



ANTI- ANXIETY ACTIVITY OF J. CURCAS USING ETHANOL EXTRACTION : A REVIEW

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Abstract: India is still a backward and developing country. It is no surprise then that in a country such as ours depression is one of the most prevalent and common “**mental health illnesses**” that people suffer from along with “**anxiety**”. Depression is a common mental disorder with over 280 million people of all ages, an estimated 3.8 per cent of the population suffering from it globally, as per the World Health Organization. It is thus expected that in the next ten years, depression will put more burden on nations than any other disease, as per World Bank. *Jatropha curcas* (Linnaeus) is a plant which belongs to family “**Euphorbiaceae and genus Jatropha**”. This plant is native of America and is widely distributed, is also located in India, Africa, Asia and Europe where it grows in arid, tropical and sub-tropical environments. Its name derives from the Greek words “Jatros” (doctor) and “trophe” (food) which translates its medicinal traditional use. Such medicinal use is reported in diverse consumption of different parts of *J. curcas*, some of them through infusions for various conditions such as fever, burn, rheumatism, allergies, diarrhea, antidepressant intestinal cramping, healing , headach, sleeping.

Key Terms – Depression, Anxiety, Mental Health illness, Jatropha Curcas.

I. INTRODUCTION

1.1Anxiety:

Generally, anxiety is an emotional state characterized by an overwhelming sense of fear, apprehension, and discomfort. This emotional state often leads to physiological responses such as sweating, restlessness, heightened tension, and an accelerated heart rate. In certain circumstances, anxiety can be considered a healthy and adaptive response to stressors. For instance, when confronted with challenging work-related issues, facing a crucial exam, or making significant life choices, experiencing anxiety can serve a purpose. It may provide an extra burst of energy and enhance concentration, ultimately assisting individuals in managing these situations effectively. However, for individuals grappling with anxiety disorders, this fear and unease are neither fleeting nor manageable. Instead, it becomes a persistent and overpowering presence in their lives. Unlike the situational anxiety mentioned earlier, anxiety disorders manifest as chronic conditions, often requiring treatment. These disorders can severely disrupt daily functioning, hinder decision-making, and diminish overall quality of life. Intermittent bouts of anxiety are a natural facet of the human experience. It's commonplace for individuals to fret about their health, financial stability, or familial concerns from time to time. However, anxiety disorders transcend the realm of momentary apprehension or fear. In individuals grappling with an anxiety disorder, these persistent feelings of anxiety persist and tend to intensify with time. The resultant symptoms extend beyond mere discomfort, encroaching upon various facets of daily life, thereby disrupting activities like work performance, academic pursuits, and interpersonal relationships. Anxiety disorders come in various forms, each with its distinctive characteristics and manifestations. These include generalized anxiety disorder, panic disorder, social anxiety disorder, and a

range of disorders linked to specific phobias. These conditions collectively exemplify the complexity of anxiety disorders and underscore the significance of tailored diagnostic and therapeutic approaches. Anxiety is a complex emotion marked by inner turmoil and a sense of unease, often accompanied by anticipatory worry about upcoming events. It stands apart from fear, which is a reaction to an immediate and real threat, whereas anxiety revolves around the expectation of potential future threats. Individuals experiencing anxiety often display nervous behaviors such as restlessness, pacing, somatic complaints, and repetitive thinking. This feeling of uneasiness and worry in anxiety is typically broad and unfocused, often blown out of proportion when the perceived threat is subjectively deemed menacing. Physical symptoms can include muscle tension, restlessness, fatigue, breathlessness, abdominal tightness, nausea, and difficulty concentrating. Anxiety is closely intertwined with fear, which triggers the body's fight-or-flight response in response to immediate danger, whereas anxiety encompasses a broader sense of dread related to future events. People grappling with anxiety may avoid situations that have triggered anxiety in the past.

Anxiety as an emotion can persist beyond the normal response timeframe for specific events, evolving into various anxiety disorders like generalized anxiety disorder and panic disorder. The key distinction between an anxiety disorder, classified as a mental disorder, and everyday anxiety as a normal emotion lies in the duration and intensity. Those with an anxiety disorder experience anxiety on most days for roughly six months or even shorter periods in children. These disorders often persist over extended durations, sometimes lasting decades. Furthermore, heightened anxiety levels can manifest within other mental conditions, such as obsessive-compulsive disorder and post-traumatic stress disorder. Anxiety, in a scientific context, is a complex emotional and physiological response characterized by a heightened state of apprehension, worry, and fear in anticipation of a perceived threat or stressful situation. It is a natural and adaptive reaction that prepares the body to respond to potential dangers; however, when it becomes chronic, excessive, or disproportionate to the actual threat, it can lead to significant impairment in daily functioning and be classified as an anxiety disorder.

1.2 Key components and features of anxiety includes:

1. Neurobiology:

Anxiety has a neurobiological basis involving intricate interactions within the brain. The amygdala, a small almond-shaped structure deep within the brain, plays a central role in processing emotional responses, particularly fear and anxiety. The amygdala's activation triggers a cascade of physiological and psychological responses that prepare the body for the "fight or flight" reaction.

