the illness, before obvious plaque development or cognitive deterioration. Furthermore, our findings open the door for the development of isoform-specific biomarkers, which has hitherto been mainly unexplored, by identifying particular annexins (e.g., ANXA1, ANXA4, ANXA5, ANXA6) with strong evolutionary relationships to AD-related proteins. To better understand their functional significance and therapeutic potential, future studies should concentrate on experimentally analyzing these protein–protein interactions, ideally in animal and cellular models that are relevant to the disease..

Materials and Methods

1. **Protein Sequence Retrieval:**

The pdb & UniProtKB database (https://www.uniprot.org/) provided the FASTA formatted protein sequences of the human,(Homo sapiens) rat (Rattus norvegicus), and chimpanzee (Pan troglodytes) amyloid precursor protein (APP), as well as the Alzheimer's disease-associated proteins Presenilin-1 (PSEN1), Formyl Peptide Receptor 2 (FPR2), and several Annexin family proteins (Annexin A1, A2, A4, A5, A6, A13). To guarantee accuracy and completeness, accession IDs were cross-checked for every species. PyMOL was used to visualize the three-dimensional structure of annexin proteins, allowing detailed examination of their conserved domains and structural features.

2. 2. Alignment of Multiple Sequences (MSA) and building of phylogenetic tree:

Multiple sequence alignment was carried out using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) to evaluate sequence conservation and divergence across APP across species, as well as between annexins and AD-associated proteins. To objective was to find conserved motifs, divergent areas, and putative pathogenic functional domains, alignments were displayed. To assess the evolutionary links between the APP proteins from various species and their resemblance to annexins and other proteins linked to Alzheimer's disease, a phylogenetic tree, or phylogram, was created. For comparison, the Maximum Likelihood (ML) and Neighbor-Joining (NJ) approaches were used. Conserved residues and domains were annotated using Jalview.

3. Analysis of the Functional Relationship:

Additionally, pairwise alignments were performed using BLASTp (NCBI Protein–Protein BLAST) with adjusted E-value thresholds (< 1e-5) to assess sequence similarity and possible evolutionary conservation in order to investigate possible functional associations between Annexin proteins and AD-related proteins (APP, PSEN1, FPR2). Additionally, Protein–protein interaction (PPI) analysis was conducted using the STRING database ((https://string-db.org/) to identify functional associations among key targets, including APP, PSEN1, and annexin family proteins (ANXA1, ANXA2, ANXA4, ANXA5, ANXA6, ANXA13). To analyze structural conservation, PROSITE was used to compare all annexin family members. The analysis revealed shared conserved domains characteristic of the annexin superfamily, supporting their functional similarity and potential co-regulation in cellular processes.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Sharmistha D, Prabha M, Siva Kiran RR, Ashoka H. Enhanced in silico QSAR-based screening of butyrylcholinesterase inhibitors using multi-feature selection and machine learning. SAR and QSAR in Environmental Research. 2025 Feb 1;36(2):79-99. https://doi.org/10.1080/1062936X.2025.2466020

2. Park LY, Cordeiro AM, Costa AC, Gattaz WF, Forlenza OV, Joaquim HP, Talib LL. Donepezil Down-Regulates Annexin A3 Protein in Alzheimer Disease Patients. Journal ISSN. 2023;2766:2276. https://www.jelsciences.com/articles/jbres1799.pdf
3. Krzyzanowska A, Garcia-Consuegra I, Pascual C, Antequera D, Ferrer I, Carro

E. Expression of regulatory proteins in choroid plexus changes in early stages of Alzheimer disease. Journal of Neuropathology & Experimental Neurology. 2015 Apr 1;74(4):359-69. Available from: https://doi.org/10.1097/NEN.0000000000000181

4. 4. Hitchcock JK, Katz AA, Schäfer G. Dynamic reciprocity: the role of annexin A2 in tissue integrity. Journal of cell communication and signaling. 2014 Jun;8(2):125-33. Available from: https://doi.org/10.1007/s12079-014-0231-0
5. Han PF, Che XD, Li HZ, Gao YY, Wei XC, Li PC. Annexin A1 involved in the regulation of inflammation and cell signaling pathways. Chinese Journal of Traumatology. 2020 Apr 1;23(2):96-101. https://www.sciencedirect.com/science/article/pii/S1008127520300432
6. Sohma H, Imai SI, Takei N, Honda H, Matsumoto K, Utsumi K, Matsuki K, Hashimoto E, Saito T, Kokai Y. Evaluation of annexin A5 as a biomarker for Alzheimer's disease and dementia with lewy bodies. Frontiers in aging neuroscience. 2013 Apr 5;5:15. https://www.frontiersin.org/journals/agingneuroscience/articles/10.3389/fnagi.2013.00015/full
7. Piljić A, Schultz C. Annexin A4 self-association modulates general membrane protein mobility in living cells. Molecular biology of the cell. 2006 Jul;17(7):3318-28. https://www.molbiolcell.org/doi/full/10.1091/mbc.e06-01-0041
8. Sadleir KR, Gomez KP, Edwards AE, Patel AJ, Ley ML, Khatri AW, Guo J, Mahesh S, Watkins EA, Popovic J, Karunakaran DK. Annexin A6 membrane repair protein protects against amyloid-induced dystrophic neurites and tau phosphorylation in Alzheimer's disease model mice. Acta Neuropathologica. 2025 May 24;149(1):51: https://link.springer.com/article/10.1007/s00401-025-02888-1
9. Ansari J, Senchenkova EY, Vital SA, Al-Yafeai Z, Kaur G, Sparkenbaugh EM, Orr AW, Pawlinski R, Hebbel RP, Granger DN, Kubes P. Targeting the AnxA1/Fpr2/ALX pathway regulates neutrophil function, promoting thromboinflammation resolution in sickle cell disease. Blood, The Journal of the American Society of Hematology. 2021 Mar 18;137(11):1538-49.https://doi.org/10.1182/blood.2020009166
10. Chua XY, Chong JR, Cheng AL, Lee JH, Ballard C, Aarsland D, Francis PT, Lai MK. Elevation of inactive cleaved annexin A1 in the neocortex is associated with amyloid, inflammatory and apoptotic markers in neurodegenerative dementias. Neurochemistry international. 2022 Jan 1;152:105251. Available from: https://www.sciencedirect.com/science/article/pii/S0197018621002977
11. Cuestas Torres DM, Cardenas FP. Synaptic plasticity in Alzheimer's disease and healthy aging. Reviews in the Neurosciences. 2020 Apr 28;31(3):245-68. https://doi.org/10.1515/revneuro-2019-0058
12. Weiss R, Bitton A, Shimon MB, Goldman SE, Nahary L, Cooper I, Benhar I, Pick CG, Chapman J. Annexin A2, autoimmunity, anxiety and depression. Journal of Autoimmunity. 2016 Sep 1;73:92-9. https://www.sciencedirect.com/science/article/pii/S0896841116300890
13. de Souza Ferreira LP, da Silva RA, Gil CD, Geisow MJ. Annexin A1, A2, A5, and A6 involvement in human

pathologies. Proteins: Structure, Function, and Bioinformatics. 2023 Sep;91(9):1191-204. https://onlinelibrary.wiley.com/doi/abs/10.1002/prot.26512

14. Ries M, Watts H, Mota BC, Lopez MY, Donat CK, Baxan N, Pickering JA, Chau TW, Semmler A, Gurung B, Aleksynas R. Annexin A1 restores cerebrovascular integrity concomitant with reduced amyloid-β and tau pathology. Brain. 2021 May 1;144(5):1526-41. Available from: https://doi.org/10.1093/brain/awab050
15. Jing J. The relevance, predictability, and utility of annexin A5 for human physiopathology. International Journal of Molecular Sciences. 2024 Mar 1;25(5):2865. Available from: https://doi.org/10.3390/ijms25052865

Vita Scientia

Research Article

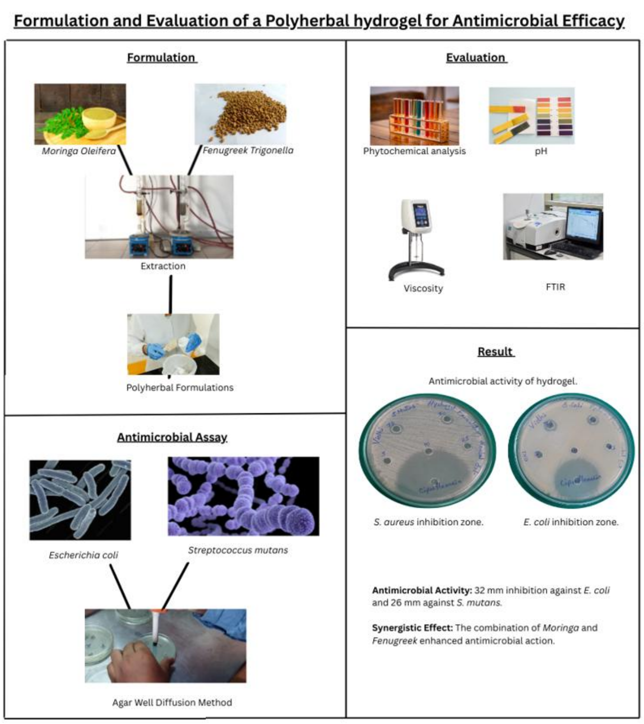
Read Online

Formulation and Evaluation of a Polyherbal hydrogel for Antimicrobial efficacy

Rakesh Surappa Anjaneya[1*], Anusha Bukkambudhi Nataraj[2], Pratiksha Chandrashekhar Chandragirivar[3], Mohd Yosuf Malik Damani[2], Ramesh Chakka[2]

[1]Department of Pharmacognosy, GM Institute of Pharmaceutical Sciences and Research, Davangere, Karnataka, India
[2]Department of Pharmacology, GM Institute of Pharmaceutical Sciences and Research, Davangere, Karnataka, India
[3]Department of Pharmaceutics, GM Institute of Pharmaceutical Sciences and Research, Davangere, Karnataka, India
[*]Correspondence: rakesh@gmipsr.ac.in

Graphical abstract



Summary

The development of natural and effective treatments for fungal infections remains a key area in dermatological research. This study explores the formulation and evaluation of a polyherbal hydrogel incorporating Moringa oleifera leaf powder and Trigonella foenum-graecum (fenugreek), both recognized for their antimicrobial properties. Using a carbopol-based gel matrix, multiple formulations were developed with varying concentrations of herbal extracts. The hydrogels were evaluated for physicochemical properties, including pH and viscosity. Antimicrobial activity was tested against Streptococcus mutans and Escherichia coli using the agar well diffusion method. All formulations demonstrated antimicrobial effects, with one formulation showing the highest zone of inhibition, likely due to its higher concentration of plant extracts. The findings suggest a synergistic interaction between the herbal constituents, supporting the potential of polyherbal hydrogels as effective topical agents for managing microbial infections.

Keywords

Polyherbal hydrogel, Moringa oleifera, Trigonella foenum-graecum, Agar well diffusion method

Introduction

Three-dimensional, extremely hydrophilic networks of polymers called hydrogels may absorb and hold vast amounts of biological fluids or water. Possessing physical and mechanical properties akin to human extracellular matrices [1]. Their biocompatibility, moisture retention, viscoelasticity, and ease of application make them ideal carriers for topical delivery systems. These networks can be natural, synthetic, or hybrid, and cross-linked chemically or physically. Cross-linking can be tailored to ensure structural integrity and stimuli-responsiveness (to pH, temperature, light, or enzymes), which enables controlled, localized drug release and reduces systemic side effects [2]. The alarming increase in antibiotic resistance and the limitations of synthetic antimicrobials have spurred interest in plant-based alternatives delivered via biocompatible systems like hydrogels, which maintain moisture, offer controlled release, and support wound healing [3]. Moringa oleifera leaf and seed extracts have demonstrated potent antimicrobial and antibiofilm activities against pathogens such as Staphylococcus aureus, including methicillin-resistant strains, via disruption of microbial membranes and inhibition of biofilm formation [4]. Similarly, Trigonella foenum graecum (fenugreek) seeds contain alkaloids, saponins, flavonoids, and coumarins that have demonstrated strong inhibitory effects on a variety of bacteria, including Gram-positive and Gram-negative, including multidrug-resistant S. aureus and E. coli at concentrations between 50–1000 µg/mL, and notably disrupt quorum sensing and biofilm formation [5]. Although several studies have formulated polyherbal gels, such as those combining neem, turmeric, garlic, basil, cinnamon, and tamarind, demonstrating improved antimicrobial efficacy and favorable physicochemical profiles [6]. There is limited investigation into hydrogels comprising Moringa and Fenugreek specifically. One notable study developed a Moringa seed-based hydrogel that exhibited antioxidant, antimicrobial, and wound-healing actions in vivo as well as in vitro excision and incision models [7]. Fenugreek gels have also been evaluated for antimicrobial and anti-inflammatory efficacy against oral pathogens, proving more effective than standard agents like doxycycline in vitro [8].

Building on this foundation, our study aims to formulate and evaluate a polyherbal hydrogel containing Moringa oleifera leaf powder and Trigonella foenum graecum seed extract, investigating its physicochemical characteristics (e.g., pH, viscosity, spread-ability, stability) and in vitro antimicrobial efficacy against clinically relevant pathogens. We ask: Can this dual herb hydrogel exhibit synergistic antimicrobial and antibiofilm properties suitable for topical application in managing skin and wound infections? The answer could pave the way for safe, affordable, plant-based antimicrobial therapies that address antimicrobial resistance and benefit resource-limited healthcare environments.

Results and Discussion

Results

Standardization of Crude Drugs:

Total ash values were found to be 3% for Trigonella foenum-graecum and 10.5% for Moringa oleifera, with acid-insoluble ash values of 1% and 1.5%, respectively. Loss on drying (LOD) was 93.5% for fenugreek and 95% for Moringa. Extractive values showed higher solubility of Moringa in water (8%) and alcohol (10.4%) compared to fenugreek (2.4% and 4.8%).

Phytochemical Screening:

Fenugreek extract tested positive for carbohydrates, alkaloids, proteins, amino acids, tannins, flavonoids, saponins, and glycosides (except glycosides negative in fenugreek by Borntrager's test). Moringa oleifera extract revealed the existence of carbohydrates, Saponins, flavonoids, and glycosides, but was negative for alkaloids and proteins.

Thin Layer Chromatography (TLC):

TLC analysis revealed that T. foenum-graecum powder and extract revealed respective Rf values of 0.33 and 0.29. while M. oleifera powder and extract exhibited Rf values of 0.73 and 0.71. This indicates the presence of specific polar compounds.

Evaluation of Hydrogel Formulations:

The table 1 summarizes the pH and viscosity measurements of three hydrogel formulations containing extracts of Moringa oleifera and Trigonella foenum-graecum.

Discussion

The standardization results indicate that both Trigonella foenum-graecum (fenugreek) and Moringa oleifera possess significant inorganic and moisture content, with Moringa displaying higher extractive values, suggesting a richer concentration of soluble phytoconstituents. Phytochemical screening revealed a wide array of bioactive compounds in both extracts, which are likely responsible for their observed biological effects. Thin-layer chromatography (TLC) profiles demonstrated consistent Rf values between powder and extract forms, indicating effective extraction and stability of key chemical constituents. Although not detailed here, FTIR spectral analysis further supported the chemical integrity of the plant extracts within the formulations.

The hydrogel formulations exhibited pH levels slightly on the acidic side, which aligns well with the natural pH of human skin, making them suitable for topical application. Viscosity analysis showed that higher extract concentrations increased the hydrogel's viscosity, potentially enhancing its retention on the skin and thereby improving therapeutic efficacy. While individual plant extracts showed limited antimicrobial activity, the polyherbal hydrogel exhibited a synergistic effect, with noticeable zones of inhibition against Streptococcus mutans and Escherichia coli. This enhanced activity may result from the combined action of diverse phytochemicals on various microbial targets, as well as the hydrogel base facilitating improved delivery and sustained release of the active constituents.

Collectively, these findings suggest that the formulated polyherbal hydrogel not only preserves the key phytochemicals of the individual extracts but also enhances their antimicrobial efficacy. This supports its potential as a promising natural topical antimicrobial agent for managing microbial infections.