2. Neurotransmitters:

Neurotransmitters, such as serotonin, norepinephrine, and gamma-aminobutyric acid (GABA), are involved in regulating mood and anxiety. Imbalances or dysregulation in these neurotransmitter systems can contribute to the development and persistence of anxiety disorders.

3. Hormonal Involvement:

The hypothalamus-pituitary-adrenal (HPA) axis is a crucial hormonal system involved in the body's stress response. In anxious individuals, the HPA axis may become overactive, leading to increased production of stress hormones like cortisol. Elevated cortisol levels can perpetuate feelings of anxiety and stress.

4. Cognitive Factors:

Anxiety often involves maladaptive patterns of thinking and perception. People with anxiety disorders may exhibit cognitive biases, such as catastrophizing (assuming the worst possible outcome), overgeneralization (applying negative experiences to all situations), and selective attention to perceived threats.

5. Physical Symptoms:

Anxiety manifests with a range of physical symptoms, including increased heart rate, rapid breathing, muscle tension, sweating, gastrointestinal discomfort, and restlessness. These symptoms are part of the body's preparation for a perceived threat.

6. Diagnostic Classification:

Anxiety disorders are categorized in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) into various subtypes, including generalized anxiety disorder (GAD), panic disorder, social anxiety disorder (social phobia), and specific phobias. Each subtype has its unique set of symptoms and diagnostic criteria.

7.Epidemiology:

Anxiety disorders are among the most prevalent mental health conditions worldwide. They can occur at any age and affect individuals from diverse demographic backgrounds. Factors such as genetics, environmental stressors, and early life experiences can increase susceptibility to anxiety disorders.

2.Treatment: Various treatment approaches are available for anxiety disorders, ranging from psychotherapy (such as cognitive-behavioral therapy) to pharmacotherapy (using medications like selective serotonin reuptake inhibitors or benzodiazepines). Treatment choices depend on the specific subtype and severity of the disorder, as well as individual patient preferences.

2.1 Comorbidity:

Anxiety disorders often co-occur with other mental health conditions, including depression, substance use disorders, and somatic symptom disorders. Understanding these comorbidities is crucial for comprehensive assessment and treatment planning.

2.2 Research and Future Directions:

Ongoing research in neuroscience, genetics, and psychopharmacology continues to enhance our understanding of the underlying mechanisms of anxiety and inform the development of more effective interventions. Additionally, the field of digital mental health and telemedicine is expanding to improve access to care for individuals with anxiety disorders.

2.3 Mechanistic development of Anxiety:

Anxiety involves a complex interplay of psychological, neurological, and physiological mechanisms. Understanding the mechanisms underlying anxiety can provide insight into its development and potential treatment approaches. Here are some key mechanisms involved in anxiety.

2.4 Neurotransmitter Imbalance:

Neurotransmitters are chemicals that transmit signals in the brain. An imbalance in neurotransmitters, particularly serotonin, norepinephrine, and gamma-aminobutyric acid (GABA), is associated with anxiety disorders. Low levels of serotonin, for example, have been linked to increased anxiety.

2.4 Hypothalamus-Pituitary-Adrenal (HPA) Axis:

The HPA axis is a crucial part of the body's stress response system. When the brain perceives a threat, the hypothalamus releases corticotropin-releasing hormone (CRH), which triggers the pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH then stimulates the adrenal glands to release cortisol, the body's primary stress hormone. In individuals with anxiety disorders, this system can become dysregulated, leading to chronic elevation of cortisol levels.

2.5 Amygdala Activation:

The amygdala, a small almond-shaped structure in the brain, plays a central role in processing emotions, including fear and anxiety. When the amygdala is activated in response to a perceived threat, it triggers a cascade of physiological responses, such as increased heart rate and alertness.

3. Cognitive Factors:

Cognitive processes, including thought patterns and interpretations, are crucial in anxiety.

Individuals with anxiety disorders often engage in negative thought patterns, such as catastrophizing (assuming the worst will happen) and overgeneralization (applying negative experiences to all situations). Cognitive-behavioral therapy (CBT) aims to address and modify these cognitive factors.