Conclusion

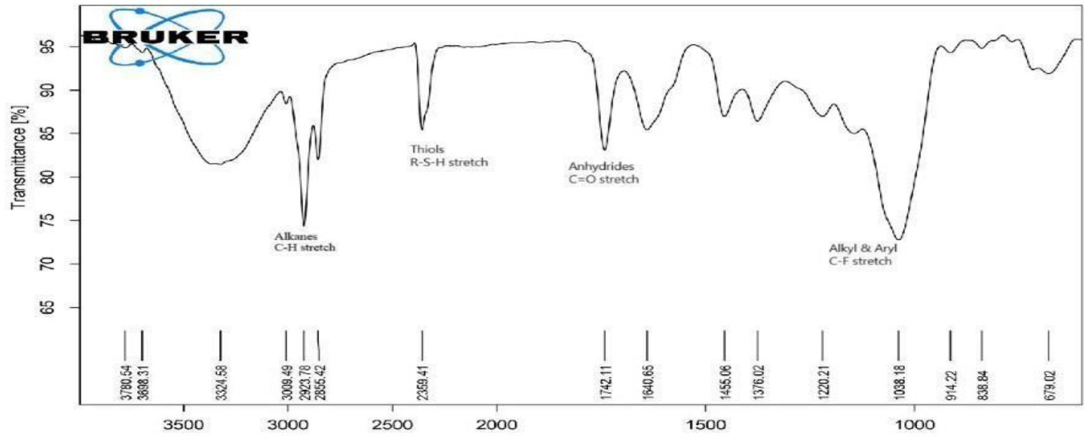
The present study successfully formulated and evaluated a polyherbal hydrogel incorporating

Parameter	Formulation 1	Formulation 2	Formulation 3
pH	6.00	5.90	5.88
Viscosity (cP)	3917.90	6422.28	8547.47

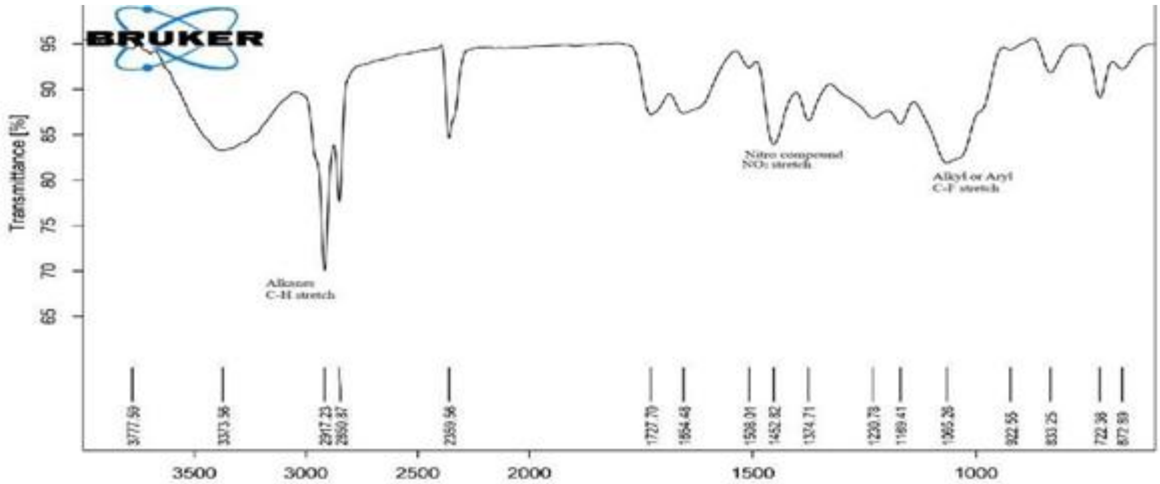
Table 1. Evaluation of pH and Viscosity of Polyherbal Hydrogel FormulationsAll formulations were prepared using standardized ethanolic extracts and evaluated under identical laboratory conditions. cP = centipoise

FTIR Spectroscopic Analysis

(A)



(B)



(C)

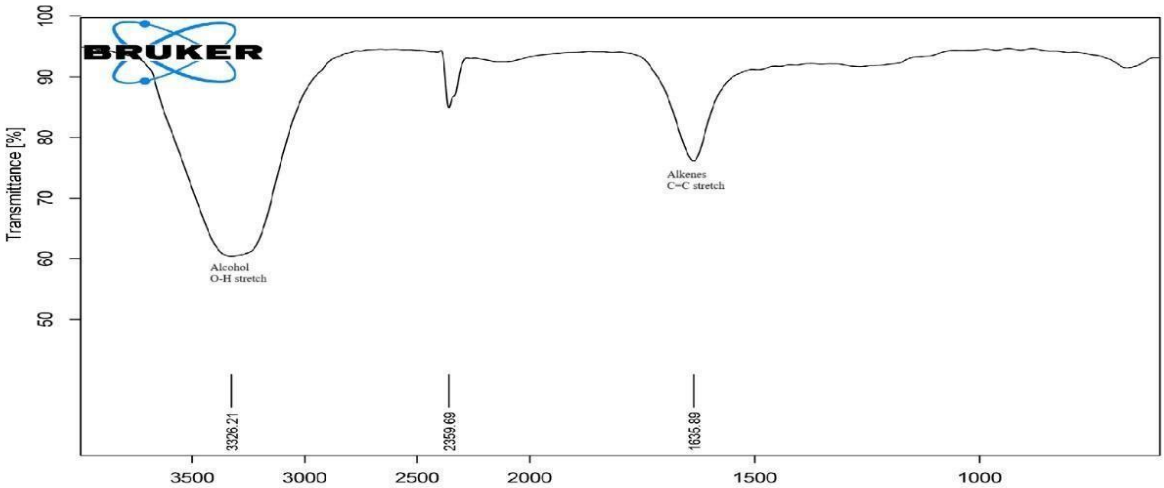


Figure 1. FTIR Spectral Analysis of Extracts and Formulation

(A)*Moringa oleifera* leaf extract showing characteristic functional group peaks. (B)*Trigonella foenum-graecum* seed extract showing peaks corresponding to alkanes and nitro compounds. (C) Hydrogel formulation confirming retention of phytoconstituents.

Antimicrobial Activity:

Antimicrobial activity of polyherbal extracts:

Table 2. Individual plant extracts' antimicrobial capabilities against *Escherichia coli* and *Streptococcus mutans*

Sl. No.	Organism	Sample	75 µl/ml	50 µl/ml	25 µl/ml	10 µl/ml	5 µl/ml	Ciprofloxacin (mm)
1	S. mutans	Sample 1	R	R	R	R	R	58
2	S. mutans	Sample 2	R	R	R	R	R	52
3	E. coli	Sample 1	10 mm	R	R	R	R	45
4	E. coli	Sample 2	12 mm	R	R	R	R	48

Footnote: Sample 1 – *fenugreek* seed extract; Sample 2 – *Moringa oleifera* leaf extract. R = resistant; Inhibition zone < 8 mm.

(A) **(B)**

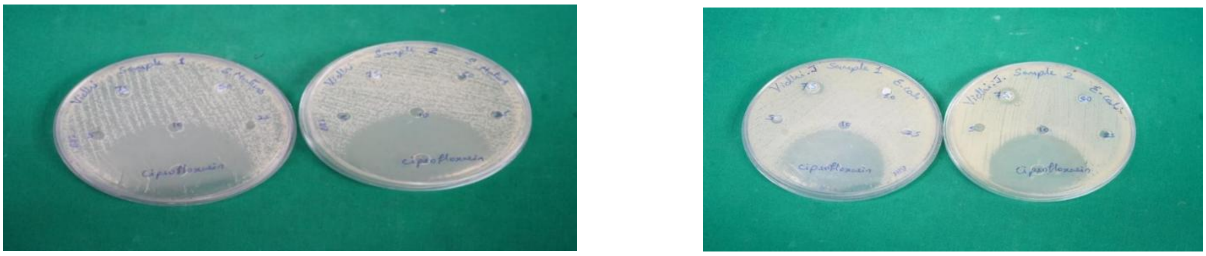


Figure 2. Antibacterial Activity of Plant Extracts Against Test Pathogens, (A) Activity against *Streptococcus mutans*. (B) Activity against *Escherichia coli*.

Antimicrobial activity of polyherbal hydrogels

Table 3. Polyherbal antimicrobial properties of hydrogel formulation in opposition to *Streptococcus mutans* and *Escherichia coli*

Sl. no.	Organism	75 µl/ml (mm)	50 µl/ml	25 µl/ml	10 µl/ml	5 µl/ml	Ciprofloxacin (mm)
1	*S. mutans*	26	R	R	R	R	45
2	*E. coli*	32	R	R	R	R	58

Footnote: The hydrogel formulation contained both *Trigonella foenum-graecum* seed extract and *Moringa oleifera* leaf extract. R = resistant; Inhibition zone < 8 mm.

Figure 3. Antimicrobial Activity of Hydrogel Formulation, (A) Zone of inhibition against *Staphylococcus aureus*. (B) Zone of inhibition against *E. coli*.

Herbal Hydrogel Formulations

Table 4. Composition of *Moringa oleifera* and *fenugreek-based* herbal hydrogel formulations

Component	Formulation 1	Formulation 2	Formulation 3
Carbopol 940 (1%w/v)	0.5	1.0	1.5
***Moringa oleifera* extract (1%w/v)**	2.0	2.0	2.0
***Fenugreek* extract (1%w/v)**	2.0	2.0	2.0
Sodium benzoate (1%w/v)	0.1	0.1	0.1
Triethanolamine to adjust PH (1N)	q.s.	q.s.	q.s.
Distilled water(upto 100ml)	q.s.	q.s.	q.s.

Footnote: q.s. = quantum satis; amount sufficient to achieve desired volume or consistency.

Trigonella foenum-graecum seed extract and Moringa oleifera leaf extract. Standardization parameters confirmed the quality and identity of the crude drugs. Phytochemical screening revealed the existence of important bioactive substances including carbohydrates, flavonoids, and saponins, which contribute to the therapeutic potential of the formulation. The presence of unique phytoconstituents in both the powder and extract forms was verified by Thin Layer Chromatography (TLC).

The prepared hydrogel formulations demonstrated acceptable pH and viscosity profiles, making them suitable for topical application. Importantly, the polyherbal hydrogel showed significant antimicrobial activity against Escherichia coli and Streptococcus mutans.

Suggesting its potential in managing skin infections or oral microbial conditions, these results support further investigation and potential clinical application of the polyherbal hydrogel as a safe and effective natural antimicrobial agent. Future studies should explore its in vivo efficacy and safety in clinical settings, along with its potential for commercialization as a cost-effective natural topical antimicrobial product.

Materials and Methods

Collection and Authentication of Plant Materials

Seeds of Trigonella foenum-graecum L. (fenugreek) and fresh leaves of Moringa oleifera Lam. were collected from Harihara, Davanagere District, Karnataka, India, during June–July. The plant materials were authenticated by Dr. Aruna Charanthimath, Head of the Department of Botany, GM Academy First Grade College, Davanagere, Karnataka, and voucher specimens were deposited in the departmental herbarium for future reference.

Processing of Raw Materials

Fresh Moringa oleifera leaves were washed with clean water, shade dried at ambient temperature away from direct sunlight, and milled into a coarse powder. T. foenum-graecum seeds were similarly cleaned and shade-dried. All dried plant materials were stored in airtight, labeled containers at room temperature to prevent degradation of phytoconstituents, in accordance with WHO guidelines on good herbal processing practices.

Extraction Procedure

Dried powders (50 g) of each plant were extracted using ethanol (95%) for 6 hours in a Soxhlet apparatus. A rotary evaporator was used to evaporate the solvent at a lower pressure yielding a semi-solid extract. This method was based on standard Soxhlet extraction techniques with minor modifications.

Ash and acid insoluble ash Value:

Total ash and acid-insoluble ash were determined by incinerating 2 g of dried powder in a muffle furnace (Remi Instruments, India) at 500 ± 25 °C. Acid-insoluble ash was determined by treating the ash with 2N HCl, filtering, and incinerating the residue [9].

Formula to calculate % of ash and acid insoluble ash value is as follow

Ash value(%)=(Weight of ash)/(Weight of sample)×100

Acid insoluble value(%)=(Weight of residue)/(Weight of sample)×100

Loss on Drying (LOD)

Approximately 2 g of the material was dried for 4 hours at 105°C in a hot air oven to calculate the LOD. The difference in weight before and after drying was used to calculate the moisture content [10]. In this process, W1 refers to the weight of the empty dish, although W2 indicates the dish's weight and the residue's weight upon drying.

Extractive Value(%)=(W2-W1)/(Weight of drug taken)×100

Extractive Value

Alcohol and water-soluble extractives were determined by macerating 5 g of powder with 100 mL of solvent for 24 h. To calculate extractive values, a 25 mL aliquot was dried by evaporation, and the residue was weighed [11]. The weight of the empty dish is denoted as W1, while W2 represents the weight of the dish along with the residue.

Extracive Value(%)=(W2-W1)/(Weight of drug taken)×100

Preliminary Phytochemical Screening

Standard testing for phytochemicals was conducted on the ethanol extracts for the presence of alkaloids, tannins, flavonoids, glycosides, carbohydrates, proteins, and saponins [12].

Thin Layer Chromatography (TLC)

TLC was used to examine the phytochemical components of the plant extracts using pre-coated silica gel GF254 plates. Mobile phase systems were selected based on the polarity and solubility of the compounds present in the extracts. Two solvent systems were employed: ethyl acetate: acetic acid: formic acid: water (10:5.5:5.5:13, v/v/v/v) and toluene: ethyl acetate: formic acid (5:4:1, v/v/v). Sample solutions were applied manually as discrete spots or bands using fine capillary tubes. The TLC plates were developed in a pre-saturated chamber lined with filter paper to ensure solvent saturation and consistent migration of the compounds. To identify fluorescent or UV-active components, the plates were air-dried after growth and examined under UV light at 254 and 366 nm. For enhanced visualization of separated compounds, the plates were post-treated with vanillin–sulfuric acid reagent and heated at 110°C to induce color development, aiding in compound differentiation [13]. RF is a number that is given as a decimal fraction and describes how a single compound behaves in TLC.

Rf=(Distance of center of spot from starting point)/(Distance of solvent front from starting point) 4.10. Evaluation of Herbal Hydrogel

pH

The pH of each formulation was determined using a digital pH meter (Eutech Instruments, Singapore). A 1% w/v dispersion of the hydrogel in distilled water was prepared, and the pH was recorded at room temperature. Formulations within the pH range of 5.5–6.5 were considered suitable for topical application

Viscosity Measurement

Viscosity of the hydrogel formulations was assessed using a Brookfield Viscometer Spindle No. 4, at 60 rpm. Approximately 50 g of each gel sample was placed in a 100 mL beaker, ensuring that it was levelled correctly. The spindle was immersed in the gel without touching the container's bottom, and viscosity was recorded after achieving a steady reading.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were recorded using an ATR-based Alpha Bruker FTIR spectrometer. Samples were scanned in the 4000–400 cm^{-1} range to identify functional groups

Antimicrobial Assay

The antimicrobial activity of extracts and hydrogels was evaluated by the agar well diffusion method against Escherichia coli and Streptococcus mutans. Brain Heart Infusion Agar was used. The wells were loaded with 5–75 µL of test solution. Ciprofloxacin (5 µg/disc) served as a positive control. The zone of inhibition was measured after 24 hours of incubation at 37 °C [14].

Acknowledgement

We thank Dr. Girish Bolakatti, Principal of GM Institute of Pharmaceutical Sciences and Research for his valuable guidance and support throughout the study. Gratitude is extended to the Department of Microbiology, Maratha Mandal College, Belgaum, and GM Institute of Pharmaceutical Sciences and Research, Davangere for providing facilities for antimicrobial studies. We also acknowledge our colleagues and staff for their technical assistance.

Author contribution

Conceptualization and experimental work: Rakesh S. A.

Manuscript writing: Anusha B. N.

Data analysis and interpretation (Results and Discussion): Prathiksha C. C.

Supervision and overall guidance: Dr. Ramesh C and Mohammed Yosuf Malik Damani

Conflict of interest

The authors declare no conflict of interest

Reference

1.Fu X, Zheng L, Wen X, et al. Functional hydrogel dressings for wound management: a comprehensive review. Mater Res Express. 2023; 10:112001. doi:10.1088/2053-1591/acfb5c.

2.Jia B, Li G, Cao E, et al. Recent progress of antibacterial hydrogels in wound dressings. Mater Today Bio. 2023; 19:100582. doi: 10.1016/j.mtbio.2023.100582.

3.Saher T, Manzoor R, Abbas K, et al. Analgesic and anti-inflammatory properties of two hydrogel formulations comprising polyherbal extract. J Pain Res. 2022; 15:1203-19. doi:10.2147/JPR.S351921.