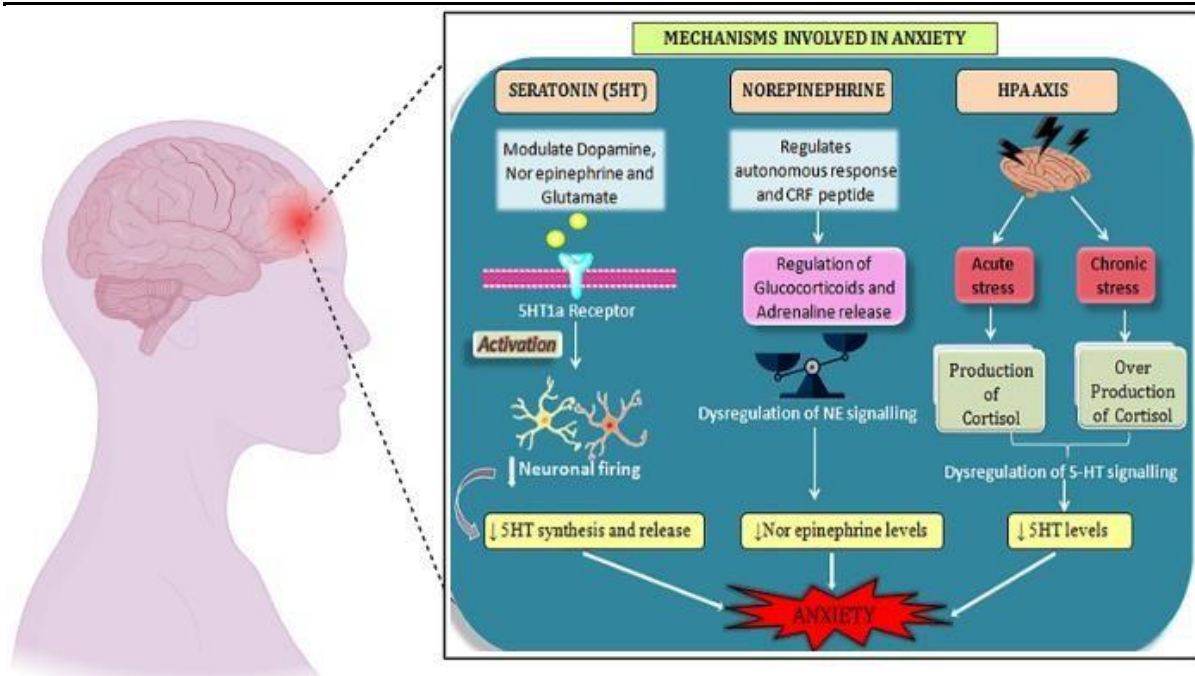


Figure 1. Proposed processes for the emergence of anxiety

3.1 Genetics:

There is evidence to suggest that genetics plays a role in the development of anxiety disorders. People with a family history of anxiety disorders may be more predisposed to develop them themselves.

3.2 Environmental Factors:

Stressful life events, trauma, and chronic exposure to stressors can contribute to the development of anxiety disorders. Early life experiences, including childhood adversity, can also increase susceptibility to anxiety.

3.3 Neural Circuitry:

Anxiety involves specific neural circuits in the brain, including the prefrontal cortex, which is responsible for decision-making and executive function, and the anterior cingulate cortex, which is involved in regulating emotional responses.

3.4 Neuroplasticity:

The brain's ability to adapt and rewire itself, known as neuroplasticity, also plays a role in anxiety. Chronic anxiety can lead to changes in brain structure and function, which may perpetuate the disorder.

3.5 Inflammatory Processes:

Emerging research suggests that inflammation in the body may be linked to anxiety disorders. Chronic inflammation can affect neurotransmitter function and neural circuits, contributing to anxiety.

3.6 Medication and Neurochemistry:

Medications used to treat anxiety disorders, such as selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines, target specific neurotransmitter systems to alleviate symptoms.

It's important to note that anxiety is a complex condition, and these mechanisms often interact and overlap. Treatment approaches, including therapy and medication, aim to address these mechanisms to alleviate anxiety symptoms and improve an individual's quality of life. Anxiety is a complex psychological and physiological phenomenon with various contributing factors. Its study involves multidisciplinary approaches encompassing neuroscience, psychology, psychiatry, and pharmacology. A comprehensive understanding of anxiety is essential for developing effective interventions and improving the quality of life for individuals affected by anxiety disorders.

4. Types of Anxiety:

Anxiety is a broad term that encompasses various types of anxiety disorders, each characterized by distinct features and symptoms. Here are some common types of anxiety disorders:

4.1 Generalized Anxiety Disorder (GAD):

People with GAD experience excessive and uncontrollable worry and anxiety about a wide range of everyday situations or events.

This worry is often accompanied by physical symptoms like restlessness, muscle tension, fatigue, and difficulty concentrating.

GAD typically lasts for at least six months and can significantly impair daily functioning.

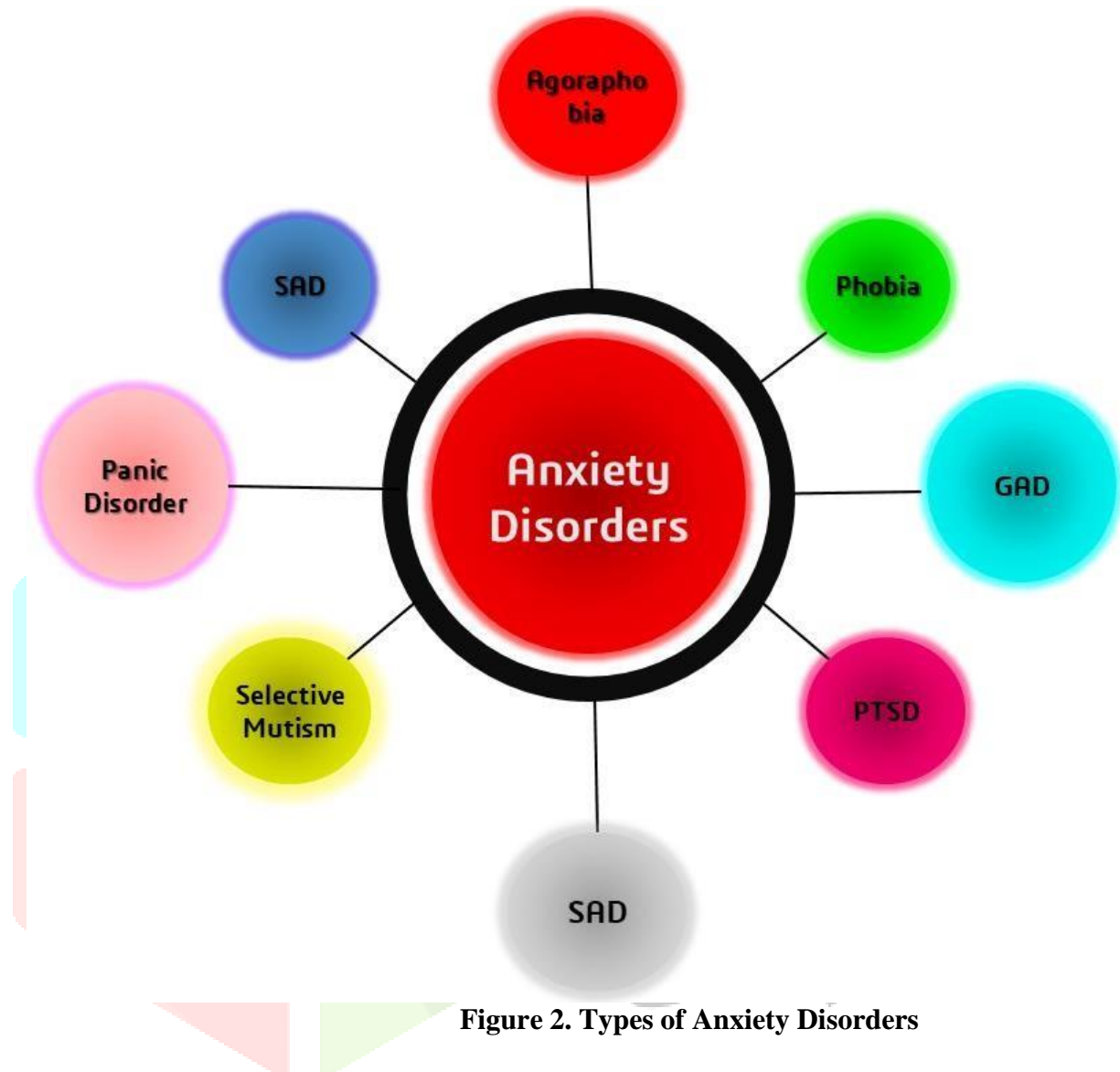


Figure 2. Types of Anxiety Disorders

4.2 Panic Disorder:

Panic disorder involves recurrent and unexpected panic attacks, which are intense episodes of fear and discomfort.

Individuals with panic disorder often fear having another panic attack, leading to avoidance behavior and changes in lifestyle.

Symptoms of panic attacks may include rapid heart rate, sweating, trembling, shortness of breath, and a sense of impending doom.

4.3 Social Anxiety Disorder (Social Phobia):

Social anxiety disorder is characterized by an intense fear of social or performance situations where one might be scrutinized or judged by others.

People with social anxiety often experience extreme self-consciousness, avoidance of social interactions, and physical symptoms like blushing, trembling, or nausea.

4.4 Specific Phobia:

Specific phobias involve an irrational and intense fear of a specific object or situation, such as heights, spiders, flying, or public speaking. These fears lead to avoidance behavior and can significantly disrupt daily life.

4.5 Obsessive-Compulsive Disorder (OCD):

OCD is characterized by the presence of obsessions (intrusive, unwanted thoughts) and compulsions (repetitive behaviors or mental acts performed to alleviate distress).

Individuals with OCD may feel compelled to engage in rituals like handwashing, counting, or checking to reduce anxiety associated with their obsessions.

4.6 Post-Traumatic Stress Disorder (PTSD):

PTSD develops after exposure to a traumatic event, such as a serious accident, natural disaster, or violence. Symptoms include intrusive memories, nightmares, flashbacks, avoidance of reminders of the trauma, negative changes in mood and cognition, and heightened arousal.

4.7 Agoraphobia:

Agoraphobia involves an intense fear of situations or places where escape or help may be difficult or embarrassing in the event of a panic attack or other anxiety symptoms. This fear can lead to avoidance of crowded places, public transportation, or open spaces. Separation Anxiety Disorder: Typically diagnosed in childhood, separation anxiety disorder involves excessive fear or worry about separation from attachment figures, often leading to distress when separated or anticipating separation.

4.8 Selective Mutism:

Selective mutism is a rare childhood anxiety disorder characterized by consistent failure to speak in specific social situations (e.g., school, with peers) despite being capable of speech in other settings.

4.9 Illness Anxiety Disorder (Hypochondriasis):

People with illness anxiety disorder have an excessive preoccupation with having a serious medical condition, despite minimal or no medical evidence to support their concerns. It's important to note that these anxiety disorders can co-occur with each other or with other mental health conditions. Treatment approaches, such as therapy (e.g., cognitive-behavioral therapy), medication, or a combination of both, are available to help individuals manage and alleviate their symptoms. Seeking professional help is crucial for accurate diagnosis and effective treatment.