4.Menon L, Chouhan O, Walke R, et al. Disruption of Staphylococcus aureus biofilms with purified Moringa oleifera leaf extract protein. Protein Pept Lett.2023;30:116-25. doi:10.2174/0929866530666230123113007.

5.Alenazy R. Antimicrobial activities and biofilm inhibition properties of Trigonella foenum-graecum methanol extracts against multidrug-resistant Staphylococcus aureus and Escherichia coli. Life (Basel). 2023; 13:703. doi:10.3390/life13030703.

6.Bhinge SD, Bhutkar MA, Randive DS, et al. Formulation development and evaluation of antimicrobial polyherbal gel. Ann Pharm Fr. 2017; 75:349-58. doi: 10.1016/j.pharma.2017.04.006.

7.Ali A, Garg P, Goyal R, et al. A novel herbal hydrogel formulation of Moringa oleifera for wound healing. Plants (Basel). 2021; 10:25. doi:10.3390/plants10010025.

8.Sindhusha VB, Rajasekar A. Preparation and evaluation of antimicrobial property and anti-inflammatory activity of fenugreek gel against oral microbes: an in vitro study. Cureus. 2023;15: e47659. doi:10.7759/cureus.47659.

9.Manikandaselvi S, Vadivel V, Brindha P. Studies on physicochemical and nutritional properties of aerial parts of Cassia occidentalis L. J Food Drug Anal. 2016; 24:508-15. doi: 10.1016/j.jfda.2016.02.003.

10.Ahn JY, Kil DY, Kong C, et al. Comparison of oven-drying methods for determination of moisture content in feed ingredients. Asian-Australas J Anim Sci. 2014; 27:1615-20. doi:10.5713/ajas.2014.14305.

11.Simran Singh P, Bal M, Mukhtar HM, et al. Standardization and pharmacological investigation on leaves of Ficus bengalensis. Int J Res Pharm Chem. 2011;1(3).

12.Pant DR, Pant ND, Saru DB, et al. Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of Pterocarpus marsupium Roxburgh. J Intercult Ethnopharmacol. 2017; 6:170-6. doi:10.5455/jice.20170403094055.

13.Danciu V, Hosu A, Cimpoiu C. Thin-layer chromatography in spices analysis. J Liq Chromatogr Relat Technol. 2018; 41:282-300. doi:10.1080/10826076.2018.1447895.

14.Mehdipour A, Ehsani A, Samadi N, et al. The antimicrobial and antibiofilm effects of three herbal extracts on Streptococcus mutans compared with chlorhexidine 0.2% (in vitro study). J Med Life. 2022; 15:526-34. doi:10.25122/jml-2021-0189.

Vita Scientia

Review Article

Read Online

Nutritional Composition and Health Benefits of Superfoods: Potential Roles in Chronic Disease Prevention and Dietary Applications

Vishal Kumar[1*], Akanksha[2], Anu Kumari[1], Nimisha Tehri[1]

[1]College of Post harvest Technology and Food Processing, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh- 250 110

[2] Centre of Food Technology, Institute of Professional Studies, University of Allahabad, Prayagraj, Uttar Pradesh- 211 002

*Correspondence: vishalkumar@bhu.ac.in

Summary

Recent progress in food science and nutrition has led to the emergence of a specialized class of food products referred to as superfoods, recognized for their potential to confer a range of health benefits. These foods are characterized by high concentrations of essential nutrients—such as vitamins, minerals, dietary fiber, and essential fatty acids—as well as bioactive phytochemicals, including polyphenols, flavonoids, carotenoids, and anthocyanins. Superfoods have garnered substantial attention due to their purported roles in enhancing immune function, mitigating oxidative stress, and reducing the risk of chronic diseases, including cardiovascular disorders, diabetes mellitus, obesity, and certain types of cancer. Despite the historical use of many superfoods in traditional dietary and medicinal practices, the scientific understanding of their mechanisms of action remains relatively limited. Their increasing popularity is largely driven by consumer interest in health and wellness, alongside marketing influences. This review aims to systematically examine the nutritional composition and functional attributes of selected superfoods, while exploring current evidence regarding their potential health-promoting effects. The findings aim to support informed dietary choices and inform the integration of superfoods into evidence-based nutritional strategies for disease prevention and health promotion.

Keywords

Bioactive, Health Promoting, flavonoids, Superfood, Therapeutic

Introduction

In recent years, accumulating scientific evidence has emphasized the profound influence of diet and its components on human health, prompting a marked shift in consumer behavior and dietary preferences [31]. Beyond satisfying fundamental physiological needs such as hunger and nutrient provision, a growing segment of consumers now perceives food as a vehicle for enhancing overall well-being, preventing diet-related disorders, and supporting both mental and physical health. This evolving perspective is deeply rooted in historical ideologies—particularly the Hippocratic principle, “Let food be thy medicine and medicine be thy food”—which has gained renewed relevance in contemporary nutritional science [52].

Foods that positively influence physiological functions, assist in the prevention of various diseases, and may serve therapeutic purposes are categorized under the term "functional foods." The concept of functional food was first established in Japan nearly three decades ago and has since garnered global recognition across scientific, regulatory, and commercial domains [53]. In addition to their role in promoting health, functional foods are increasingly acknowledged for their potential to reduce healthcare expenditures, particularly in aging populations, and are considered economically significant within the food industry.

More recently, the term "superfoods" has gained prominence, often used to describe a subset of functional foods that are distinguished by their high nutrient density and purported health benefits [39]. Interest in superfoods has grown considerably, especially in Western societies, due to their rich concentrations of essential macro- and micronutrients, which contribute to their perceived role in disease prevention and overall health maintenance. According to the Oxford English Dictionary, a superfood is defined as a food product considered especially beneficial to human health and well-being due to its high nutritional content [28].

The scope of the term "superfood" has expanded to include certain traditional food items that have undergone enhancement through non-genetic processing techniques aimed at improving their functional attributes [17]. While superfoods share functional attributes with functional foods, they differ in several key respects. Typically, superfoods are characterized as minimally processed, naturally occurring food items with a longstanding history of traditional use. These foods often originate from remote or indigenous regions and are associated with both culinary and medicinal applications. Their increasing popularity is attributed not only to their potent health benefits but also to their perceived authenticity and cultural distinctiveness [43].

Furthermore, superfoods particularly those classified as superfruits—are often regarded as dual-purpose entities, functioning both as dietary and medicinal agents. This classification is attributed to their complex composition of synergistic bioactive compounds that contribute collectively to health promotion [48]. Superfoods are frequently represented in the produce sections of supermarkets, primarily among fruits and vegetables rich in phytochemicals. These include antioxidants, micronutrients, and other biologically active compounds, which, when consumed, may enhance health outcomes by increasing the intake of protective dietary constituents.

A recent study demonstrated that a Google search for the term “superfoods” yielded approximately 57 relevant entries within the first 15 pages of search results, indicating widespread usage in consumer-facing domains [45]. Notably, the term "superfoods" is predominantly encountered in marketing materials, food packaging, media discourse, and discussions surrounding innovative or alternative ingredients aimed at improving consumer health, rather than in rigorous scientific literature [47]. As of August 2018, a database search revealed that “superfoods” returned 191 entries including research articles, book chapters, and reviews while “superfruits” produced 85 results on the ScienceDirect platform. In contrast, “functional foods” generated 210,226 entries on Wiley and 382,852 results on ScienceDirect for the period between 1998 and 2017, highlighting the more established scientific foundation of functional foods compared to superfoods [44].

Superfoods especially exotic superfruits are frequently characterized by high levels of bioactive compounds such as anthocyanins, flavonoids, and phenolic acids. These compounds are recognized for their potent antioxidant capacities and have been linked to protective effects against chronic diseases, including cardiovascular disorders and type 2 diabetes mellitus. The mechanisms underlying these effects often involve modulation of critical clinical biomarkers, such as blood pressure, body mass index, waist circumference, fasting blood glucose, and plasma triacylglycerol concentrations [46].

Brazil Nuts

Brazil nuts are among the most commonly consumed tree nuts in South America and are derived from the Brazil nut tree (Bertholletia excelsa), a large species native to the Amazon rainforest. This tree thrives in well-drained, compact soils typically found along the Amazon River and is predominantly located in countries such as Brazil, Venezuela, Colombia, Ecuador, and Peru. The fruit of Bertholletia excelsa is generally round or pear-shaped, with a woody, thick outer shell approximately 0.5 cm in thickness and a diameter of about 6 cm. Each fruit contains between 12 and 24 angular, three-sided seeds (commonly referred to as nuts). These seeds are irregularly cylindrical in shape,nutritionl light cream in color, and enclosed within a hard, woody capsule. The seed capsule itself resembles a large grapefruit in size and can weigh up to 2 kilograms [51].

Brazil nuts hold significant economic value, particularly for local and indigenous communities in South America. Harvesting typically occurs during the rainy season, after which the nuts are transported to processing facilities. The processing sequence includes several key stages: initial sorting and grading, drying, shell removal, and size classification.

The first stage—manual or visual sorting—is critical for the removal of nuts that are mold-contaminated or discolored. Following this, the nuts are grouped based on size. After undergoing controlled drying and cooling, the final product is sealed using heat and vacuum packaging techniques to ensure quality preservation and extend shelf life [32]. Brazil nut kernels are of significant interest in food science research due to their rich content of proteins, lipids, and minerals, particularly selenium, which exhibits strong antioxidant properties. As a result, the food industry has increasingly focused on extracting oil from these nuts using hydraulic pressing methods. However, several by-products generated during this process—such as press cake, defatted meal, oilcake flour, nut shell powder, and skin residues—remain largely underutilized. These by-products are typically rich in plant proteins, dietary fiber, essential amino acids, polyphenols, and residual lipids, making them promising candidates for various value-added applications. Potential uses include incorporation into functional foods, protein-enriched flours, dietary fiber supplements, and nutraceutical formulations, as well as animal feed, organic fertilizers, and biodegradable packaging materials. Comprehensive characterization and valorization of these by-products could support sustainable resource utilization and promote circular economy practices within the nut processing industry.

The global recognition of Brazil nuts stems from their high caloric density and substantial nutritional value, prompting numerous studies aimed at isolating and characterizing their key functional and nutritional components. Among these, the lipid fraction is of particular industrial interest due to its economic viability and high yield potential. Brazil nuts are regarded as promising candidates for future commercial applications, offering favorable cost-benefit ratios and significant potential for advancement in experimental research and product development [38].

Nutritional Composition of Brazil Nuts

Macro-Nutrients

The macronutrient composition of Brazil nuts includes approximately 3.5% water, 66.4% total lipids, 14.3% protein, and 12.3% carbohydrates. In terms of lipid composition, Brazil nuts (Bertholletia excelsa) contain approximately 66.4% total lipids by dry weight, making them one of the richest natural sources of dietary fat among tree nuts. The fatty acid profile of these lipids is predominantly composed of unsaturated fatty acids, including approximately 25% monounsaturated fatty acids (MUFAs) and 21% polyunsaturated fatty acids (PUFAs), along with about 15% saturated fatty acids (SFAs) [56]. It is important to note that these percentages refer specifically to the relative composition of fatty acids within the total lipid fraction, not to the overall nut weight. The remaining portion of the lipid content may include minor fatty acids and non-triglyceride lipid components such as phytosterols, phospholipids, and fat-soluble vitamins (e.g., vitamin E). Nuts, in general, are among the most concentrated dietary sources of unsaturated fatty acids, and Brazil nuts are particularly valued for their favorable ratio of MUFAs and PUFAs, which are known to confer cardioprotective and anti-inflammatory health benefits.

Among tree nuts, Brazil nuts exhibit one of the highest saturated fat contents—second only to coconuts—and surpass macadamia nuts in this regard. They also contain a notable proportion of omega-3 fatty acids, primarily α-linolenic acid, which constitutes approximately 7% of total fat content. The predominant lipid in Brazil nuts is monounsaturated fat, mainly oleic acid, followed by saturated fats such as palmitic and stearic acids, and polyunsaturated fats including linoleic acid (omega-6) [25, 37].

Micro-Nutrients

Brazil nuts are recognized for their high concentrations of trace elements, which play a crucial role in human nutrition. Elements such as chromium (Cr), copper (Cu), and iron (Fe) function as essential cofactors in numerous metabolic and physiological processes. Typically, the elemental concentration in Brazil nuts follows the general descending order: magnesium (Mg) > calcium (Ca) > iron (Fe) > copper (Cu) > chromium (Cr) > arsenic (As) > selenium (Se). Notably, Brazil nuts are one of the richest dietary sources of selenium; however, their selenium content varies significantly due to differences in soil selenium levels, tree uptake, and geographic origin—ranging from very high in selenium-rich soils (e.g., eastern Amazon) to much lower in deficient regions. This variability has important implications for dietary recommendations, as both deficiency and excess intake may pose health risks [30].[30].Among various tree nuts, Brazil nuts demonstrate significantly higher selenium concentrations compared to cashew nuts, walnuts, and pecans[21].Brazil nuts are regarded as one of the most concentrated dietary sources of selenium, supplying approximately 160% of the United States Recommended Dietary Allowance (US RDA) for this essential trace element per serving [50].Brazil nuts are rich in sulfur-containing amino acids, which can enhance the absorption of selenium and other essential minerals [40]. Nuts contain a substantial quantity of bound phenolic compounds, measured at approximately (123.1 ± 18.4) mg per 100 grams [50]. Brazil nuts contain appreciable levels of tocopherols, with concentrations of α-tocopherol, β-tocopherol, and δ-tocopherol reported as (5.73 ± 1.54) mg, (7.87 ± 2.15) mg, and (0.77 ± 0.66) mg per 100 grams, respectively (Maguire et al., 2004[38]). Phytosterols such as β-sitosterol, stigmasterol, and campesterol constitute approximately 95% of the total phytosterol content considered beneficial in the human diet. Brazil nuts are a notable source of these compounds, containing significant amounts of campesterol (26.9 ± 4.4 µg/g oil), β-sitosterol (1325.4 ± 68.1 µg/g oil), and stigmasterol (577.5 ± 34.3 µg/g oil). Additionally, Brazil nuts have been reported to possess the highest levels of squalene, with a concentration of (1377.8 ± 8.4) µg/g oil [50].

Health Benefits of Brazil Nuts

Scientific studies have demonstrated that the regular consumption of Brazil nuts offers various health benefits, largely attributed to their rich content of both macro- and micronutrients, which serve as sources of functional and nutritional compounds. In this context, the substantial presence of amino acids, proteins, and selenium facilitates the formation of affinity interactions among these components, resulting in the development of an organic complex with enhanced bioavailability. Consumption of Brazil nuts significantly increases plasma selenium levels; however, this has minimal direct effects on HDL functionality, lipid profiles, or apolipoproteins in humans [41]. Nonetheless, the nuts' rich content of unsaturated fatty acids, antioxidants, and fiber may support cardiovascular health through alternative mechanisms such as reducing oxidative stress and inflammation.1A single consumption of Brazil nuts by healthy individuals was associated with a sustained reduction in inflammatory markers [23]. Selenium, a trace mineral abundantly present in Brazil nuts, plays a critical role in maintaining human health. It is essential for optimal thyroid gland function and the proper operation of the immune system. Selenium serves as a key component of antioxidant enzymes, contributing to the body's defense against oxidative stress. Due to its potent natural anti-carcinogenic properties, selenium has garnered significant research interest. Experimental studies in animal models have demonstrated that elevated selenium levels may exert protective effects against cancer development, potentially through mechanisms involving its role in antioxidant defense via selenoproteins like glutathione peroxidase, as well as in DNA repair and apoptosis regulation [9].

Various studies investigating the anti-proliferative effects of different nut extracts on cell cultures have demonstrated dose-dependent inhibition and cytotoxicity against Caco-2 human colon carcinoma and HepG2 human liver carcinoma cell lines. Nuts exhibited a more pronounced inhibitory effect on the proliferation of Caco-2 cells compared to HepG2 cells. The total phenolic content present in Brazil nuts is thought to contribute partially to the observed reduction in cancer cell proliferation, indicating that the antiproliferative effects may be attributed to specific phenolic compounds or groups of phenolics within the nut extracts [50].