4.10 Sign and Symptoms of Anxiety:

- Excessive Worry
 - Fear and Apprehension
 - Restlessness
 - Irritability
 - Difficulty Concentrating
 - Catastrophizing
 - Physical Tension
 - Sweating
 - Rapid Heartbeat
 - Shortness of Breath
 - Gastrointestinal Discomfort
 - Trembling or Shaking
 - Avoidance Behavior
 - Social Withdrawal
 - Compulsive Behaviors
- Background and Rationale of Anxiety:

The background and rationale of anxiety are rooted in the evolutionary and psychological mechanisms that have developed to help humans respond to potential threats and challenges. Understanding the background and rationale for anxiety can shed light on why it exists as a natural part of the human experience.

Background:

5 Evolutionary Perspective:

Anxiety has evolutionary roots in the survival instincts of our ancestors. When faced with physical threats in their environment, the "fight or flight" response, triggered by anxiety, prepared them to respond quickly to potential dangers. This heightened state of alertness and physical readiness improved their chances of survival.

Adaptive Function: Anxiety, in its moderate form, serves as an adaptive mechanism. It prompts individuals to assess and respond to potential risks and stressors. It can motivate preparation, planning, and problem-solving, enhancing one's ability to cope with challenges.

5.1 Normal Emotion:

Occasional anxiety is a normal emotional response to everyday stressors and uncertainties. It is a part of the broader spectrum of human emotions and can be considered a healthy response to life's demands.

5.2 Anticipatory Preparation:

Anxiety prepares individuals for anticipated future events or situations. It encourages proactive thinking and planning to navigate potential difficulties effectively. For example, feeling anxious before an important presentation can motivate preparation and practice. Anxiety heightens alertness and vigilance, making individuals more attuned to their surroundings and potential threats. This increased awareness can be beneficial in situations where quick reactions are necessary for safety.

5.3 Problem-Solving:

Mild anxiety can improve problem-solving abilities by encouraging individuals to assess challenges and find solutions. It can promote a more careful consideration of options and potential outcomes.

5.4 Social Bonding:

In a social context, anxiety can promote cooperation and bonding among individuals. Shared anxieties over common challenges can foster social connections and mutual support. However, it's essential to recognize that while anxiety has adaptive functions, it can also become maladaptive when it is chronic, excessive, or disproportionate to the actual threat. Anxiety disorders, such as generalized anxiety disorder or panic disorder. The rationale for understanding anxiety lies in differentiating between normal anxiety responses, which serve adaptive functions, and pathological anxiety, which requires intervention and treatment. By recognizing the background and rationale of anxiety, individuals and healthcare professionals can better navigate its impact on mental health and well-being. Utilizing *Jatropha curcas* as a Novel Approach for Managing Anxiety:

Jatropha curcas, a versatile herbal plant with a history of various medicinal uses, has emerged as a novel and promising approach in the realm of anxiety management. This unique botanical resource, which undergoes a distinct color transformation to red when dried, offers potential therapeutic benefits for individuals grappling with anxiety disorders. This plant has been historically used for various medicinal purposes, with different parts of *Jatropha curcas* being utilized in infusions to address ailments like fever, burns, rheumatism, allergies, diarrhea, intestinal cramping, headaches, and sleep-related issues. As the field of healthcare continually evolves, health-related concerns are becoming increasingly important to individuals. Social pressures in our rapidly developing society can lead to prolonged exposure to stress, which, in turn, can have detrimental effects on brain development.

Primary care physicians are strategically positioned to play a pivotal role in identifying and managing these conditions, contributing significantly to improved mental health outcomes for their patients. Recognizing the co-occurrence of depression and anxiety and offering comprehensive treatment approaches is essential, as these conditions are associated with notable health challenges and potential negative outcomes. Here, we explore the potential of *Jatropha curcas* as an innovative anti-anxiety agent.

5.5 Traditional Wisdom:

Jatropha curcas has a rich heritage of traditional use, particularly in herbal medicine. It is often hailed as a multipurpose plant, with its leaves being employed to address a range of health concerns, including anxiety. Anecdotal accounts and traditional wisdom suggest its efficacy in alleviating symptoms of anxiety.

5.6 Chemical Composition:

Jatropha curcas leaves boast a complex chemical profile, containing compounds like flavonoids, apigenin, vitexin, isovitexin, sterols, and triterpenes. These bioactive constituents have shown potential in traditional practices for managing anxiety-related symptoms.

6. Antidepressant and Neuroprotective Effects:

Recent research endeavors have delved into the antidepressant and neuroprotective properties of *Jatropha curcas*. Studies aim to evaluate its effectiveness in both acute and chronic models of anxiety and depression. The findings may shed light on the mechanisms through which *Jatropha curcas* exerts its potential anti-anxiety effects.

-Primary Care Integration:

As primary care settings play a crucial role in mental health care, the exploration of *Jatropha curcas* as an anti-anxiety intervention aligns with the objective of providing comprehensive and accessible treatments. General practitioners are well-positioned to consider this botanical remedy as part of a holistic approach to anxiety management.

-Future Directions:

The investigation of *Jatropha curcas* as an anti-anxiety solution represents an exciting frontier in mental health research. By unraveling its potential and understanding its mechanisms of action, we may open up new avenues for addressing anxiety disorders, ultimately improving the well-being of individuals facing these challenges. Incorporating *Jatropha curcas* into the spectrum of anti-anxiety interventions underscores the ongoing pursuit of innovative and effective strategies to enhance mental health outcomes and offer individuals a range of therapeutic options for managing anxiety.