Amla

Amla (Emblica officinalis Gaertn.), commonly referred to as Indian gooseberry, is a well-known medicinal plant belonging to the family Euphorbiaceae and is extensively utilized in Ayurvedic medicine due to its broad spectrum of therapeutic properties.. It is well-regarded for its rich bioactive compound profileand is a source of various bioactive constituents. The fruit contains a diverse range of phytochemicals, including tannins, amino acids, mucic acid, flavone glycosides, alkaloids, sesquiterpenoids, phenolic glycosides, flavonol glycosides, phenolic acids, carbohydrates, and norsesquiterpenoids. These compounds contribute to its pharmacological efficacy and nutritional value [46]. Emblica officinalis is indigenous to the Indian subcontinent and is predominantly distributed across tropical and subtropical regions. Its natural habitat extends throughout South and Southeast Asia, including countries such as Sri Lanka, Pakistan, Uzbekistan, Malaysia, and China [33]. All parts of Emblica officinalis are employed in the prevention and treatment of various ailments; however, the fruit, characterized by its globular shape, yellowish-green coloration, and smooth, fleshy texture, holds particular importance in traditional and folk medicine. In addition to its medicinal applications, the fruit is widely used in culinary practices, including the

Superfood	Key Components	Health Benefits
Brazil Nuts	Selenium, Phenolic compounds	Antioxidant activity, thyroid support, anti-cancer

Blueberries	Anthocyanins, Vitamin C	Anti-inflammatory, cognitive function, heart health
Kale	Vitamins A, C, K; Fiber	Bone health, immune support, detoxification
Quinoa	Complete protein, Fiber, Magnesium	Muscle repair, digestion, cardiovascular health
Salmon	Omega-3 fatty acids, Vitamin D	Heart health, brain function, anti-inflammatory
Turmeric	Curcumin	Anti-inflammatory, antioxidant, joint health
Chia Seeds	Omega-3 fatty acids, Fiber	Digestive health, cardiovascular support
Spinach	Iron, Folate, Vitamin K	Blood health, energy metabolism, bone health

Table 1-Superfoods: Components and Health Benefits *Source-World Health Organization. https://www.who.int/)*

Superfood	Nutrient/Compound	Health Benefits	Reference
Moringa	Vitamin C, calcium, polyphenols	Antioxidant, anti-inflammatory, supports immunity and bone health	[24]
Chia Seeds	Omega-3, fiber, antioxidants	Supports heart health, digestion, and blood sugar control	[44]
Quinoa	Complete protein, fiber, iron	Supports muscle repair, digestion, and anemia prevention	[36]
Spirulina	Protein, B12, chlorophyll	Enhances energy, detoxification, and immune response	[7]
Blueberries	Anthocyanins, vitamin C, fiber	Boosts brain function, reduces oxidative stress, supports heart health	[20]
Turmeric	Curcumin	Anti-inflammatory, antioxidant, may reduce risk of chronic diseases	[1]
Flaxseeds	Lignans, omega-3, fiber	Supports hormonal balance, heart health, and digestive health	[8]
Goji Berries	Polysaccharides, vitamin A, antioxidants	Supports eye health, immune system, and healthy skin	[3]
Matcha Green Tea	Catechins, especially EGCG	Enhances metabolism, concentration, and protects against cell damage	[14]

Table 2 Superfood and its bioactive components

preparation of chutneys, pickles, and vegetable dishes. It is also traditionally processed into murabba, a sweet fruit preserve made by soaking ripe amla fruits in concentrated sugar syrup—a method that may contribute to the preservation and stability of key bioactive compounds, such as ascorbic acid and polyphenols, during storage and processing [57]. Furthermore, ripe amla fruits are used to produce fresh juice, which is often marketed as a concentrate for the convenient preparation of diluted beverages [4]. Amla fruit juice contains a significantly high concentration of vitamin C, measuring approximately (478.56) mg per 100 mL. This level is notably higher compared to the vitamin C content found in several other fruits, including apple, pomegranate, lime, Pusa Navrang grape, and Perlette grape.

Nutritional Composition of Amla

Macro-Nutrients

The macronutrient composition of Emblica officinalis primarily includes carbohydrates (82.91 g/100 g), protein (6.04 g/100 g), dietary fiber (2.78 g/100 g), and fat (0.51 g/100 g). The amino acid profile of the fruit reveals the presence of several key amino acids, with glutamic acid (29.6%) being the most abundant, followed by proline (14.6%), aspartic acid (8.1%), alanine (5.4%), and lysine (5.3%). In the dried pulp portion of the fruit—separated from the seed—additional constituents have been identified, including gallic acid (1.32%), albumin (13.08%), tannins (13.75%), gum (3.83%), moisture (17.08%), crude cellulose (4.12%), and various minerals [16].

Micro-Nutrients

Emblica officinalis is recognized as a rich source of bioactive constituents, particularly alkaloids, tannins, and phenolic compounds. Its fruit juice contains an exceptionally high concentration of vitamin C, approximately (478.56) mg per 100 mL. When incorporated into other fruit-based products, amla significantly enhances their bioactive compound profile, particularly by increasing their vitamin C content [19]. The phytochemical analysis of Emblica officinalis fruit juice has revealed the presence of several bioactive compounds. Per 100 mL of juice, the concentrations of key phytoconstituents were reported as follows: chlorogenic acid (17.43 mg), gallic acid (37.95 mg), quercetin (2.01 mg), and ellagic acid (71.20 mg). These compounds contribute to the fruit's antioxidant and therapeutic properties [6].A variety of phytochemical constituents have been isolated and identified in Emblica officinalis. These include ellagic acid, gallic acid, 3,6-di-O-galloyl-D-glucose, 1-O-galloyl-β-D-glucose, 3-ethylgallic acid, quercetin, chebulagic acid, 1,6-di-O-galloyl-β-D-glucose, corilagin, chebulinic acid, and isostrictiniin. These compounds are known for their significant pharmacological and antioxidant activities, contributing to the therapeutic potential of the fruit [49].The flavonoid profile of Emblica officinalis includes compounds such as kaempferol 3-O-α-L-(6'-O-methyl)-rhamnopyranoside, quercetin, and kaempferol 3-O-α-L-(6'-O-ethyl)-rhamnopyranoside. These flavonoids contribute to the fruit's antioxidant capacity and are associated with various health-promoting properties [15].The mineral composition of Emblica officinalis fruit per 100 g fresh weight includes phosphorus (159 mg/100 g), calcium (129 mg/100 g), magnesium (46 mg/100 g), iron (11 mg/100 g), potassium (2.54 mg/100 g), chromium (0.82 mg/100 g), zinc (0.23 mg/100 g), copper (0.22 mg/100 g), and nicotinic acid (0.2 mg/100 g). These minerals contribute to the fruit's nutritional and therapeutic value [46].

Health Benefits of Amla

The therapeutic significance of Emblica officinalis is attributed to its diverse pharmacological activities. The plant exhibits a broad spectrum of bioactive effects, including anti-inflammatory, antioxidant, adaptogenic, anticancer, nootropic, antidiabetic, antimicrobial, and immunomodulatory properties [29].In addition to its therapeutic efficacy against a range of diseases, Emblica officinalis has been shown to contribute to the prevention of osteoporosis, hyperlipidemia, and several other health conditions [34].The potent antioxidant activity of Emblica officinalis is attributed to the presence of compounds structurally related to ascorbic acid, including pedunculagin, emblicanin A, emblicanin B, punigluconin, and gallic acid [46].A clinical trial involving administration of Emblica officinalis powder over a 21-day period demonstrated significant reductions in fasting blood glucose and 2-hour postprandial glucose levels in diabetic subjects. Additionally, treatment with daily doses of (1, 2) or (3) g of E. officinalis powder resulted in decreased serum triglycerides (TG) and total cholesterol levels. Furthermore, an increase in high-density lipoprotein cholesterol (HDL-C) and a decrease in low-density lipoprotein cholesterol (LDL-C) were observed in both healthy and diabetic volunteers receiving (2) or (3) g of E. officinalis powder daily [2]. The antidiabetic effects of Emblica officinalis are primarily attributed to its key phytoconstituents, including corilagin, gallotannins, gallic acid, and ellagic acid. These compounds exert their therapeutic action largely through antioxidant-mediated free radical scavenging mechanisms [27].Numerous preclinical studies in animal models have demonstrated that Emblica officinalis possesses cardioprotective and anticoagulant properties, suggesting its potential utility in the prevention and management of various cardiovascular disorders. This protective effect is primarily attributed to its tannin content, specifically ellagic acid, emblicanin A and B, and corilagin [11]. Various extracts of Triphala, an Ayurvedic formulation containing a high concentration of Emblica officinalis, have been evaluated for their antimutagenic potential using the Ames test with Salmonella typhimurium strains TA100 and TA98. The assay tested against direct-acting mutagens such as sodium azide and NPD, as well as indirect-acting pro-mutagens like 2-aminofluorene, in the presence of the hepatic S9 microsomal fraction derived from phenobarbital-induced rat liver. The results indicated that E. officinalis effectively inhibited mutagenicity induced by both direct and indirect mutagens [22]. Multiple studies have investigated the hepatoprotective effects of Emblica officinalis against carbon tetrachloride (CCl_4)-induced acute liver injury. Results demonstrated that treatment with E. officinalis significantly reduced focal necrosis and inflammatory infiltration in hepatic tissue. Furthermore, histological analysis revealed restoration of normal liver architecture in treated subjects [46].Emblica officinalis exerts anticancer effects by inhibiting activator protein-1 (AP-1) and targeting the translation of viral oncogenes involved in the progression of cervical cancer. This mechanism highlights its potential therapeutic application in the treatment of human papillomavirus (HPV)-induced cervical malignancies [26].

Jackfruit

Jackfruit (Artocarpus heterophyllus Lam.), a member of the Moraceae family, is primarily cultivated in India, followed by Bangladesh and various regions of Southeast Asia. It is considered one of the most prominent evergreen tree species found in tropical climates and is extensively grown across Asia, particularly in India. A medium-sized jackfruit tree typically attains a height ranging from 28 to 80 feet. The fruit commonly develops on both the main trunk and lateral branches. On average, the fruit weighs between 3.5 and 10 kilograms, although in some cases, it can reach up to 25 kilograms. Jackfruit is regarded as a non-seasonal fruit with considerable potential to contribute to food security, particularly in regions experiencing shortages of staple food grains, and is therefore often referred to as the "poor man's food." Once the fruit reaches maturity, it must be consumed promptly to prevent the development of undesirable off-flavors. To ensure optimal quality and suitability for processing, it is generally recommended to harvest the fruit at a semi-ripe, firm stage before full ripening occurs on the tree. Post-harvest, the fruit should be stored under appropriate conditions until it softens and reaches a suitable state for consumption or processing [35]. Jackfruit seeds are recognized as a rich source of essential nutrients and are consumed either in their raw form or after undergoing various processing methods. They are commonly incorporated into a variety of culinary applications, including the preparation of traditional dishes, and their flour is frequently employed in baking formulations. The unripe jackfruit is typically utilized as a vegetable ingredient in savory preparations such as curries and salads, contributing both nutritional value and culinary versatility. In contrast, the ripe fruit is consumed in diverse forms, including raw, thermally processed (often prepared as a dessert with coconut milk), or as value-added products such as jackfruit-based confectioneries and edible fruit leathers [42]. In India, jackfruit seeds are traditionally consumed as a dessert, commonly prepared by boiling them in sugar syrup. Jackfruit is widely recognized for its high nutritional value and ranks third in terms of annual production among fruits in South India, following banana and mango. Both the seeds and pulp of jackfruit exhibit a superior bioactive compound profile particularly to calcium, protein, thiamine, and iron content, when compared to other tropical fruits such as papaya, pineapple, mango, orange, and banana [5].

Nutritional Composition of Jackfruit

Macro-Nutrients

Jackfruit (Artocarpus heterophyllus Lam.) is recognized for its nutritional value, particularly as a source of macronutrients. The edible portion of unripe (young) jackfruit, on a per 100 g basis, comprises carbohydrates ranging from (9.4 to 11.5) g, fats between (0.1) and (0.6) g, proteins from (2.0) to (2.6) g, dietary fiber in the range of (2.6) to (3.6) g, and provides an energy value of approximately (50) to (210) kJ, with a moisture content of (76.2) to (85.2) g. In contrast, the ripe fruit exhibits higher carbohydrate levels, varying from (16.0) to (25.4) g per 100 g, while fat and protein contents are slightly lower, ranging from (0.1) to (0.4) g and (1.2) to (1.9) g, respectively. The fiber content in ripe jackfruit decreases to (1.0–1.5) g, energy content ranges from (88) to (410) kJ, and water content varies between (72.0) and (94.0) g per 100 g [13]. Jackfruit is characterized by a relatively low caloric density, providing approximately 94 kilocalories per 100 grams of edible portion [32]. Several studies have reported variability in the protein and carbohydrate composition of jackfruit seeds across different cultivars, even when cultivated under identical regional conditions [5].The protein and carbohydrate content of jackfruit seeds varies among different species, ranging from (5.3%) to (6.8%) and (37.4%) to (42.5%), respectively. Histological and chemical analyses have confirmed a substantial presence of starch within both the seeds and perianth regions of the fruit. The edible pulp of ripe jackfruit contains approximately (1.9) g of protein per 100 g. Research indicates that as the fruit matures, there is a corresponding increase in dietary fiber and starch content in the flesh. Additionally, jackfruit is recognized as a valuable source of essential amino acids, including cysteine, arginine, leucine, histidine, methionine, lysine, tryptophan, and threonine[36] .Jackfruit is recognized as a notable source of various minerals, containing calcium (31.28 mg), magnesium (36.96 mg), copper (0.38 mg), iron (3.26 mg), manganese (0.56 mg), and lead (0.20 mg) per 100 grams of the fruit [36]..Jackfruit exhibits a notably high potassium content, measuring 303 mg per 100 grams of fruit. The fruit also contains a diverse array of chemical compounds, primarily including morin, flavone pigments, cynomacurin, dihydromorin, isoartocarpin, artocarpin, cycloartocarpin, coxydihydroartocarpesin, artocarpesin, norartocarpetin, artocarpetin, artocarpanone, and cycloartinone [42]. Phytochemicals, particularly phenolic compounds, constitute a significant portion of jackfruit and play a crucial role in the development of value-added products. These compounds have potential applications in the food and nutraceutical industries aimed at promoting and sustaining human health.

Micro-Nutrients

Jackfruit is a rich source of various micronutrients, including significant levels of riboflavin, vitamin C, vitamin A, thiamine, potassium, calcium, sodium, iron, niacin, and zinc [32].).In addition to its bioactive compound profile, jackfruit is recognized as an abundant source of various bioactive compounds, including flavonoids, carotenoids, tannins, and volatile sterols [5].Phytochemicals, particularly phenolic compounds, constitute a significant portion of jackfruit and play a crucial role in the development of value-added products. These compounds have potential applications in the food and nutraceutical industries aimed at promoting and sustaining human health [18]. Jackfruit contains a total phenolic content of (0.36) mg GAE per 100 g dry weight (milligrams of gallic acid equivalent per gram of dry weight). Vitamin C is a significant constituent, present at concentrations ranging from (12) to (14) mg per 100 g of fresh fruit. Flavonoids, carotenoids, and related polyphenols, including glutathione and α-lipoic acid, represent a major category of nonenzymatic antioxidants. In addition to carotenoids, compounds such as lutein, lycopene, and beta-carotene are recognized as potent antioxidant [42].The predominant carotenoids identified in jackfruit include all-trans-lutein, all-trans-β-carotene, all-trans-neoxanthin, 9-cis-neoxanthin, and 9-cis-violaxanthin, with their respective proportions ranging from (24–44%), (24–30%,) (4–19%), (4–9%), and (4–10%) [10].

Health Benefits of Jackfruit

The primary benefit of jackfruit consumption is attributed to its high vitamin C content. Since the human body cannot synthesize sufficient quantities of vitamin C, it is essential to obtain this nutrient through dietary sources. Vitamin C contributes to the neutralization of free radicals via its antioxidant properties, supports the maintenance of healthy gingival tissues, and plays a vital role in enhancing immune system function [18]. Jackfruit is rich in phytonutrients, including saponins, lignans, and isoflavones, which contribute to a variety of health-promoting effects. The fruit demonstrates antiulcer, antiaging, antihypertensive, and anticancer activities by inhibiting cancer cell proliferation, protecting against gastric ulcers, regulating blood pressure, and preventing cellular degradation, thereby promoting youthful skin appearance. Additionally, the niacin (vitamin B3) content in jackfruit plays a crucial role in energy metabolism, hormone synthesis, and the proper functioning of the nervous system [42]. The potassium content in jackfruit has been shown to aid in the regulation of blood pressure by counteracting the effects of sodium, which is known to elevate blood pressure and adversely affect cardiovascular health. Additionally, potassium contributes to the proper functioning of nerves and muscles and helps prevent bone loss. Furthermore, vitamin B6 present in jackfruit plays a role in reducing blood homocysteine levels, thereby lowering the risk of cardiovascular diseases [12]. The presence of various micronutrients in jackfruit contributes to its potential health benefits. Iron (0.5 mg/100 g) plays a crucial role in facilitating efficient blood circulation by preventing anemia. Copper (10.45 mg/kg) is essential for thyroid gland metabolism, particularly in the synthesis and uptake of thyroid hormones. Magnesium, present at concentrations of (27 mg/100 g) in the fruit and (54 mg/100 g) in the seed, is a key nutrient involved in calcium absorption and works synergistically with calcium to strengthen bone structure and prevent bone-related disorders such as osteoporosis. Additionally, jackfruit supports regular bowel movements and helps prevent constipation due to its high dietary fiber content (3.6 g/100 g). It also protects the colonic mucous membrane by aiding in the removal of carcinogenic compounds from the large intestine [42]. Jackfruit exhibits a wide range of beneficial health effects, including anti-inflammatory, antioxidant, anticancer, and antibacterial properties. It also demonstrates potential in inhibiting melanin biosynthesis, exerting hypoglycemic activity, possessing antineoplastic effects, enhancing sexual function, and promoting wound healing [5].

Conclusion

Superfoods are predominantly plant-derived foods characterized by high nutritional density, providing substantial health benefits with relatively low caloric content. These foods are rich sources of vitamins, dietary fiber, minerals, and antioxidants. Due to their significant bioactive and nutritional properties, superfoods have the potential to contribute to the prevention and management of chronic diseases. Isolation and extraction of bioactive compounds from these foods, followed by their incorporation into various food matrices, present promising opportunities for the development of functional foods within the food processing industry. Increasing scientific evidence supporting the health-promoting effects of superfoods is expected to enhance consumer interest in these products. To substantiate the claimed benefits of superfoods, future research should prioritize well-designed human clinical trials. Additionally, regulatory agencies should establish standardized definitions for superfoods to facilitate clearer understanding and consistent communication within the scientific community and among consumers.

Authors contribution

Vishal Kumar: Conceptualization, original draft, corresponding author
Akanksha: Literature collection and drafting
Anu Kumari: Literature analysis and editing
Nimisha Tehri: Critical review and supervision

Conflict of Interest

The authors declare no conflict of interest.

References

1.Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. The molecular targets and therapeutic uses of curcumin in health and disease. 2007 Jan 1:1-75.

2.Akhtar MS, Ramzan A, Ali A, Ahmad M. Effect of Amla fruit (Emblica officinalis Gaertn.) on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. International journal of food sciences and nutrition. 2011 Sep 1;62(6):609-16.

3.Amagase H, Nance DM. A randomized, double-blind, placebo-controlled, clinical study of the general effects of a standardized Lycium barbarum (Goji) juice, GoChi™. The Journal of Alternative and Complementary Medicine. 2008 May 1;14(4):403-12.

4.Baliga MS, Dsouza JJ. Amla (Emblica officinalis Gaertn), a wonder berry in the treatment and prevention of cancer. European Journal of Cancer Prevention. 2011 May 1;20(3):225-39.

5.Baliga MS, Shivashankara AR, Haniadka R, Dsouza J, Bhat HP. Phytochemistry, nutritional and pharmacological properties of Artocarpus heterophyllus Lam (jackfruit): A review. Food research international. 2011 Aug 1;44(7):1800-11.

6.Bansal V, Sharma A, Ghanshyam C, Singla ML. Coupling of chromatographic analyses with pretreatment for the determination of bioactive compounds in Emblica officinalis juice. Analytical Methods. 2014;6(2):410-8.

7.Belay A, Ota Y, Miyakawa K, Shimamatsu H. Current knowledge on potential health benefits of Spirulina. Journal of applied Phycology. 1993 Apr;5(2):235-41.

8.Bloedon LT, Szapary PO. Flaxseed and cardiovascular risk. Nutrition reviews. 2004 Jan 1;62(1):18-27.

9.Combs Jr GF. Impact of selenium and cancer-prevention findings on the nutrition-health paradigm. Nutrition and cancer. 2001 May 1;40(1):6-11.

10.De Faria AF, De Rosso VV, Mercadante AZ. Carotenoid composition of jackfruit (Artocarpus heterophyllus), determined by HPLC-PDA-MS/MS. Plant foods for human nutrition. 2009 Jun;64(2):108-15.

11.D'souza J, D'souza P, Shivashankara A, Mathai R, Jimmy R, Palatty P, Ravi R, Simon P, Baliga M. Cardioprotective effects of Indian gooseberry (Emblica officinalis Gaertn) and its phytochemicals: A review. Current Nutrition & Food Science. 2014 Jun 10;10(2):141-9.

12.Fernando MR, Wickramasinghe SN, Thabrew MI, Ariyananda PL, Karunanayake EH. Effect of Artocarpus heterophyllus and Asteracanthus longifolia on glucose tolerance in normal human subjects and in maturity-onset diabetic patients. Journal of ethnopharmacology. 1991 Mar 1;31(3):277-82.

13.Goswami C, Chacrabati R. Jackfruit (Artocarpus heterophylus). InNutritional composition of fruit cultivars 2016 Jan 1 (pp. 317-335). Academic Press.

14.Graham HN. Green tea composition, consumption, and polyphenol chemistry. Preventive medicine. 1992 May 1;21(3):334-50.

15.Habib-ur-Rehman, Yasin KA, Choudhary MA, Khaliq N, Atta-ur-Rahman, Choudhary MI, Malik S. Studies on the chemical constituents of Phyllanthus emblica. Natural Product Research. 2007 Jul 20;21(9):775-81.

16.Hasan MR, Islam MN, Islam MR. Phytochemistry, pharmacological activities and traditional uses of Emblica officinalis: A review. International Current Pharmaceutical Journal. 2016 Jan 18;5(2):14-21.

17.Hefferon K. Let Thy Food Be Thy Medicine: Plants and Modern Medicine. Oxford University Press; 2012 Aug 16.

18.Jagtap UB, Panaskar SN, Bapat VA. Evaluation of antioxidant capacity and phenol content in jackfruit (Artocarpus heterophyllus Lam.) fruit pulp. Plant foods for human nutrition. 2010 Jun;65(2):99-104.

19.Jain SK, Khurdiya DS. Vitamin C enrichment of fruit juice based ready-to-serve beverages through blending of Indian gooseberry (Emblica officinalis Gaertn.) juice. Plant Foods for human nutrition. 2004 Apr;59(2):63-6.

20.Joseph JA, Shukitt-Hale B, Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. The American journal of clinical nutrition. 2005 Jan 1;81(1):313S-6S.

21.Kannamkumarath SS, Wrobel K, Wrobel K, Vonderheide A, Caruso JA. HPLC–ICP–MS determination of selenium distribution and speciation in different types of nut. Analytical and bioanalytical chemistry. 2002 Jul;373(6):454-60.

22.Kaur S, Michael H, Arora S, Härkönen PL, Kumar S. The in vitro cytotoxic and apoptotic activity of Triphala—an Indian herbal drug. Journal of ethnopharmacology. 2005 Feb 10;97(1):15-20.

23.Kluczkovski AM, Martins M, Mundim SM, Simões RH, Nascimento KS, Marinho HA, Junior AK. Properties of Brazil nuts: A review. African Journal of Biotechnology. 2015 Apr 13;14(8):642-8.

24.Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Moringa oleifera seeds and oil: Characteristics and uses for human health. International journal of molecular sciences. 2016 Dec 20;17(12):2141.

25.Maguire LS, O'sullivan SM, Galvin K, O'connor TP, O'brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. International journal of food sciences and nutrition. 2004 May 1;55(3):171-8.

26.Mahata S, Pandey A, Shukla S, Tyagi A, Husain SA, Das BC, Bharti AC. Anticancer activity of Phyllanthus emblica Linn.(Indian gooseberry): inhibition of transcription factor AP-1 and HPV gene expression in cervical cancer cells. Nutrition and cancer. 2013 Jan 1;65(sup1):88-97.

27.Mehta S, Singh RK, Jaiswal D, Rai PK, Watal G. Anti-diabetic activity of Emblica officinalis in animal models. Pharmaceutical Biology. 2009 Nov 1;47(11):1050-5.

28.Meyerding SG, Kürzdörfer A, Gassler B. Consumer preferences for superfood ingredients—The case of bread in Germany. Sustainability. 2018 Dec 7;10(12):4667.

29.Mirunalini S, Krishnaveni M. Therapeutic potential of Phyllanthus emblica (amla): the ayurvedic wonder. Journal of basic and clinical physiology and pharmacology. 2010 Feb;21(1):93-105.

30.Moodley R, Kindness A, Jonnalagadda SB. Elemental composition and chemical characteristics of five edible nuts (almond, Brazil, pecan, macadamia and walnut) consumed in Southern Africa. Journal of Environmental Science and Health, Part B. 2007 Jun 11;42(5):585-91.

31.Mozaffarian, D. (2016). Dietary and policy priorities for cardiovascular disease, diabetes, and obesity: a comprehensive review. Circulation, 133(2): 187–225.

32.Mukprasirt A, Sajjaanantakul K. Physico-chemical properties of flour and starch from jackfruit seeds (Artocarpus heterophyllus Lam.) compared with modified starches. International Journal of Food Science and Technology. 2004 Mar;39(3):271-6.

33.Pacheco A, Scussel V. Aflatoxins evaluation on in-shell and shelled dry Brazil nuts for export analysed by LC-MS/MS-2006 and 2007 harvests. World Mycotoxin Journal. 2009 Aug 1;2(3):295-304.

34.Pardeshi S, Dhodapkar R, Kumar A. Molecularly imprinted microspheres and nanoparticles prepared using precipitation polymerisation method for selective extraction of gallic acid from Emblica officinalis. Food chemistry. 2014 Mar 1;146:385-93.

35.Patel SS, Goyal RK. Emblica officinalis Geart.: a comprehensive review on phytochemistry, pharmacology and ethnomedicinal uses.

36.Ranasinghe RA, Maduwanthi SD, Marapana RA. Nutritional and health benefits of jackfruit (Artocarpus heterophyllus Lam.): a review. International journal of food science. 2019;2019(1):4327183.

37.Ryan E, Galvin K, O'connor TP, Maguire AR, O'brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of brazil, pecan, pine, pistachio and cashew nuts. International journal of food sciences and nutrition. 2006 Jan 1;57(3-4):219-28.

38.Santos OV, Corrêa NC, Soares FA, Gioielli LA, Costa CE, Lannes SC. Chemical evaluation and thermal behavior of Brazil nut oil obtained by different extraction processes. Food Research International. 2012 Jul 1;47(2):253-8.

39.Siró I, Kápolna E, Kápolna B, Lugasi A. Functional food. Product development, marketing and consumer acceptance—A review. Appetite. 2008 Nov 1;51(3):456-67.

40.SM SUN S, Altenbach SB, Leung FW. Properties, biosynthesis and processing of a sulfur-rich protein in Brazil nut (Bertholletia excelsa HBK). European Journal of Biochemistry. 1987 Feb;162(3):477-83.

41.Strunz CC, Oliveira TV, Vinagre JC, Lima A, Cozzolino S, Maranhão RC. Brazil nut ingestion increased plasma selenium but had minimal effects on lipids, apolipoproteins, and high-density lipoprotein function in human subjects. Nutrition Research. 2008 Mar 1;28(3):151-5.

42.Swami SB, Thakor NJ, Haldankar PM, Kalse SB. Jackfruit and its many functional components as related to human health: a review. Comprehensive Reviews in Food Science and Food Safety. 2012 Nov;11(6):565-76.

43.Tacer-Caba Z. The concept of superfoods in diet. InThe role of alternative and innovative food ingredients and products in consumer wellness 2019 Jan 1 (pp. 73-101). Academic Press.

44.Ullah R, Nadeem M, Khalique A, Imran M, Mehmood S, Javid A, Hussain J. Nutritional and therapeutic perspectives of Chia (Salvia hispanica L.): a review. Journal of food science and technology. 2016 Apr;53(4):1750-8.

45.Van den Driessche JJ, Plat J, Mensink RP. Effects of superfoods on risk factors of metabolic syndrome: a systematic review of human intervention trials. Food & function. 2018;9(4):1944-66.

46.Variya BC, Bakrania AK, Patel SS. Emblica officinalis (Amla): A review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms. Pharmacol Res. 2016;111:180-200..

47.Weitkamp E, Eidsvaag T. Agenda building in media coverage of food research: Superfoods coverage in UK national newspapers. Journalism Practice. 2014;8(6):871-86.

48.Wolfe D. Superfoods: the food and medicine of the future. Berkeley (CA): North Atlantic Books; 2009

49.Yang F, Yaseen A, Chen B, Li F, Wang L, Hu W, et al. Chemical constituents from the fruits of Phyllanthus emblica L. Biochem Syst Ecol. 2020;92:104122

50.Yang J. Brazil nuts and associated health benefits: A review. LWT Food Sci Technol. 2009;42(10):1573-80

51.Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G., & Faria, J. A. F. (2020). Functional foods and nondairy probiotic food development: Trends, concepts, and products. Comprehensive Reviews in Food Science and Food Safety, 19(3): 2021–2040.

52.Jaiswal P, Kumar P, Singh VK, Singh DK. A review on phytochemical and pharmacological properties of Amla (Emblica officinalis) and its traditional uses. Asian J Pharm Clin Res. 2016;9(6):1–8.

53.Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G., & Faria, J. A. F. (2020). Functional foods and nondairy probiotic food development: Trends, concepts, and products. Comprehensive Reviews in Food Science and Food Safety, 19(3): 2021–2040

Vita Scientia

Review Article

Read Online

Polycystic ovarian syndrome (PCOS) and crosstalk between PCOS and Psychological disorders

Vishal Bodke[1], Akash S Mali[2]*
Department of Pharmaceutics, Konkan Gyanpeeth Rahul Dharkar college of pharmacy and Research institute, Karjat, Maharashtra, India.
Faculty of Science, Charles University, Prague, Czech Republic.
*Correspondence: akashmit97@gmail.com

Summary

Polycystic Ovarian Syndrome (PCOS) is a hormonal disorder marked by elevated androgen levels. Common symptoms include ovarian cysts, irregular menstrual cycles, hirsutism, acne, and obesity. Affecting approximately 8–13% of women of reproductive age, up to 70% of PCOS cases remain undiagnosed globally. It is the leading cause of anovulation and a major contributor to female infertility. Women with PCOS are also at increased risk for diabetes, hypertension, coronary artery disease, anxiety, depression, and endometrial cancer. Infertility, often associated with PCOS, presents a significant emotional and psychological challenge for couples. Moreover, the use of Assisted Reproductive Techniques (ART) can exacerbate mental health issues, particularly anxiety and depression. This review offers a comprehensive exploration of PCOS, with a specific focus on its intersection with psychological disorders.

Keywords

PCOS, androgens, infertility, anxiety, contraception, depression.

Introduction

Polycystic Ovary Syndrome (PCOS) is one of the most prevalent endocrine disorders among women of reproductive age and constitutes a major public health concern [1]. An estimated 8–13% of women in this age group are affected, with approximately 70% of cases remaining undiagnosed [2]. Prevalence is notably higher in certain ethnic groups, who often experience more severe metabolic complications [3]. The disorder's physical and psychological consequences—especially regarding obesity, infertility, and body image—frequently contribute to mental health challenges and social stigma [4]. PCOS significantly impairs health-related quality of life (HRQoL) and mental well-being, especially among young women. It often persists as a chronic condition beyond adolescence [5]. Contributing factors to psychological distress include noticeable physical changes, obesity, and menstrual irregularities. Although frequently underestimated, the psychological burden of PCOS can be severe, heightening the risk of anxiety, depression, and suicidal ideation [6,7]. Geographic and cultural differences can further influence the psychosocial impact of PCOS. Clinically, it is characterized by oligo- or anovulation and hyperandrogenism, which can lead to infertility and metabolic complications [8]. PCOS increases the risk of reproductive and metabolic disorders such as endometrial cancer, gestational complications, and psychological disturbances. Some symptoms can be mitigated through lifestyle changes, medication, and fertility treatments [9]. Although its exact etiology remains unclear, genetic predisposition and comorbid conditions like type 2 diabetes are significant risk factors [10]. The disorder affects 8–13% of women, with a disproportionately higher prevalence in certain ethnicities, where metabolic complications are also more common [11]. Both the biological and psychological effects of PCOS—especially those linked to body weight, self-image, and fertility—can provoke mental health issues and reinforce stigma [12]. While there is no definitive cure, symptom management is possible. Women experiencing irregular periods, infertility, acne, or hirsutism are advised to consult healthcare providers. Lifestyle interventions, including a nutritious diet and regular exercise, can assist with weight management and reduce the risk of developing type 2 diabetes [13]. Hormonal contraceptives may help regulate menstrual cycles and manage symptoms, while other medications can target acne and excessive hair growth [14]. PCOS may present anatomically as polycystic ovaries or biochemically as hyperandrogenemia. Elevated androgen levels can disrupt follicular development, leading to microcysts, anovulation, and menstrual irregularities [15].