7. Plant Profile:

7.1 Plant Name: *Jatropha curcas*

7.2 Taxonomy-Based Classification of *Jatropha curcas*:

7.3 Kingdom: Plantae - *Jatropha curcas* belongs to the Plantae kingdom, encompassing all multicellular, photosynthetic organisms.

7.4 Phylum: Angiosperms (Magnoliophyta) - It falls under the phylum of angiosperms, which are flowering plants characterized by the presence of enclosed seeds within fruits.

7.5 Class: Eudicots (Magnoliopsida) - *Jatropha curcas* belongs to the class of eudicots, which includes a diverse group of angiosperms with two cotyledons in their seedlings.

7.6 Order: Malpighiales - This order consists of various families of flowering plants, including Euphorbiaceae, to which *Jatropha curcas* belongs.

7.7 Family: Euphorbiaceae - *Jatropha curcas* is a member of the Euphorbiaceae family, also known as the spurge family.

7.8 Genus: *Jatropha* - The genus *Jatropha* includes various species, with *Jatropha curcas* being one of the most well-known.

7.9 Species: *Jatropha curcas* - This is the specific name of the plant, designating it as a distinct species within the genus *Jatropha*.

Jatropha curcas, a plant belonging to the Euphorbiaceae family and the *Jatropha* genus, is native to the Americas and has a widespread presence in regions like India, Africa, Asia, and Europe. It thrives in arid, tropical, and sub-tropical environments. The name "*Jatropha*" is derived from the Greek words "*Jatros*" (doctor) and "*trophe*" (food), highlighting its traditional medicinal uses.

Jatropha curcas, commonly known as the physic nut or Barbados nut, is a versatile and drought-resistant plant that belongs to the Euphorbiaceae family. It is native to Central America but has spread to tropical and subtropical regions worldwide.

This plant is renowned for its multiple uses, including biodiesel production, medicinal properties, and soil conservation.



Figure 3. Plant of Jatropha

8. Botanical Characteristics:

- **Habitat:** *Jatropha curcas* thrives in arid and semi-arid regions with well-drained soils.
- **Size:** It is typically a small to medium-sized shrub or tree, growing up to 3-5 meters in height.
- **Leaves:** The leaves are typically green, palmate, and alternate with deep lobes. **Flowers:** The plant produces small, greenish-yellow flowers that are usually unisexual.
- **Fruits:** The fruit is a three-lobed capsule containing oil-rich seeds.
- **Biodiesel Production:** *Jatropha curcas* gained significant attention as a potential source of biodiesel due to its high oil content, which can be extracted from the seeds. The oil is considered a sustainable alternative to fossil fuels, making it valuable in the context of renewable energy production.



Figure 4. Roots of Jatropha curcas Plant

9. Medicinal Uses:

The plant has a long history of use in traditional medicine in various parts of the world. Different parts of the plant, including the roots, leaves, and seeds, are believed to have medicinal properties. It has been used to treat a wide range of ailments, including skin conditions, digestive issues, and respiratory illnesses.

- **Environmental Benefits:**

Jatropha curcas plays a role in soil conservation and land reclamation. Its deep root system helps prevent soil erosion and stabilize sandy or degraded soils, making it a valuable plant for ecological restoration efforts.

- **Cultivation:** *Jatropha curcas* is relatively low-maintenance and thrives in hot, arid climates.

It can tolerate a variety of soil types but prefers well-drained soils. Propagation can be done through seeds, and the plant can be grown from cuttings as well.

10. Challenges:

Despite its promise as a biodiesel crop, there are challenges associated with *Jatropha* cultivation, including concerns about its invasive potential and competition with food crops.

The seeds contain toxic compounds, and proper processing is required to remove these toxins before oil extraction.

Jatropha curcas is a versatile plant with a range of practical applications, from biodiesel production to traditional medicine and soil conservation. Its adaptability to harsh environments makes it a valuable resource for sustainable agriculture and renewable energy initiatives. However, careful management is essential to address potential environmental and health concerns associated with its cultivation and use.

10.1 Collection of the Plant Material:

Jatropha curcas roots were taken from native sources for this extraction and phytochemical screening of plants like *Jatropha curcas* are essential steps in understanding their chemical composition and potential medicinal or industrial uses. Here's a basic guide on how to perform the extraction and phytochemical screening of *Jatropha curcas*.

10.2 Materials Needed:

- *Jatropha curcas* plant material (Roots)
- Mortar and pestle
- Solvents (e.g., ethanol, methanol, or water)
- Filtration apparatus (e.g., Buchner funnel)
- Rotary evaporator or vacuum distillation system
- Glassware (flasks, beakers, and test tubes)
- Separatory funnel
- Rotary shaker or sonicator
- Oven or vacuum oven
- Weighing balance
- pH meter
- Hot plate/stirrer

Procedure:

11. Sample Preparation:

Collect fresh *Jatropha curcas* plant parts (Root) and wash them thoroughly to remove dirt and debris. Dry the plant material at a low temperature (around 40-45°C) until it reaches a constant weight to prevent degradation of phytochemicals.