PATHOPHYSIOLOGY IN PCOS

Hormonal Imbalance

PCOS is marked by elevated androgen levels, such as testosterone, which disrupt the normal process of folliculogenesis—the maturation of ovarian follicles. These hormonal imbalances underlie many of the pathological symptoms observed in women with PCOS [16]. Abnormal hormonal profiles include imbalances in insulin, growth hormone (GH), ghrelin, LEAP-2, gonadotropin-releasing hormone (GnRH), and the luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio, along with elevated androgens and altered oestrogen levels [17]. Such disturbances are closely linked to a range of metabolic dysfunctions, including insulin resistance, type 2 diabetes, obesity, infertility, and irregular menstruation [18]. Specifically, increased insulin levels, reduced GH, elevated ghrelin, and leptin resistance are associated with a greater risk of diabetes and obesity in women with PCOS. Reproductive issues stem from altered levels of GH, LEAP-2, high LH, increased LH/FSH ratio, elevated androgens, and low oestrogen levels [19].

Insulin Resistance

A large proportion of women with PCOS exhibit insulin resistance, meaning their cells do not respond effectively to insulin. This results in compensatory hyperinsulinemia and elevated blood glucose levels [20]. Insulin, a central hormone in glucose and lipid metabolism, also acts as a mitogenic agent. It modulates activity in several organs along the hypothalamic-pituitary-ovarian (HPO) axis [21]. In steroidogenic tissues such as the ovaries and adrenal cortex, insulin promotes steroid hormone production. Hyperinsulinemia mimics LH activity and enhances GnRH secretion, driving excess androgen production [22]. Furthermore, insulin resistance lowers levels of sex hormone-binding globulin (SHBG), a protein that regulates circulating testosterone. Improving insulin sensitivity through therapeutic interventions can decrease androgen levels and significantly improve PCOS symptoms [23].

Hyperandrogenism

Hyperandrogenism is a hallmark feature of PCOS and manifests in symptoms such as hirsutism, acne, and androgenic alopecia. Around 75–90% of PCOS patients with oligomenorrhea display elevated androgen levels, which often worsen over time [24]. Androgens are overproduced primarily by the ovaries and, to a lesser extent, the adrenal glands. Elevated levels of free (unbound) testosterone serve as a reliable marker of hyperandrogenism. Dysfunction in ovarian or adrenal steroidogenesis pathways contributes to this overproduction [25]. Excess androgens impair follicular maturation, resulting in the development of multiple small ovarian cysts and menstrual irregularities [26]. During the early gonadotropin-dependent stages of follicular development, high androgen levels lead to excessive recruitment of primordial follicles and an increase in antral follicles. The hypothalamus secretes GnRH, which in turn stimulates the anterior pituitary to release gonadotropins [27]. LH binds to receptors in ovarian theca cells, triggering androgen production, while FSH promotes oestrogen synthesis in granulosa cells by converting androgens into oestrogens necessary for follicle growth. However, in PCOS, neuroendocrine abnormalities disrupt this balance. Increased GnRH secretion disproportionately elevates LH levels over FSH, causing an elevated LH:FSH ratio—commonly observed in PCOS. Genetic variations, particularly in the CYP family of genes, affect steroidogenesis and are closely linked to hyperandrogenism [28].

Genetic and Epigenetic Factors

PCOS has a multifactorial origin involving both genetic and environmental influences. Epigenetic mechanisms—modifications in gene expression without altering the DNA sequence—also contribute to its pathogenesis. While some familial studies suggest an autosomal dominant inheritance pattern, the prevailing evidence supports a multifactorial aetiology [29]. The genetic architecture of PCOS is complex and non-Mendelian, involving multiple loci. Genome-wide association studies (GWAS) and next-generation sequencing have identified several genes associated with PCOS. Approximately 241 genetic variants are believed to contribute to its development [30,31]. These include single nucleotide polymorphisms (SNPs) that affect gene expression and function, particularly in genes regulating steroidogenesis, ovarian theca cell activity, and hypothalamic pituitary signalling. Key genes implicated in PCOS include INSR, IRS1, GHRL, LDLR, MC4R, ADIPOQ, UCP1, UCP2, UCP3, FTO, PCSK9, FBN3, NEIL2, FDFT1, CYP11, CYP17, CYP21, HSD17, STAR, POR, AKR1C3, AMH, AMHR2, INHBA, AR, SHBG, LHR, FSHR, FSHβ, SRD5A, GATA4, THADA, YAP1, ERBB2, DENND1A, and FEM1B, among others [32]. Understanding the genetic and epigenetic underpinnings of PCOS may pave the way for more personalized and effective interventions.

Obesity in PCOS

Obesity is both a clinical feature and a biochemical aggravator of PCOS, particularly among genetically predisposed individuals. Between 38% to 88% of women with PCOS are overweight or obese [33]. Data from the Northern Finland Birth Cohort (NFBC) 1966 demonstrate a strong correlation between body mass index (BMI) and PCOS features across all life stages [34]. Even modest weight loss (approximately 5%) can lead to significant improvements in reproductive health, androgen levels, and metabolic parameters in women with PCOS. Insulin resistance is also highly prevalent, affecting 50–90% of individuals with the condition [35]. Despite its frequency, the precise mechanisms underlying insulin resistance in PCOS remain incompletely understood [36].

Impact of Obesity on the Immune System

Obesity is characterized as a systemic metabolic disorder that disrupts metabolic homeostasis and provokes chronic low-grade inflammation. A growing body of research has demonstrated a significant correlation between immune system dysfunction and obesity [37] (Fig. 1). In obese individuals, adipose tissue becomes infiltrated with immune cells, including neutrophils, M1 macrophages, and T cells. Adipose tissue macrophages (ATMs) play a central role in mediating systemic inflammation. The elevated secretion of monocyte chemoattractant protein-1 (MCP-1/CCL2) and leukotriene B4 (LTB4) by adipocytes promotes macrophage recruitment and infiltration [38]. Macrophages and adipocytes secrete pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, which activate the nuclear factor kappa B (NF-κB) signalling pathway, further amplifying inflammation. Leptin, an adipokine

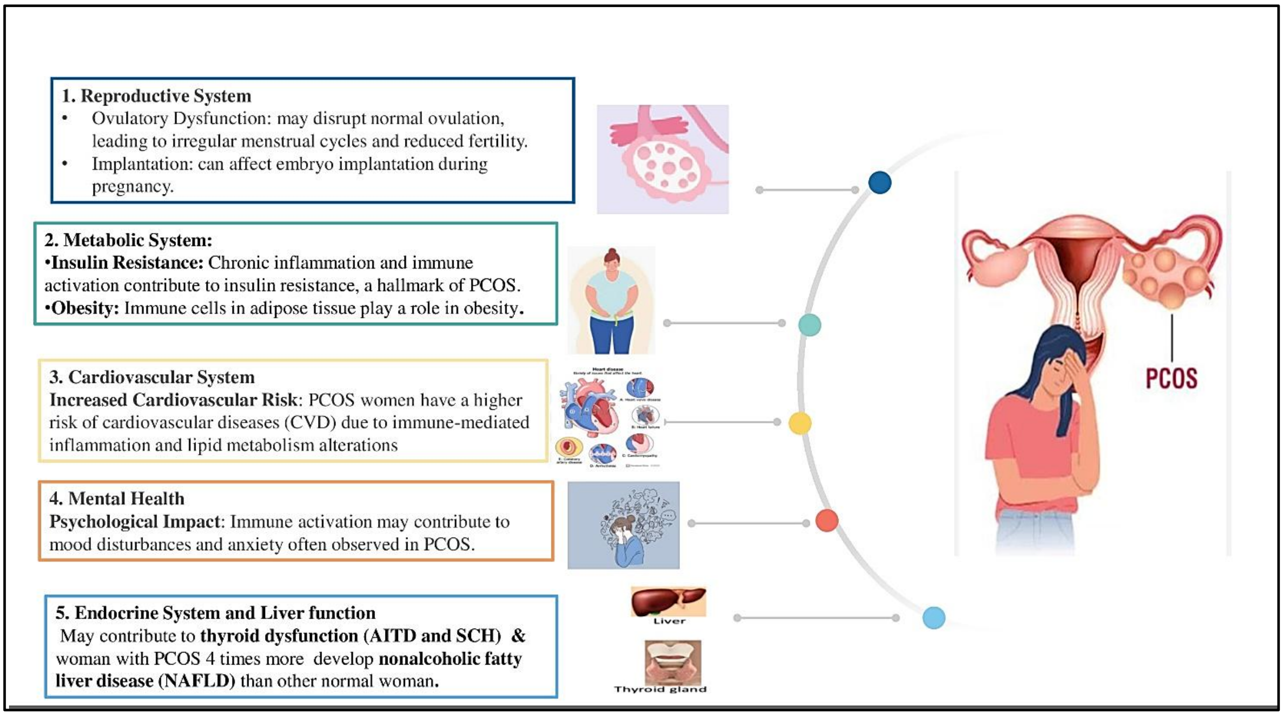


Figure 1. Organ system dysfunction associated with immunological dysregulation in women with PCOS

secreted by adipose tissue, also contributes to immune dysfunction in obesity [39]. Additionally, CD4+ T cells within adipose tissue produce interferon-gamma (IFN-γ). Both macrophages and adipocytes in obese tissue often overexpress MHC class II and costimulatory molecules (CD80 and CD86), functioning as antigen-presenting cells (APCs) that stimulate CD4+ T cell proliferation and differentiation into Th1 cells. These Th1 cells release excessive IFN-γ, worsening the inflammatory environment. Leptin further promotes IFN-γ secretion and MHC class II expression, exacerbating immune imbalance and chronic inflammation in obese individuals [40].

PCOS and Hypertension

Hypertension is characterized by consistently elevated blood pressure levels, impacting a significant portion of the population and leading to severe health complications. In women with Polycystic Ovary Syndrome (PCOS), the prevalence of systemic arterial hypertension (SAH) is notably higher, with studies indicating a 40% increased likelihood of SAH in this group compared to women without the condition [41]. SAH plays a crucial role in the progression of PCOS and associated cardiovascular issues. Clinical research has established a significant link between hypertension and the endocrine system, suggesting that any dysfunction within the endocrine system is likely to correlate with hypertension [42]. In cases of PCOS, endocrine abnormalities are frequently observed, contributing to the development of hypertension. Additionally, insulin resistance and metabolic disorders are prevalent among women with PCOS, further elevating the risk of hypertension [43]. Insulin resistance leads to hyperinsulinemia and an increase in luteinizing hormone (LH) production, which in turn raises androgen levels, resulting in persistently high blood pressure in these patients. Critical clinical studies indicate a high incidence of hypertension among females with PCOS. Therefore, it is essential for women with this condition to regularly monitor their blood pressure to ensure timely management and intervention [44].

Symptoms of PCOS

Symptoms of polycystic ovarian syndrome can vary widely between individuals and may evolve over time. They often emerge without a clear cause. Common manifestations include heavy, prolonged, irregular, or absent menstrual periods; infertility; acne or oily skin; excessive hair growth on the face or body; male-pattern hair loss; and weight gain, particularly abdominal obesity [45]. PCOS significantly increases the risk of type 2 diabetes, hypertension, dyslipidemia, coronary artery disease, and endometrial cancer. Psychological consequences such as anxiety, depression, and poor body image are also prevalent [44]. Symptoms like infertility, obesity, and hirsutism often carry societal stigma, impacting personal relationships, employment, and social functioning [46].

Treatment of PCOS

PCOS is a chronic condition, and available treatments primarily aim to manage symptoms and reduce the risk of long-term complications (Fig. 3). Combination oral contraceptives (COCs), containing both oestrogen and progestin, are the most prescribed medications for regulating menstrual cycles in women with PCOS [47]. COCs also help alleviate hirsutism and acne by suppressing ovarian androgen production. Alternatives such as contraceptive patches and vaginal rings are also available. Progesterone-releasing intrauterine devices (IUDs) are another option for reducing uterine bleeding and protecting against endometrial cancer, although they are less effective for acne and hirsutism [48]. Medications Beyond permanent methods like surgical sterilization, hormonal contraceptives remain the most effective form of birth control. COCs inhibit ovulation by suppressing gonadotropins (FSH and LH), thereby preventing follicular maturation and the mid-cycle LH surge required for ovulation [49–51]. Even in cases where ovulation occurs, these contraceptives may prevent implantation or impair gamete transport. Progesterone also thickens cervical mucus, reducing sperm mobility. Non-contraceptive benefits of COCs include cycle regulation, reduced menstrual flow, and decreased risks of endometriosis and certain cancers [52]. However, COCs are contraindicated in women with a history of thromboembolism, cerebrovascular disease, migraines, liver dysfunction, or cardiovascular issues. Smoking further elevates the risk of adverse cardiovascular events [53]. Forms of oral contraception include

Combination pill: Taken daily for 21 days, followed by 7 days of inactive pills [54]. Extended-cycle pill: Hormonal pills for 12 weeks, followed by 1 week of inactive pills, resulting in quarterly menstruation [55].

Minipill: Progestin-only pills taken daily without a hormone-free interval [56].

Non-Oral Hormonal Contraception Contraceptive patch (Ortho Evra®): Applied weekly to various body sites for three consecutive weeks [57]. Vaginal ring (NuvaRing®): Inserted for three weeks, followed by a one-week break [58]. Male condom: A barrier method worn over the penis before intercourse and disposed of afterward [58].

Emergency Contraception (EC) EC, also called the "morning-after pill," contains high doses of oestrogen and/or progestin to inhibit FSH and LH release, preventing ovulation and implantation. It should be taken within 72 hours of unprotected intercourse, with a second dose administered 12 hours later [59,60].

Hormonal Injections Depot medroxyprogesterone acetate (Depo-Provera®) is

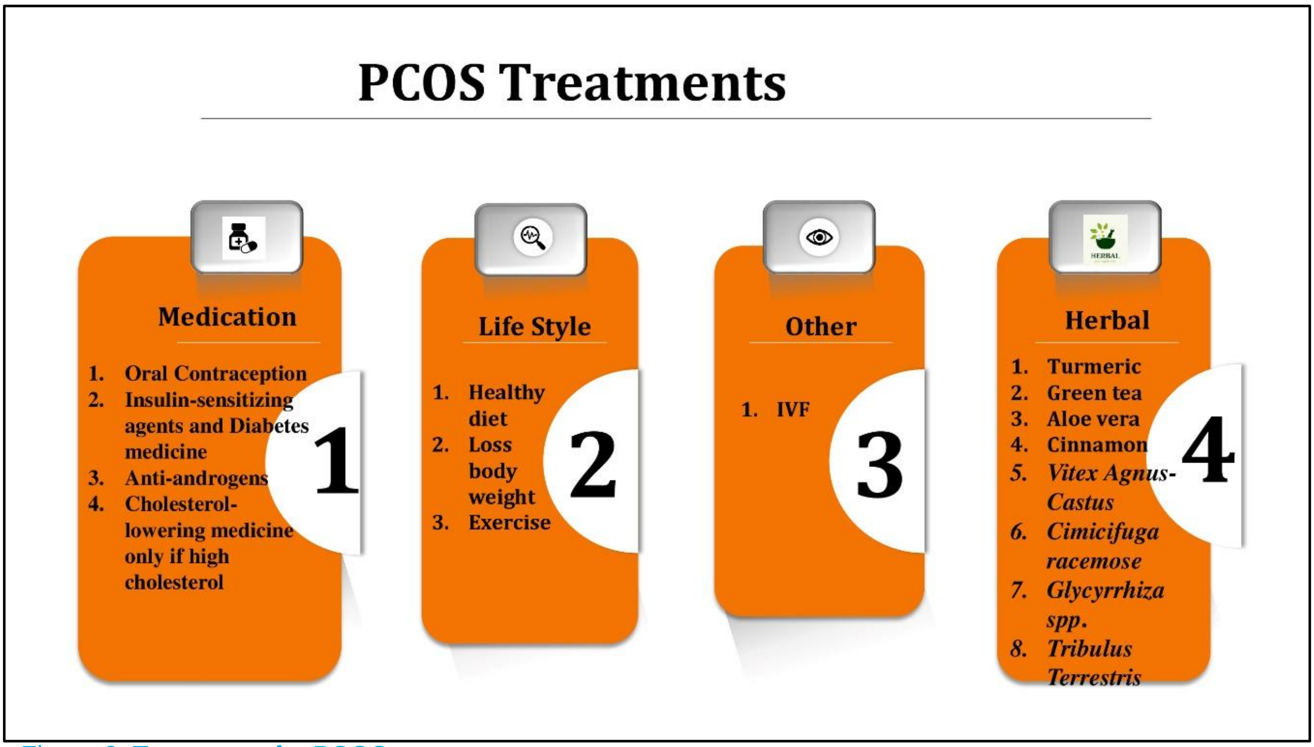


Figure 2. Treatments for PCOS

administered every three months to inhibit ovulation.

Insulin-Sensitizing Agents and Diabetes Medications given the role of insulin resistance in PCOS pathogenesis, treatments like metformin and thiazolidinediones (TZDs) help reduce insulin resistance, restore ovulatory function, and lower androgen levels. Metformin improves glucose uptake, decreases hepatic glucose production, and enhances insulin sensitivity in peripheral tissues. It has also been shown to induce weight loss and normalize menstrual cycles without significant lifestyle modifications [61,62].

Lifestyle Modifications

Lifestyle interventions such as improved diet and regular physical activity are first-line treatments for addressing PCOS-related metabolic and reproductive complications [63]. These changes enhance insulin sensitivity, promote ovulation, reduce androgen levels, regulate menstrual cycles, and improve mental health. Even a modest 5–10% reduction in body weight can yield substantial improvements in reproductive function, insulin resistance, and depressive symptoms [64].

Other Treatment Options Hair Removal: Techniques include laser therapy, electrolysis, shaving, and depilatory creams. Assisted Reproductive Technologies (ART): Procedures like in vitro fertilization (IVF) are available for women struggling with infertility [65].

CROSSTALK BETWEEN PCOS AND ANXIETY

Neuroendocrinology offers insights into why anxiety is more prevalent in women with PCOS. The chronic hyperandrogenic, oligo-ovulatory state disrupts the hormonal progression of the menstrual cycle. Persistent anovulation results in deficient progesterone levels and unopposed oestrogen exposure, creating a neurohormonal environment associated with mood instability [66]. Up to 40% of women with PCOS experience anxiety or depression, and some studies report that they are up to six times more likely to suffer from moderate to severe anxiety than the general female population [67]. Meta-analyses show increased odds of depressive symptoms (OR 3.78; 95% CI 3.03–4.72) and anxiety symptoms (OR 5.62; 95% CI 3.22–9.80) in women with PCOS. Factors such as elevated testosterone, insulin resistance, and obesity contribute to these mental health challenges [68]. Oestrogen tends to have excitatory and potentially anxiety-inducing effects on the brain, whereas progesterone exerts a calming, anti-anxiety influence [69,70]. Prolonged exposure to unopposed oestrogen can lead to mood volatility, irritability, and heightened anxiety in susceptible individuals [71]. Treatment with natural progesterone has shown promise in alleviating such symptoms when standard anti-anxiety medications prove ineffective [72]. Anxiety and depression are long known to affect fertility, though exact mechanisms remain unclear [73]. Anxiety becomes pathological when its intensity is disproportionate to the trigger and interferes with daily functioning [74]. According to the World Health Organization, anxiety disorders are projected to become the second leading cause of disability worldwide, following cardiovascular diseases [75,76]. Indian studies reveal a broad range of anxiety prevalence in PCOS patients—from 15.45% to 100% [77]. Infertility is a major psychological stressor for women, giving rise to several anxiety-inducing factors: Societal Pressure and Expectations: Cultural emphasis on motherhood can lead to feelings of inadequacy [78,79]. Loss of Control: Disrupted life plans and bodily autonomy often cause distress [80]. Relationship Strain: Fertility treatments and uncertainty can challenge partnerships [81]. Fear of the Unknown: Uncertainty around treatment outcomes can create chronic anxiety [82]. Hormonal Imbalances: Hormones directly influence mood and emotional regulation [83]. Support from healthcare professionals, mental health counsellors, and PCOS support groups is vital [84]. Additionally, PCOS is associated with a higher prevalence of not only anxiety and depression but also eating disorders, personality disorders, and bipolar spectrum conditions [85,86]. These mental health issues significantly reduce quality of life (QoL) in PCOS patients, and untreated anxiety can delay timely care and worsen outcomes [87].

Future aspects

PCOS is a multifactorial condition with significant long-term consequences. Diagnosis remains challenging due to its heterogeneous nature and evolving diagnostic criteria. The integration of personalized treatment strategies will likely improve outcomes. Early intervention is essential to mitigate comorbidities and improve the quality of life for women of reproductive age experiencing infertility or other complications.

Conclusion

Polycystic Ovary Syndrome (PCOS) is a complex endocrine-metabolic disorder defined by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. It arises from a combination of genetic predisposition, environmental factors, and hormonal imbalances, including insulin resistance and chronic inflammation. Mental health challenges, especially anxiety and depression, are significantly more common in women with PCOS, influenced by hormonal, psychological, and social factors. While current treatments manage symptoms, there remains a pressing need for comprehensive approaches that address both physiological and emotional well-being. Future research should further explore the biological links between PCOS and mental health to inform more targeted, effective therapies.

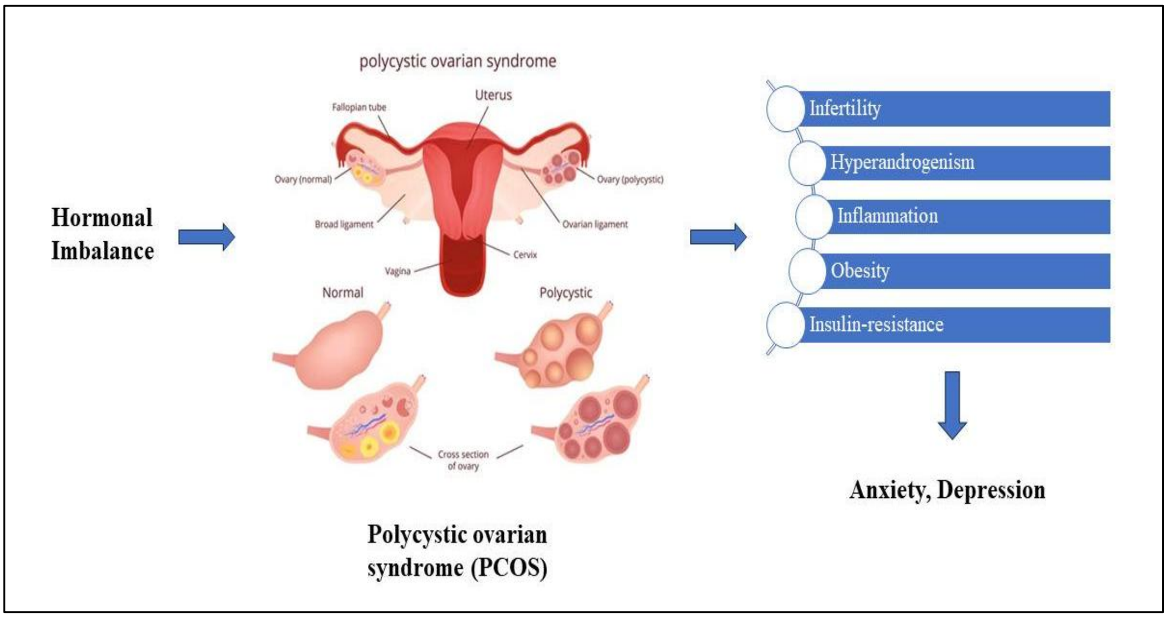


Figure 3. Relation of PCOS with anxiety

References

[1] E. Joham et al., "Polycystic ovary syndrome," The Lancet Diabetes & Endocrinology, vol. 10, no. 9, pp. 668–680, Sep. 2022, doi: 10.1016/S2213-8587(22)00163-2.

[2] J. Yang and C. Chen, "Hormonal changes in PCOS," Journal of Endocrinology, vol. 261, no. 1, p. e230342, Jan. 2024, doi: 10.1530/JOE-23-0342.

[3] S. F. Witchel, S. E. Oberfield, and A. S. Peña, "Polycystic Ovary Syndrome: Pathophysiology, Presentation, and Treatment With Emphasis on Adolescent Girls," Journal of the Endocrine Society, vol. 3, no. 8, pp. 1545–1573, Aug. 2019, doi: 10.1210/js.2019-00078.

[4] World Health Organization: WHO and World Health Organization: WHO, "Polycystic ovary syndrome," Jun. 28, 2023. https://www.who.int/news-room/fact-sheets/detail/polycystic-ovary-syndrome

[5] V. De Leo, M. C. Musacchio, V. Cappelli, M. G. Massaro, G. Morgante, and F. Petraglia, "Genetic, hormonal and metabolic aspects of PCOS: an update," Reprod. Biol. Endocrinol., vol. 14, no. 1, p. 38, Dec. 2016, doi: 10.1186/s12958-016-0173-x.

[6] S. Singh et al., "Polycystic Ovary Syndrome: Etiology, Current Management, and Future Therapeutics," J. Clin. Med., vol. 12, no. 4, p. 1454, Feb. 2023, doi: 10.3390/jcm12041454.

[7] F. Tabassum, C. Jyoti, H. H. Sinha, K. Dhar, and M. S. Akhtar, "Impact of polycystic ovary syndrome on quality of life of women in correlation to age, basal metabolic index, education and marriage," PLOS ONE, vol. 16, no. 3, p. e0247486, Mar. 2021, doi: 10.1371/journal.pone.0247486.

[8] J. Bulsara, P. Patel, A. Soni, and S. Acharya, "A review: Brief insight into Polycystic Ovarian syndrome," Endocrine and Metabolic Science, vol. 3, p. 100085, Jun. 2021, doi: 10.1016/j.endmts.2021.100085.

[9] J. Collée, M. Mawet, L. Tebache, M. Nisolle, and G. Brichant, "Polycystic ovarian syndrome and infertility: overview and insights of the putative treatments," Gynecological Endocrinology, vol. 37, no. 10, pp. 869–874, Oct. 2021, doi: 10.1080/09513590.2021.1958310

[10] C. Dennett and J. Simon, “The Role of Polycystic Ovary Syndrome in Reproductive and Metabolic Health: Overview and Approaches for Treatment,” Diabetes Spectrum, vol. 28, no. 2, pp. 116–120, May 2015, doi: 10.2337/diaspect.28.2.116.

[11] Y. A. Hajam, H. A. Rather, Neelam, R. Kumar, M. Basheer, and M. S. Reshi, “A review on critical appraisal and pathogenesis of polycystic ovarian syndrome,” Endocrine and Metabolic Science, vol. 14, p. 100162, Mar. 2024, doi: 10.1016/j.endmts.2024.100162.

[12] P. Sharma, M. Jain, M. Tripathi, M. Sharma, and A. Halder, “An Update on the Genetics of Polycystic Ovary Syndrome: PCOS and Genetics,” JER, pp. 217–240, Jan. 2024, doi: 10.18311/jer/2023/34654.

[13] D. Anderson, C. M. B. Solorzano, and C. R. McCartney, “Childhood obesity and its impact on the development of adolescent PCOS,” Semin Reprod Med, vol. 32, no. 3, pp. 202–213, May 2014, doi: 10.1055/s-0034-1371092.

[14] L. Cole and P. R. Kramer, “Chapter 6.8 - Ovarian Cystic Disorders,” in Human Physiology, Biochemistry and Basic Medicine, L. Cole and P. R. Kramer, Eds., Boston: Academic Press, 2016, pp. 219–221. doi: 10.1016/B978-0-12-803699-0.00046-3

[15] M. C., N. K. M., U. K. P., J. K., S. K. V., and D. K. P., “Polycystic Ovary Syndrome (Pcos)– An Overview,” Int J Curr Pharm Sci, vol. 10, no. 6, p. 5, Nov. 2018, doi: 10.22159/ijcpr.2018v10i6.30969.

[16] N. Madnani, K. Khan, P. Chauhan, and G. Parmar, “Polycystic ovarian syndrome,” Indian J Dermatol Venereol Leprol, vol. 79, p. 310, May 2013, doi: 10.4103/0378-6323.110759.

[17] Y. Li et al., “Multi-system reproductive metabolic disorder: significance for the pathogenesis and therapy of polycystic ovary syndrome (PCOS),” Life Sci, vol. 228, pp. 167–175, Jul. 2019, doi: 10.1016/j.lfs.2019.04.046.

[18] L.-H. Zeng et al., “Polycystic Ovary Syndrome: A Disorder of Reproductive Age, Its Pathogenesis, and a Discussion on the Emerging Role of Herbal Remedies,” Front. Pharmacol., vol. 13, p. 874914, Jul. 2022, doi: 10.3389/fphar.2022.874914.

[19] S. Siddiqui, S. Mateen, R. Ahmad, and S. Moin, “A brief insight into the etiology, genetics, and immunology of polycystic ovarian syndrome (PCOS),” J Assist Reprod Genet, vol. 39, no. 11, pp. 2439–2473, Nov. 2022, doi: 10.1007/s10815-022-02625-7.

[20] J. Wang, T. Yin, and S. Liu, “Dysregulation of immune response in PCOS organ system,” Front. Immunol., vol. 14, p. 1169232, May 2023, doi: 10.3389/fimmu.2023.1169232.

[21] H. E. Cabre, L. M. Gould, L. M. Redman, and A. E. Smith-Ryan, “Effects of the Menstrual Cycle and Hormonal Contraceptive Use on Metabolic Outcomes, Strength Performance, and Recovery: A Narrative Review,” Metabolites, vol. 14, no. 7, p. 347, Jun. 2024, doi: 10.3390/metabo14070347.

[22] T. Tanbo, J. Mellembakken, S. Bjercke, E. Ring, T. Åbyholm, and P. Fedorcsak, “Ovulation induction in polycystic ovary syndrome,” Acta Obstet Gynecol Scand, vol. 97, no. 10, pp. 1162–1167, Oct. 2018, doi: 10.1111/aogs.13395.

[23] Talmor and B. Dunphy, “Female Obesity and Infertility,” Best Practice & Research Clinical Obstetrics & Gynaecology, vol. 29, no. 4, pp. 498–506, May 2015, doi: 10.1016/j.bpobgyn.2014.10.014.

[24] M. Rababa’h, B. R. Matani, and A. Yehya, “An update of polycystic ovary syndrome: causes and therapeutics options,” Heliyon, vol. 8, no. 10, p. e11010, Oct. 2022, doi: 10.1016/j.heliyon.2022.e11010.

[25] S. Palomba, T. T. Piltonen, and L. C. Giudice, “Endometrial function in women with polycystic ovary syndrome: a comprehensive review,” Human Reproduction Update, vol. 27,

no. 3, pp. 584–618, Apr. 2021, doi: 10.1093/humupd/dmaa051.

[26] U. A. Ndefo, A. Eaton, and M. R. Green, “Polycystic Ovary Syndrome,” Polycystic Ovary Syndrome.

[27] R. Hart, “PCOS and infertility,” Panminerva Med, vol. 50, no. 4, pp. 305–314, Dec. 2008.

[28] D. Armanini, M. Boscaro, L. Bordin, and C. Sabbadin, “Controversies in the pathogenesis, diagnosis and treatment of PCOS: focus on insulin resistance, inflammation, and hyperandrogenism,” International Journal of Molecular Sciences, vol. 23, no. 8, p. 4110, Apr. 2022, doi: 10.3390/ijms23084110.

[29] X. Zeng, Y.-J. Xie, Y.-T. Liu, S.-L. Long, and Z.-C. Mo, “Polycystic ovarian syndrome: Correlation between hyperandrogenism, insulin resistance and obesity,” Clinica Chimica Acta, vol. 502, pp. 214–221, Mar. 2020, doi: 10.1016/j.cca.2019.11.003.