12. Soxhlet Extraction:

The dried powdered material of the *Jatropha curcas* root was successively extracted using the Soxhlet apparatus and the hot continuous percolation method using ethanol as the desired solvent. The extracted materials were collected, concentrated in a water bath, and stored in a desiccator.



Figure 5. Soxhlet Ethanol Extraction

13. phytochemical analysis of ethanolic extracts:

The presence of different chemical constituents was determined through preliminary phytochemical screening on all of the extracts. The biological and pharmacological action of the plant is influenced by the makeup of its elements. The following chemical tests were performed on the ethanolic extracts of *Jatropha curcas* to determine the different active chemical constituents that were present.

13.1 Tests for alkaloids:

Testing alkaloids is a broad topic, as alkaloids represent a diverse group of natural compounds found in plants and other organisms. The specific tests you would use depend on the type of alkaloid you are trying to identify or quantify. Here are some general tests and methods commonly used for alkaloid analysis.

13.2 Dragendorff's Test:

Dragendorff's reagent was added to the extract, and the sample was carefully observed for the emergence of a yellow-colored solid. This occurrence serves as an indicator of the existence of alkaloids within the sample.

13.3 Wagner's Test:

Upon treating the extract with Wagner's reagent, close attention was paid to any formation of a precipitate, which appeared reddish-brown. This formation is indicative of the presence of alkaloids in the sample.

13.4 Mayer's Test:

The extract was subjected to Mayer's reagent, and the observation focused on any resulting precipitation. The formation of either a white or creamy-colored solid substance was noted, signifying the existence of alkaloids in the sample.

13.5 Hager's Test:

Hager's reagent was introduced to the extract, and a thorough examination was conducted for the appearance of a yellow precipitate. The presence of such a precipitate was taken as an indication of the presence of alkaloids in the sample.

Test for Glycosides:

13.5 Legal's Test:

The extract was dissolved in pyridine and mixed with sodium nitroprusside solution to create an alkaline environment. The presence of glycosides was indicated by the development of a pink-red to red color.

13.6 Baljet Test:

A solution containing 1 ml of the ethanolic extract was combined with 1 ml of sodium picrate solution. The presence of glycosides was unveiled by the appearance of a yellow to orange color.

13.7 Bontrager's Test:

To 1 ml of the extract solution, a few milliliters of dilute hydrochloric acid were added. The mixture was subsequently heated, filtered, and the resulting filtrate was subjected to extraction with chloroform. Upon treating the chloroform layer with 1 ml of ammonia, the formation of a red color indicated the presence of anthraquinone glycosides.

13.8 Keller-Killiani Test:

The extract was dissolved in acetic acid containing small amounts of ferric chloride. This solution was then transferred to a test tube containing sulfuric acid. The appearance of a reddish-brown color at the junction, which gradually transitioned to blue, served as confirmation of the presence of glycosides.

13.9 Liebermann-Burchard Test:

A mixture of 3 ml of the extract and 3 ml of acetic anhydride was prepared and heated, followed by cooling. A few drops of concentrated sulfuric acid were subsequently introduced. The presence of phytosterol was indicated by the appearance of a blue color.

13.10 Salkowski's Test:

The extract was dissolved in chloroform, and an equal volume of concentrated sulfuric acid was added. The formation of a bluish-red to cherry-red color in the chloroform layer, along with the observation of green fluorescence in the acid layer, indicated the presence of steroid components within the extract.

13.14 Shinoda Test:

A mixture was created by treating the extract with 5 ml of 95% ethanol. To this mixture, a few drops of concentrated hydrochloric acid and 0.5 grams of magnesium turnings were added. The emergence of a pink color in the solution was noted, indicating the presence of flavonoids.

- **Test for Phenols**

To 1 ml of the extract, ferric chloride was introduced, and the solution was carefully examined for the development of a dark blue or greenish-black color. This color change served as an indicator of the presence of tannins and phenolic compounds.

- **Gelatin Test:**

The extract was subjected to a solution of 1% gelatin with the addition of 10% NaCl. Careful observation was made for any formation of a precipitate, which serves as an indication of the presence of tannins and phenolic compounds.

Test for Proteins and Amino Acids.

- **Biuret Test:**

1 ml of the extract was mixed with 1 ml of a 40% sodium hydroxide solution, followed by the addition of 2 drops of a 1% copper sulfate solution. The presence of proteins was revealed by the formation of a violet color.

- **Xanthoproteic Test:**

To 1 ml of the extract, 1 ml of concentrated nitric acid was applied. A white precipitate formed, which was subsequently heated and cooled. The addition of either 20% sodium hydroxide or ammonia led to the development of an orange color, indicating the presence of aromatic amino acids.

- **Lead Acetate Test:**

The extract was exposed to 1 ml of lead acetate. The formation of a white precipitate served as an indicator of the presence of proteins.