[30] M. E. S. Manique and A. M. A. P. Ferreira, “Polycystic Ovary Syndrome in Adolescence: Challenges in diagnosis and management,” Revista Brasileira Ginecologia E Obstetrícia, vol. 44, no. 04, pp. 425–433, Apr. 2022, doi: 10.1055/s-0042-1742292.

[31] M. Dapas and A. Dunaif, “Deconstructing a Syndrome: Genomic insights into PCOS causal mechanisms and classification,” Endocrine Reviews, vol. 43, no. 6, pp. 927–965, Jan. 2022, doi: 10.1210/endrev/bnac001.

[32] E. Diamanti-Kandarakis, H. Kandarakis, and R. S. Legro, “The role of genes and environment in the etiology of PCOS,” Endocrine, vol. 30, no. 1, pp. 19–26, Jan. 2006, doi: 10.1385/endo:30:1:19.

[33] V. Bruni, A. Capozzi, and S. Lello, “The Role of Genetics, Epigenetics and Lifestyle in Polycystic Ovary Syndrome Development: the State of the Art,” Reproductive Sciences, vol. 29, no. 3, pp. 668–679, Mar. 2021, doi: 10.1007/s43032-021-00515-4.

[34] C. J. Glueck and N. Goldenberg, “Characteristics of obesity in polycystic ovary syndrome: Etiology, treatment, and genetics,” Metabolism, vol. 92, pp. 108–120, Mar. 2019, doi: 10.1016/j.metabol.2018.11.002.

[35] L. Barrea, G. Muscogiuri, G. Pugliese, G. De Alteriis, A. Colao, and S. Savastano, “Metabolically Healthy Obesity (MHO) vs. Metabolically Unhealthy Obesity (MUO) Phenotypes in PCOS: Association with Endocrine-Metabolic Profile, Adherence to the Mediterranean Diet, and Body Composition,” Nutrients, vol. 13, no. 11, p. 3925, Nov. 2021, doi: 10.3390/nu13113925.

[36] H. Cena, L. Chiovato, and R. E. Nappi, “Obesity, polycystic ovary syndrome, and infertility: a new avenue for GLP-1 receptor agonists,” The Journal of Clinical Endocrinology & Metabolism, vol. 105, no. 8, pp. e2695–e2709, May 2020, doi: 10.1210/clinem/dgaa285.

[37] E. Silvestris, G. De Pergola, R. Rosania, and G. Loverro, “Obesity as disruptor of the female fertility,” Reproductive Biology and Endocrinology, vol. 16, no. 1, Mar. 2018, doi: 10.1186/s12958-018-0336-z.

[38] A. E. Joham, N. Naderpoor, and O. Celik, “Editorial: Mechanisms involved in the development of obesity with PCOS,” Frontiers in Endocrinology, vol. 15, Jul. 2024, doi: 10.3389/fendo.2024.1444299.

[39] A. P. Snider and J. R. Wood, “Obesity induces ovarian inflammation and reduces oocyte quality,” Reproduction, vol. 158, no. 3, pp. R79–R90, Sep. 2019, doi: 10.1530/rep-18-0583.

[40] R. Pasquali and C. Oriolo, “Obesity and androgens in women,” Frontiers of Hormone Research, pp. 120–134, Jan. 2019, doi: 10.1159/000494908.

[41] S. Özkan, Ö. Ç. Yılmaz, and B. Yavuz, “Increased masked hypertension prevalence in patients with polycystic ovary syndrome (PCOS),” Clinical and Experimental

Hypertension, vol. 42, no. 8, pp. 681–684, May 2020, doi: 10.1080/10641963.2020.1772815.

[42] D. Fahs, D. Salloum, M. Nasrallah, and G. Ghazeeri, “Polycystic ovary Syndrome: Pathophysiology and controversies in diagnosis,” Diagnostics, vol. 13, no. 9, p. 1559, Apr. 2023, doi: 10.3390/diagnostics13091559.

[43] S. Sirmans and K. Pate, “Epidemiology, diagnosis, and management of polycystic ovary syndrome,” Clinical Epidemiology, p. 1, Dec. 2013, doi: 10.2147/clep.s37559.

[44] O. Osibogun, O. Ogunmoroti, and E. D. Michos, “Polycystic ovary syndrome and cardiometabolic risk: Opportunities for cardiovascular disease prevention,” Trends in Cardiovascular Medicine, vol. 30, no. 7, pp. 399–404, Oct. 2020, doi: 10.1016/j.tcm.2019.08.010.

[45] S. F. Witchel, A. C. Burghard, R. H. Tao, and S. E. Oberfield, “The diagnosis and treatment of PCOS in adolescents: an update,” Current Opinion in Pediatrics, vol. 31, no. 4, pp. 562–569, Aug. 2019, doi: 10.1097/mop.0000000000000778.

[46] H. F. Escobar-Morreale, “Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment,” Nature Reviews Endocrinology, vol. 14, no. 5, pp. 270–284, Mar. 2018, doi: 10.1038/nrendo.2018.24.

[47] J. M. Weiss, S. Tauchert, A. K. Ludwig, and K. Diedrich, “Treatment strategies in PCOS patients,” Reproductive BioMedicine Online, vol. 10, pp. 67–74, Jan. 2005, doi: 10.1016/S1472-6483(11)60393-3.

[48] S. Akre, K. Sharma, S. Chakole, and M. B. Wanjari, “Recent Advances in the Management of Polycystic Ovary Syndrome: A Review Article,” Cureus, vol. 14, no. 8, p. e27689, doi: 10.7759/cureus.27689.

[49] C. Ep and K. S, “Advances in contraception research and development,” Current opinion in obstetrics & gynecology, vol. 32, no. 6, Dec. 2020, doi: 10.1097/GCO.0000000000000666.

[50] J. Davis, “Advances in contraception,” Obstet Gynecol Clin North Am, vol. 27, no. 3, pp. 597–610, vii, Sep. 2000, doi: 10.1016/s0889-8545(05)70158-3.

[51] R. S. Legro et al., “Diagnosis and Treatment of Polycystic Ovary Syndrome: An Endocrine Society Clinical Practice Guideline,” The Journal of Clinical Endocrinology & Metabolism, vol. 98, no. 12, pp. 4565–4592, Dec. 2013, doi: 10.1210/jc.2013-2350.

[52] Hanson, E. Johnstone, J. Dorais, B. Silver, C. M. Peterson, and J. Hotaling, “Female infertility, infertility-associated diagnoses, and comorbidities: a review,” J Assist Reprod Genet, vol. 34, no. 2, pp. 167–177, Feb. 2017, doi: 10.1007/s10815-016-0836-8.

[53] E. S. Dason, O. Koshkina, C. Chan, and M. Sobel, “Diagnosis and management of polycystic ovarian syndrome,” CMAJ, vol. 196, no. 3, pp. E85–E94, Jan. 2024, doi: 10.1503/cmaj.231251.

[54] K. M. Hoeger, A. Dokras, and T. Piltonen, “Update on PCOS: Consequences, Challenges, and Guiding Treatment,” J Clin Endocrinol Metab, vol. 106, no. 3, pp. e1071–e1083, Mar. 2021, doi: 10.1210/clinem/dgaa839.

[55] S. Singh et al., “Polycystic Ovary Syndrome: Etiology, Current Management, and Future Therapeutics,” JCM, vol. 12, no. 4, p. 1454, Feb. 2023, doi: 10.3390/jcm12041454.

[56] Badawy and Elnashar, “Treatment options for polycystic ovary syndrome,” IJWH, p. 25, Feb. 2011, doi: 10.2147/IJWH.S11304.

[57] eBioMedicine, “Polycystic ovary syndrome: deciphering mechanisms to facilitate management and treatment,” eBioMedicine, vol. 94, p. 104754, Aug. 2023, doi: 10.1016/j.ebiom.2023.104754.

[58] X. Che, Z. Chen, M. Liu, and Z. Mo, “Dietary Interventions: A Promising Treatment for Polycystic Ovary Syndrome,” Ann Nutr Metab, vol. 77, no. 6, pp. 313–323, 2021, doi: 10.1159/000519302

[59] S. Cowan et al., “Lifestyle management in polycystic ovary syndrome – beyond diet and physical activity,” BMC Endocr Disord, vol. 23, no. 1, p. 14, Jan. 2023, doi: 10.1186/s12902-022-01208-y.

[60] H. J. Teede, “Recommendations From the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome*,” The Journal of Clinical Endocrinology, vol. 108, no. 10, 2023.

[61] M. Szczuko et al., “Nutrition Strategy and Life Style in Polycystic Ovary Syndrome—Narrative Review,” Nutrients, vol. 13, no. 7, p. 2452, Jul. 2021, doi: 10.3390/nu13072452.

[62] A. Dokras et al., “Weight loss and lowering androgens predict improvements in Health-Related quality of life in women with PCOS,” The Journal of Clinical Endocrinology & Metabolism, vol. 101, no. 8, pp. 2966–2974, Aug. 2016, doi: 10.1210/jc.2016-1896.

[63] S. Alesi, C. Ee, L. J. Moran, V. Rao, and A. Mousa, “Nutritional supplements and complementary therapies in polycystic ovary syndrome,” Advances in Nutrition, vol. 13, no. 4, pp. 1243–1266, Jul. 2022, doi: 10.1093/advances/nmab141.

[64] P. Jin and Y. Xie, “Treatment strategies for women with polycystic ovary syndrome,” Gynecological Endocrinology, vol. 34, no. 4, pp. 272–277, Oct. 2017, doi: 10.1080/09513590.2017.1395841.

[65] Y. Shang, H. Zhou, M. Hu, and H. Feng, “Effect of diet on insulin resistance in polycystic ovary Syndrome,” The Journal of Clinical Endocrinology & Metabolism, vol. 105, no. 10, pp. 3346–3360, Jul. 2020, doi: 10.1210/clinem/dgaa425.

[66] J. V. Kolhe, A. S. Chhipa, S. Butani, V. Chavda, and S. S. Patel, “PCOS and Depression: common links and potential targets,” Reproductive Sciences, vol. 29, no. 11, pp. 3106–3123, Oct. 2021, doi: 10.1007/s43032-021-00765-2.

[67] P. Chaudhari, K. Mazumdar, and P. D. Mehta, “Anxiety, Depression, and Quality of Life in Women with Polycystic Ovarian Syndrome,” Indian Journal of Psychological Medicine, vol. 40, no. 3, pp. 239–246, May 2018, doi: 10.4103/IJPSYM.IJPSYM_561_17.

[68] L. G. Cooney and A. Dokras, “Depression and Anxiety in Polycystic Ovary Syndrome: Etiology and Treatment,” Curr Psychiatry Rep, vol. 19, no. 11, p. 83, Nov. 2017, doi: 10.1007/s11920-017-0834-2.

[69] G. Herzog, “Menstrual disorders in women with epilepsy,” Neurology, vol. 66, no. 66_suppl_3, Mar. 2006, doi: 10.1212/WNL.66.66_suppl_3.S23.

[70] L. Damone, A. E. Joham, D. Loxton, A. Earnest, H. J. Teede, and L. J. Moran, “Depression, anxiety and perceived stress in women with and without PCOS: a community-based study,” Psychol. Med., vol. 49, no. 09, pp. 1510–1520, Jul. 2019, doi: 10.1017/S0033291718002076.

[71] Dokras, “Mood and anxiety disorders in women with PCOS,” Steroids, vol. 77, no. 4, pp. 338–341, Mar. 2012, doi: 10.1016/j.steroids.2011.12.008.

[72] W. K. Almeshari, A. K. Alsubaie, R. I. Alanazi, Y. A. Almalki, N. Masud, and S. H. Mahmoud, “Depressive and Anxiety Symptom Assessment in Adults with Polycystic Ovarian Syndrome,” Depression Research and Treatment, vol. 2021, pp. 1–8, Apr. 2021, doi: 10.1155/2021/6652133.

[73] Ambreen, A. Sheikh, N. Faryad, S. Batool, and F. U. A. Ahmed, “Depression and Anxiety in Women with Polycystic Ovary Syndrome and Its Biochemical Associates,” Journal of South

Asian Federation of Obstetrics and Gynaecology, vol. 8, no. 1, pp. 44–47, Mar. 2016, doi: 10.5005/jp-journals-10006-1384.

[74] P. Gdańska, E. Drozdowicz-Jastrzębska, B. Grzechocińska, M. Radziwon-Zaleska, P. Węgrzyn, and M. Wielgoś, “Anxiety and depression in women undergoing infertility treatment,” Ginekol Pol, vol. 88, no. 2, pp. 109–112, Feb. 2017, doi: 10.5603/GP.a2017.0019.

[75] J. Zhuang, X. Wang, L. Xu, T. Wu, and D. Kang, “Antidepressants for polycystic ovary syndrome,” Cochrane Database of Systematic Reviews, vol. 2013, no. 6, May 2013, doi: 10.1002/14651858.CD008575.pub2.

[76] R. Wang, R. Zhao, and D. Qin, “Depression in polycystic ovary syndrome: Focusing on pathogenesis and treatment,” Frontiers in Psychiatry.

[77] P. Gdańska, E. Drozdowicz-Jastrzębska, B. Grzechocińska, M. Radziwon-Zaleska, P. Węgrzyn, and M. Wielgoś, “Anxiety and depression in women undergoing infertility treatment,” Ginekol Pol, vol. 88, no. 2, pp. 109–112, Feb. 2017, doi: 10.5603/GP.a2017.0019.S. A. Buriro, S. A. Memon, Z. Iqbal, I. Chandio, H. Bano, and D. S. Thebo, “Analysis of Anxiety, Depression and Perceived Stress in Women With Polycystic Ovary Syndrome (PCOS),” 2023.

[78] Ethirajulu et al., “Insulin Resistance, Hyperandrogenism, and Its Associated Symptoms Are the Precipitating Factors for Depression in Women With Polycystic Ovarian Syndrome,” Cureus, Sep. 2021, doi: 10.7759/cureus.18013.

[79] T. Jannink et al., “Anxiety, depression, and body image among infertile women with and without polycystic ovary syndrome,” Human Reproduction, vol. 39, no. 4, pp. 784–791, Apr. 2024, doi: 10.1093/humrep/deae016.

[80] A. Deeks, M. E. Gibson-Helm, and H. J. Teede, “Anxiety and depression in polycystic ovary syndrome: a comprehensive investigation,” Fertility and Sterility, vol. 93, no. 7, pp. 2421–2423, May 2010, doi: 10.1016/j.fertnstert.2009.09.018.

[81] L. Doretto, F. C. Mari, and A. C. Chaves, “Polycystic Ovary Syndrome and Psychotic Disorder,” Front. Psychiatry, vol. 11, p. 543, Jun. 2020, doi: 10.3389/fpsyt.2020.00543.

[82] P. Dybciak, E. Humeniuk, D. Raczkiewicz, J. Krakowiak, A. Wdowiak, and I. Bojar, “Anxiety and Depression in Women with Polycystic Ovary Syndrome,” Medicina, vol. 58, no. 7, p. 942, Jul. 2022, doi: 10.3390/medicina58070942.

[83] Ethirajulu et al., “Insulin Resistance, Hyperandrogenism, and Its Associated Symptoms Are the Precipitating Factors for Depression in Women With Polycystic Ovarian Syndrome,” Cureus, Sep. 2021, doi: 10.7759/cureus.18013.

[84] A. L. Damone, A. E. Joham, D. Loxton, A. Earnest, H. J. Teede, and L. J. Moran, “Depression, anxiety and perceived stress in women with and without PCOS: a community-based study,” Psychological Medicine, vol. 49, no. 09, pp. 1510–1520, Aug. 2018, doi: 10.1017/s0033291718002076.

[85] S. Alur-Gupta and A. Dokras, “Considerations in the Treatment of Depression and Anxiety in Women with PCOS,” Seminars in Reproductive Medicine, vol. 41, no. 01/02, pp. 037–044, Mar. 2023, doi: 10.1055/s-0043-1777720.

[86] D. Rodriguez-Paris et al., “Psychiatric disorders in women with polycystic ovary syndrome,” Psychiatria Polska, vol. 53, no. 4, pp. 955–966, Aug. 2019, doi: 10.12740/pp/onlinefirst/93105.

87. C. Brutocao, F. Zaiem, M. Alsawas, A. S. Morrow, M. H. Murad, and A. Javed, "Psychiatric disorders in women with polycystic ovary syndrome: a systematic review and meta-analysis," Endocrine, vol. 62, no. 2, pp. 318–

325, Jul. 2018, doi: 10.1007/s12020-018-1692-3