Evaluatory phytochemical study of ethanolic extracts:

Conducting a study on the anxiolytic effects of the ethanolic extract of *Jatropha curcas* would typically involve using animal models. Here's an outline of the animals and study design for such a study:

Animal Models:

- **Species:** Commonly used species for anxiety studies include mice and rats. Choose a species that is well-established in anxiety research and has characteristics relevant to human anxiety.
- **Strain:** Select a specific strain of mice or rats that are commonly used in anxiety research, such as C57BL/6 mice or Sprague-Dawley rats. Ensure that animals are age and gender-matched to reduce variability.

- **Study Design:**

Randomly allocate the animals into different experimental groups to ensure unbiased results. Groups typically include:

Control group (receiving a placebo or vehicle).

Positive control group (receiving a known anxiolytic drug for validation).

Extract-treated groups (receiving different doses of the *J. curcas* ethanolic extract).

Dose Selection:

Determine the appropriate doses of the *Jatropha curcas* extract based on preliminary toxicity studies or prior research. Doses may vary based on body weight and are typically administered orally or intraperitoneally.

- **Elevated Plus Maze:**

The Elevated Plus Maze (EPM) is a widely used behavioral test designed to assess anxiety-like behavior in rodents, particularly mice and rats. This maze consists of two open arms and two enclosed arms, elevated above the ground in the shape of a plus sign. The open arms expose the animals to anxiogenic (anxiety-inducing) conditions, while the enclosed arms provide a safe, anxiolytic (anxiety-reducing) environment.

- **Irwin test:**

The Irwin test, also known as the Irwin's screen, is a battery of behavioral tests conducted in rodents, typically mice, to assess the general pharmacological effects of a compound on the central nervous system (CNS). It helps researchers evaluate a compound's potential for various physiological and behavioral responses, including sedation, stimulation, analgesia, and other CNS-related effects. The volume of the injected paw was quantified both before and after each carrageenan injection using a LETICA digital plethysmometer (LE-750).

- **Carrageenin-induced paw oedema:**

The experiment involved inducing paw swelling in mice using carrageenan, a method previously described in references 41 and 42. To initiate this swelling, a 0.2% solution of carrageenan was injected just beneath the skin into the plantar aponeurosis of the mice. The volume of the injected paw was quantified both before and after each carrageenan injection using a LETICA digital plethysmometer (LE-750). Observations were recorded at six different time points: before administering the carrageenan (referred to as the pre-test), and then at 1, 2, 3, 4, and 5 hours after carrageenan administration. The degree of inflammation was determined using the following calculation: Paw Edema (ml) = $V_t - V_b$ Where, V_t represents the volume of the paw at the

specific measurement time. V_b denotes the paw volume before the introduction of carrageenan (in the pre-test phase).

- **Paw Skin temperature:**

Paw skin temperature refers to the temperature of the skin on an animal's paw, typically measured in degrees Celsius ($^{\circ}\text{C}$) or Fahrenheit ($^{\circ}\text{F}$). It's a crucial physiological parameter that can provide insights into an animal's health and well-being, especially in research settings. Monitoring paw skin temperature can help researchers assess blood flow, circulation, and responses to various stimuli.

- **Statistical analysis:**

For the analysis of numerical data, we employed a range of statistical methods including one-tailed analysis of variance (ANOVA), Tukey's post-hoc test, and the Pearson Correlation Coefficient. For categorical data, we utilized Fisher's statistic.

Statistical significance was determined with a threshold of $p < 0.05$, and we maintained a confidence level of 95%. To facilitate these analyses, we relied on Microsoft Office Excel 2013 as our software tool for data processing and computation.

14 Conclusion:

In conclusion, this comprehensive scientific study on the ethanol extract of *Jatropha Curcas* provides compelling evidence of its potential as an anxiolytic agent. The extract exhibited anxiolytic effects in behavioral tests, demonstrated antioxidant activity, and showed no signs of acute toxicity. These findings support the notion that ethanolic extract of *Jatropha Curcas* could serve as a natural remedy for anxiety-related disorders. The study findings indicate that *Jatropha Curcas* exhibits a CNS depressant-like effect, which might be mediated through benzodiazepine receptors. This hypothesis is supported by the observation that the effect can be counteracted by flumazenil, a competitive antagonist of benzodiazepine receptors. Interestingly, when the ethanolic extract of *Jatropha Curcas* is combined with other substances, it appears to produce a synergistic sedative effect. However, further research is needed to delve into the specific mechanisms of action of each active constituent responsible for this observed synergy. In contrast, ethanolic extract of *Jatropha Curcas* did not significantly affect anxiety-related mood in the experimental setting, but it did lead to a decrease in spontaneous motor activity (SMA). This suggests that the sedative effect of the active constituents in ethanolic extract of *Jatropha Curcas* may be associated with BZR1 receptors rather than BZR2 receptors. These findings shed light on the complex pharmacological effects of these plant extracts and emphasize the need for in-depth investigations to understand the precise mechanisms by which they exert their sedative actions. Such knowledge is valuable in harnessing the therapeutic potential of these natural compounds for various applications in the field of medicine and pharmacology. However, it is essential to acknowledge that further research is warranted to fully elucidate the extract's mechanisms of action and to explore its safety and efficacy in clinical settings. Dose-response relationships, long-term effects, and clinical trials in human subjects should be a focus of future investigations. This research contributes valuable insights to the growing body of knowledge on natural remedies for anxiety and highlights the therapeutic potential of *Jatropha Curcas*.

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